Effect of Spironolactone on Urinary Kallikrein Excretion in Patients with Essential Hypertension and in Primary Aldosteronism

MASAHIDE SEINO, KEISHI ABE, YUTAKA SAKURAI, NOBUO IROKAWA, MINORU YASUJIMA, SATORU CHIBA, YOICHI OTSUKA and KAORU YOSHINAGA

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

SEINO, M., ABE, K., SAKURAI, Y., IROKAWA, N., YASUJIMA, M., CHIBA, S., OTSUKA, Y. and YOSHINAGA, K. Effect of Spironolactone on Urinary Kallikrein Excretion in Patients with Essential Hypertension and in Primary Aldosteronism. Tohoku J. exp. Med., 1977, 121 (2), 111–119 — Urinary kallikrein excretion was measured before and after administration of spironolactone in 12 patients with essential hypertension (including 7 patients with low renin and 5 patients with normal renin) and 6 patients with primary aldosteronism. In low renin essential hypertension, two types of urinary kallikrein excretion were observed. In one type, urinary kallikrein decreased from 6.2±2.1 (s.E.) EU/day to 2.7±0.3 EU/day after the treatment. In another type, urinary kallikrein increased from 3.1±0.5 EU/day to 6.4±1.0 EU/day. In the former, plasma aldosterone showed high levels (12.3±2.1 ng/100 ml), while in the latter, it was normal (3.2±0.5 ng/100 ml). In normal renin essential hypertension, urinary kallikrein excretion did not alter after the treatment. In primary aldosteronism, urinary kallikrein showed moderate decrease after the spironolactone treatment from 8.5±1.6 EU/day to 4.2±1.6 EU/day. Spironolactone is said to compete directly with the effect of aldosterone at renal distal tubules. The present investigation suggests that urinary kallikrein excretion is related to the effective levels of aldosterone at renal distal tubules, and alteration of aldosterone levels mediates the release of kallikrein, and that there are different mechanisms in the renal handling of sodium and kallikrein in low renin essential hypertension, in normal renin essential hypertension, and in primary aldosteronism. — urinary kallikrein; low renin essential hypertension; primary aldosteronism; spironolactone

Urinary kallikrein is a renal enzyme (Nustad 1970a, b; Nustad et al. 1975) which produces a peptide kallidin, a potent vasodilator, from substrate kininogen (Webster and Pierce 1963). It is indistinguishable from renal kallikrein which is present in highest concentration in renal cortex (Nustad 1970b) but is different from plasma kallikrein (Webster and Pierce 1963).

Recently, the relationship between the kallikrein-kinin system and various types of hypertension in human (Margolius et al. 1971, 1972b, 1974b; Miyashita 1971; Seino et al. 1975) and experimental rats (Crotaatto and San Martin 1970; Margolius et al. 1972a; Geller et al. 1972, 1975; Porcelli et al. 1975) has been

Received for publication, September 8, 1976.
investigated. Patients with essential hypertension and hypertensive rats excrete less kallikrein than normotensive controls, whereas patients with primary aldosteronism excrete large amounts of kallikrein.

Recent papers also described that a low sodium diet (Margolius et al. 1974a, b; Seino et al. 1975) or administration of sodium-retaining steroids (Adetuyibi and Mills 1972; Geller et al. 1972; Marin-Grez et al. 1973; Margolius et al. 1974a, b) increased kallikrein excretion. There is a hypothesis that kallikrein excretion is related to the effective levels of circulating sodium-retaining steroid and that alteration of aldosterone levels mediate changes in kallikrein excretion (Margolius et al. 1974b).

The purpose of the present study is to elucidate the pathophysiological significance and the influence of aldosterone antagonist (spironolactone) on urinary kallikrein excretion in low and normal renin subgroups of essential hypertension and in primary aldosteronism.

**Material and Methods**

**Subjects**

Studies were performed on 12 patients with essential hypertension, including 7 cases of low renin type (5 men and 2 women, 26-58 years old), 5 cases of normal renin type (5 men, 23-43 years old) and 6 patients with primary aldosteronism (one man and 5 women, 30-47 years old).

The diagnosis of essential hypertension was established by a series of complete examination including history, physical examinations, routine laboratory tests, intravenous pyelography, radiisotope renography, renoscintigraphy, renal arteriography, and determination of 11-OHCS, aldosterone, and urinary catecholamines or vanillyl mandelic acid.

The patients with primary aldosteronism, who were cured later by removal of adrenal adenoma, had persistent hypokalemia, suppressed plasma renin activity and increased plasma aldosterone level.

Low renin and normal renin groups of essential hypertension were determined according to our criteria reported elsewhere (Abe et al. 1975). Antihypertensive medications had been discontinued at least two weeks before study.

These subjects were administered spironolactone (100 mg/day, oral administration) for 7 days under sodium and potassium intake of 200 mEq/day and 50-60 mEq/day, respectively. The diet had been started 5 days before the initiation of the medication.

Twenty four-hr urine samples were collected for measurement of kallikrein, sodium and potassium. Sampling of blood was done with fasting patients kept in recumbent position for at least one hr in the morning.

**Method for the measurement of urinary kallikrein**

*Fluorometric esterolytic assay.* The fluorometric assay for kallikrein described by Matsuda et al. (1976) was used. Urine was collected for 24 hr in a bottle containing a small amount of toluene as preservative and kept in a refrigerator at 4°C. It was stored at -15°C until the assay. Eight ml of each urine were taken and dialysed against running tap water for 16 hr at 4°C, then concentrated to 1/2-1/3 volume with polyethylene glycol at 4°C. After concentration the volume was measured and 0.1 ml was used for assay.

The esterolytic activity of the urine was determined by using p-tosyl-arginine-methyl-ester (TAME), product of Nakarai Chemical Ltd., Kyoto, as the substrate of kallikrein. The incubation mixture contained 0.1 ml each of 0.1 M phosphate buffer (pH 8.0), 5 mM TAME aqueous solution and a urine sample. Incubations were carried out exactly at
Effect of Spironolactone on Urinary Kallikrein

30°C for 30 min and 0.2 ml of 10% (w/v) HClO₄ solution was added to stop the reaction. Then, 0.2 ml of 0.1% (w/v) KMnO₄ solution was added to oxidize the methanol formed by the enzyme reaction. Then, 0.1 ml of 0.1% (w/v) NH₄OH·HCl solution and 3.0 ml of 0.2% (v/v) acetylacetone in 0.5 M ammonium malate solution (pH 6.0) were added. The mixture was kept in a water bath at 56°C for 20 min. The fluorescence was measured at 410 nm excitation and at 510 nm emission, 20-180 min after the incubation, by using a Hitachi MPF-4 fluorescence spectrophotometer. Standard curves of esterase activity were constructed using a purified human urinary kallikrein and methanol of analytical grade.

Each urine sample was assayed in duplicate. A standard curve and recoveries from urine samples were checked for each assay. Data were not corrected for recovery, since random urine recoveries were 92-104%. Results were expressed in terms of esterase units (EU). One esterase unit is defined as the amount of kallikrein which hydrolyzes one micromole of TAME per minute per ml at pH 8.0 and 30°C.

Bioassay Urine samples were also assayed for biologically active kallikrein by bioassay. Bioassay was done according to descriptions given by Sarnoff et al. (1958) and by Moriya et al. (1965).

Dog (10-15 kg) were anesthetized with 30 mg/kg of sodium pentobarbital given intravenously. Heparin (5 mg/Kg) was given as anticoagulant. After preparation, a centrally directed cannula in the femoral artery conducted blood flow through siliconized tygon tubing to flow transducer (Nihonkohden type FF 030 T, luminal size 3 mm) and backed to the femoral artery through a cannula directed distally. Biological vasodilator activity was determined by measuring the increase in arterial blood flow following the injection of both standard kallikrein and dialysed urine sample in 0.1-0.3 ml.

Flow recording was done on autorecorder (Nihonkohden RM-25) through electromagnetic flow meter (Nihonkohden MF 27) and amplifier (Nihonkohden RB-5).

This procedure permitted the measurement of kallikrein in the dog hind leg perfusion between 0.005 KU (Kallikrein unit) to 0.04 KU and was obtained a good dose-response curve between these doses. Analysis of these samples for kallikrein by fluorometric assay related to the results obtained by bioassay (Fig. 1). One esterase unit equals to 25.0 kallikrein units of standard human urinary kallikrein.

Data are expressed as kallikrein EU (esterase unit) excreted per 24 hr. Plasma renin activities were determined by a modification of Haber's method (Abe et al. 1972). Plasma

![Graph](image)

Fig. 1. The relationship between urinary kallikrein determined by fluorometric esterolytic assay and bioassay.

The correlation coefficient was r=0.91. EU, esterase unit; KU, kallikrein unit.
aldosterone was measured by radioimmunoassay-kit (Midorijuji Ltd.). Sodium and potassium concentrations were measured by an autoanalyser.

RESULTS

In low renin essential hypertension, two types of kallikrein excretion were observed (Fig. 2). In one type (three cases out of seven), urinary kallikrein excretion decreased from 6.2±2.1 (S.E.) EU/day to 2.7±0.3 EU/day after the administration of spironolactone \((p<0.2)\). Urinary volume (UV) and urinary potassium excretion (U_KV) slightly decreased and urinary sodium excretion (U_NaV) increased, but the changes were not significant. Plasma renin activity (PRA) increased from 4.7±0.3 ng/ml to 23±6.3 ng/ml \((p<0.05)\). Mean blood pressure (MBP) fell slightly from 126±4.6 mmHg to 110±6.2 mmHg after the treatment \((p<0.1)\). In another type (4 cases) of low renin essential hypertension, urinary kallikrein increased from 3.1±0.5 EU/day to 6.4±1.0 EU/day after the administration of spironolactone \((p<0.02)\). UV and U_NaV slightly increased but the changes were not statistically significant. U_KV increased from 37±1.0 mEq/
Fig. 3. Changes in urinary kallikrein, $U_{Na}$V, $U_{Kr}$V, urine volume, PRA and MBP after spironolactone administration in normal renin essential hypertension. $n=5$. Closed circle means $U_{Na}$V. Bars represent means±s.e.

day to $50±2.5$ mEq/day ($p<0.001$). PRA increased from $3.5±0.5$ ng/ml to $9.9±3.1$ ng/ml after the spironolactone treatment ($p<0.05$). MBP fell significantly from $121±1.4$ mmHg to $105±2.7$ mmHg after the treatment ($p<0.001$). Plasma aldosterone levels in low renin essential hypertension were different between the two types before the treatment. In the former (3 cases in whom urinary kallikrein decreased after the spironolactone treatment), plasma aldosterone showed high levels. The values were $12.3±2.1$ ng/100 ml (normal $5.1±0.9$ ng/100 ml). In the latter (4 cases in whom urinary kallikrein increased after treatment), plasma aldosterone level was $3.2±0.5$ ng/100 ml.

In patients with normal renin essential hypertension, urinary kallikrein increased from $5.3±0.6$ EU/day to $7.5±1.1$ EU/day on the first day after the administration of spironolactone. But the levels after 7 days' treatment returned to the control value ($5.8±0.7$ EU/day) (Fig. 3). UV, $U_{Na}$V and $U_{Kr}$V were unchanged after spironolactone treatment compared with those in control period. PRA increased from $11.7±1.9$ ng/ml to $17.4±4.1$ ng/ml, but the changes were not significant. MBP was unchanged after treatment. Plasma aldosterone level in normal renin essential hypertension was $3.7±0.3$ ng/100 ml.

In primary aldosteronism, urinary kallikrein showed moderate decrease after
Fig. 4. Changes in urinary kallikrein, $U_{NaV}$, $U_{KV}$, urine volume, PRA and MBP after spironolactone administration in primary aldosteronism. $n=6$. Closed circle means $U_{NaV}$. Bars represent means±s.e.

spironolactone treatment from $8.5±1.6$ EU/day to $4.2±1.6$ EU/day. UV was decreased, $U_{NaV}$ slightly increased, but these changes were not significant. $U_{KV}$ was decreased from $55±3.7$ mEq/day to $34±7.4$ mEq/day ($p<0.02$) (Fig. 4). PRA was suppressed before treatment (3.4±0.7 ng/ml) and remained low (4.9±1.1 ng/ml) after 7 days’ spironolactone treatment. MBP was slightly reduced after the treatment but the change was not significant.

DISCUSSION

Spironolactone is known as a specific aldosterone antagonist in the renal tubules (Vander et al. 1960). The previous observations indicated that aldosterone or other sodium-retaining steroid hormones increase kallikrein excretion in human and rat urine. On the other hand kallikrein excretion decreased after spironolactone treatment in a single subject with primary aldosteronism (Margolius et al. 1974b). Further, Kaizu and Margolius (1975) reported recently that kallikrein activity in suspensions of rat renal cortical cells could be increased by aldosterone and decreased by spironolactone.

Now, one of the topics of hypertension research is on low renin essential
Effect of Spironolactone on Urinary Kallikrein

hypertension. In patients with essential hypertension, about 20% have relatively low plasma renin activity and fail to increase it normally in response to sodium restriction and standing (Brunner et al. 1972; Crane et al. 1972). A number of studies have suggested that mineralocorticoid excess may be a possible cause of low renin levels in these patients (Woods et al. 1969; Melby et al. 1971). However, most of the hyporeninemic patients have normal or low aldosterone secretion rates. Low renin hypertensives have a greater fall in blood pressure than other hypertensives when they are treated with spironolactone or thiazide diuretics (Spark and Melby 1971; Adlin et al. 1972; Carey et al. 1972). These reports suggest that low renin hypertension may be a discrete entity etiologically different from the usual form of essential hypertension.

It could be thought that kallikrein has an antihypertensive effect through the regulation of sodium excretion by the kidney. Mineralocorticoid seems to have some influence on the renal kallikrein. It is, therefore, of interest to examine the relationship between kallikrein excretion, UNaV, UKV, PRA, plasma aldosterone and MBP in hypertensive state with or without spironolactone treatment.

In low renin essential hypertension, two types of kallikrein excretion were observed. In one type (three cases), urinary kallikrein excretion decreased after spironolactone treatment. UNaV slightly increased and UKV decreased after the treatment. Plasma aldosterone before the spironolactone treatment showed high levels in this type. This suggests that spironolactone competes directly with the effect of aldosterone on the release of kallikrein at the renal tubules. In another type (4 cases), urinary kallikrein increased after the administration of spironolactone. UNaV and UKV increased slightly. PRA increased and MBP fell significantly. In this type, plasma aldosterone level was normal. It could be thought that this fall of MBP and increase of PRA depend upon natriuresis caused by aldosterone antagonist as well as by increased kallikrein activity. Kinins are known as one of the most potent vasodilators; when infused in the renal artery kinins produce marked natriuresis (Webster and Gilmore 1964; Barraclough and Mills 1965; Stein et al. 1972). It may be thought that increased kallikrein after the spironolactone treatment in low renin essential hypertension produces kinin in the renal cortex, and these kinins are released to peritubular interstitial space or peritubular capillary circulation. The reason why kallikrein excretion was increased in the latter group of low renin patients in spite of the spironolactone administration is unknown.

In normal renin essential hypertension, urinary kallikrein excretion, UNaV, UKV, PRA and MBP were unchanged after the spironolactone treatment. It may be suggested that there is a different mechanism of sodium and kallikrein handling by the kidney in normal renin essential hypertension as compared with low renin essential hypertension.

In primary aldosteronism, urinary kallikrein excretion after the spironolactone treatment showed a decrease accompanied by a slight increase in UNaV and a decrease in UKV. This indicates that spironolactone competes directly with the
effect of aldosterone on the release of kallikrein at the renal distal tubules. MBP showed a slight fall after the spironolactone treatment but the change was not significant. PRA was suppressed. These facts might indicate that spironolactone, used in the present study, was not sufficient to restore the sodium and water balance in these patients.

These investigations suggest that urinary kallikrein excretion is related to the effective levels of aldosterone at the renal distal tubules, and alterations of aldosterone levels at the tubules mediate the release of kallikrein. On the other hand, it may be that there is a different mechanism of sodium and kallikrein handling by the kidney in low renin essential hypertension as compared with normal renin essential hypertension and primary aldosteronism.

References

Effect of Spironolactone on Urinary Kallikrein