Urinary kallikrein excretion and plasma renin activity in patients with essential hypertension and primary aldosteronism

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Summary

1. The 24 h urinary excretion of kallikrein has been studied in 40 normotensive control subjects and in 74 age-matched patients with essential hypertension under similar conditions. By use of the renin-sodium index, hypertensive patients were divided into two subgroups: low-renin hypertension and normal-renin hypertension patients. Urinary kallikrein determinations were also obtained from six hypertensive patients with primary aldosteronism.

2. Urinary kallikrein was significantly lower both in patients with normal-renin and low-renin essential hypertension. Urinary kallikrein excretion was very high in the patients with primary aldosteronism.

3. In nine hypertensive patients β-adrenoceptor-blocking therapy caused a significant decrease of plasma renin activity, but had no significant effect on urinary kallikrein excretion.

4. The results support the concept that low urinary kallikrein is likely to be a marker of essential hypertension. Under certain conditions its excretion is positively related to mineralocorticoid hormone concentrations but it is not primarily related to the renin–angiotensin system.

Key words: aldosteronism, hypertension, renin activity, urinary kallikrein.

Abbreviations: PRA, plasma renin activity; BAEE, benzoylarginine ethyl ester.

Introduction

The role of renal kallikrein and kinins in arterial hypertension has been emphasized by Margolius, Geller, Pisano & Sjoerdsma (1971). Subsequently, further investigations confirmed the close relationship between urinary kallikrein and sodium metabolism in physiological and pathological conditions (Adetuyibi & Mills, 1972; Carretero & Oza, 1973; Margolius, Horwitz, Geller, Alexander, Gill, Pisano & Keiser, 1974a) and also in experimental hypertension (Croxatto & San Martin, 1970; Margolius, Geller, de Jong, Pisano & Sjoerdsma, 1972). The kallikrein–kinin system, because of its potent vasodilator activity, may regulate renal sodium excretion by influencing peritubular forces, acting therefore as a natriuretic factor. It has been demonstrated by Margolius, Horwitz, Pisano & Keiser (1974b) that urinary excretion of kallikrein is significantly diminished in patients with essential hypertension, increased in patients with secondary hypertension (primary aldosteronism and phaeochromocytoma) and normal in hypertensive patients with renal artery stenosis.

In a preliminary report (Lechi, Mengoli, Covi, Lechi & Zatti, 1973) we have confirmed the existence of a lower urinary kallikrein excretion in patients with essential hypertension. However, this group of patients does not behave homogeneously,
some hypertensive patients excreting a normal amount of kallikrein, or even higher than in normal subjects, and the mean excretion of urinary kallikrein was only significantly lower in normokalaemic hypertensive patients, but was normal in hypokalaemic patients.

In order to clarify the relationship between urinary kallikrein excretion and the metabolic profiles of arterial hypertension, we have investigated the enzyme excretion in relation to plasma renin activity. In this study we have not considered hypokalaemic hypertensive patients since in these an alteration of the renin—angiotensin—aldosterone system might have occurred. It is well known that plasma renin activity is within normal limits in most patients with essential hypertension. In about 30% of patients, however, the activity is abnormally high or low (Laragh, 1973; Drayer, Kloppenborg & Benraad, 1975), but it is unclear if these groups represent a well-defined pathological entity (Dunn & Tannen, 1974; Thurston & Swales, 1976). If the urinary kallikrein excretion is really related to mineralocorticoid secretion and sodium balance, it would be interesting to investigate kallikrein excretion in types of hypertension where there may be some disorders of the renin—angiotensin system.

**Patients and control subjects**

Kallikrein excretion was measured in 74 patients with essential hypertension, six patients with primary aldosteronism and 40 normotensive subjects under 65 years of age. All hypertensive patients showed a diastolic blood pressure between 100 and 140 mmHg, after repetitive measurements under basal conditions, and after at least 2 days in hospital. At the time of the study patients were either untreated or had treatment withdrawn (i.e. diuretics, other hypotensive agents and potassium supplements) for at least 1 week, diuretics being withdrawn for much longer than 1 week in most cases. All patients had normal renal function: serum urea concentration less than 7.8 mmol/l, serum creatinine less than 106 µmol/l and glomerular filtration rate (measured as endogenous creatinine clearance) more than 80 ml/min.

Hypertensive patients with normal and low plasma renin activity (PRA) were classified according to the PRA-sodium index suggested by Laragh (1973). PRA was plotted against the 24 h sodium excretion in a group of 26 normotensive control subjects on a normal, high or low sodium diet after 3 h in the upright position. This plot revealed a hyperbolic relation similar to that found by Laragh (1973) and by Woods, Pittman, Pulliam, Werk, Waider & Allen (1976). When expressed as the natural-log (In) plot of PRA (Fig. 1), there were 56 hypertensive patients with normal PRA (30 men and 26 women), and 18 with low PRA (five men and 13 women).

Nine patients with essential hypertension were treated with propranolol (120 mg/day). PRA and urinary kallikrein excretion were measured before and after 2 weeks treatment. During this period, all patients were hospitalized and dietary sodium intake was kept constant at 140 mmol/24 h.

Primary aldosteronism was diagnosed on the basis of a reduced PRA in the presence of elevated urinary aldosterone excretion and selective adrenal venography. Surgical intervention confirmed the diagnosis of adenoma in two patients and of bilateral hyperplasia in two others. The remaining patients underwent medical therapy.

The control population consisted of 24 men and 16 women. These subjects had no evidence of renal, hepatic or cardiac disease and, moreover, alterations of water and electrolyte metabolism were carefully excluded by clinical and laboratory
investigations. Systolic blood pressure was below 130 mmHg and diastolic blood pressure below 90 mmHg in repeated measurements.

Methods
Blood samples for measurement of PRA were taken between 08.00 and 09.00 hours after overnight fasting and recumbency and subsequently after 3 h erect. All hypertensive patients and control subjects were studied in hospital. Blood was collected into precooled EDTA tubes; the plasma was immediately separated at 4°C, frozen and stored at -20°C. PRA was measured by a radi-immunoassay estimation of the rate of generation of angiotensin I and expressed as pmol h⁻¹ ml⁻¹ by the method of Boyd, Fitz, Adamson & Peart (1969).

Collections of urine for 24 h were obtained in the same day and, during collections, urine was kept in plastic containers at 4°C. Urine samples were then stored at -20°C until assayed; when not immediately assayed, samples were kept frozen for no more than 2 weeks. Urinary kallikrein was assayed by the method based on esterolytic activity of the enzyme on the synthetic substrate benzoylarginine ethyl ester (BAEE) and expressed as bio- logical units. Kininogenin (EC 3.4.21.8) activity was determined by the method of Trautschold, Werle & Schweitzer (1970), which permits the determination of about 0.1 unit of kallikrein. It has been demonstrated by Margolius et al. (1974a) that esterolytic assay gives results in agreement with those of kininogenasic activity bioassay. The ratio between kininogenasic activity and esterolytic activity on synthetic substrates has been demonstrated to be constant by Pierce (1970) and by Nustad (1970).

Plasma and urinary sodium and potassium concentrations were measured by flame photometer, creatine concentration by a colorimetric method (Auto-analyzer, Technicon).

Mean values are given ±1 SEM, and significance of difference was evaluated by unpaired Student’s t-test.

Results
The ages of the normal-renin subgroup of hypertensive patients (43 ± 2 years) and normal subjects (41 ± 2 years) were not significantly different, but the patients of the low-renin subgroup were older (53 ± 2 years). Mean plasma potassium values were very similar in the three groups: control subjects, 4.15 ± 0.06 mmol/l, normal-renin hypertension, 4.16 ± 0.05 mmol/l, and low-renin hypertension, 4.15 ± 0.08 mmol/l.

No difference for age and sex was found in kalli- krein excretion either in control or in hypertensive patients. Kallikrein excretion was slightly, but not significantly, lower in control women (147.0 ± 10.4 units/24 h) than in control men (151.3 ± 15.3 units/24 h), but it was higher (P > 0.1) in hypertensive women (92.7 ± 10.6 units/24 h) than in hypertensive men (83.9 ± 8.7 units/24 h). Kalli- krein excretion in control subjects ranged from 45 to 288 units/24 h, mean 149.6 ± 9.9 units/24 h. Compared with control subjects (Fig. 2) a highly significant difference was found in hypertensive patients whose urinary kallikrein excretion ranged from 0 (not detectable) to 232 units/24 h, mean 88.5 ± 6.9 units/24 h (P < 0.001). No significant difference was found for urinary kallikrein excre- tion among patients with normal and low-renin hypertension (Table 1). Very high levels of urinary kallikrein excretion were found in the six patients with primary aldosteronism (mean 394.2 ± 76.3 units/24 h).

Table 2 shows the results in patients treated with propranolol. Before treatment the recumbent blood pressure averaged 176 ± 5 mmHg systolic and 110 ± 3 mmHg diastolic and dropped to 158 ± 5/101 ± 4 mmHg on treatment. A significant reduction of PRA was observed, but no change of urinary kalli- krein excretion took place.

Discussion
It has been suggested that some patients with low-renin hypertension might have either an abnormal mineralocorticoid secretion (Spark & Melby, 1971) or an extracellular fluid volume expansion due to an impaired renal ability to excrete sodium nor- mally (Jose, Crout & Kaplan, 1970). Nevertheless, this has not been confirmed by others and the reasons for a reduced PRA and its responsiveness to stimulation in essential hypertension are still uncertain. PRA is known to be affected by influen- ces other than sodium balance, notably age (Schalekamp, Krauss, Schalekamp-Kuyken, Kolster & Birkenhöger, 1971; Hayduk, Krause, Kaufmann, Huenges, Schillmöller & Unbehau, 1973).

Since it has been reported that urinary kallikrein excretion increases both after administration of mineralocorticoid and after expansion of extracellular fluid volume (Edwards, Adetuyibi & Mills, 1973; Margolius et al., 1974a), an abnormality in
renal sodium handling in patients with low-renin hypertension might be reflected in their kallikrein excretion. However, we have not been able to find a relationship between PRA and urinary kallikrein excretion in hypertensive patients, and, urinary kallikrein was not significantly different in patients with low- and normal-PRA hypertension.

These results and the finding that inhibition of renin by propranolol did not alter urinary kallikrein excretion do not support the hypothesis that kallikrein production is directly regulated by renal production of renin, as suggested by Johnston, Matthews & Dax (1976).

In patients with primary aldosteronism urinary kallikrein excretion is very high. This has been related to a chronic escape phenomenon, kallikrein having a natriuretic effect (Margolius et al., 1971; Edwards et al., 1973). However, very high levels of urinary kallikrein excretion have also been found in patients with Bartter’s disease (Lechi, Covi, Lechi, Mantero & Scuro, 1976), where PRA is extremely high and extracellular fluid volume is contracted. Consequently, urinary kallikrein excretion in these patients appears to be related to high mineralocorticoid hormone concentrations and not to expansion of extracellular fluid volume (Margolius

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**FIG. 2.** Urinary kallikrein excretion in control subjects, patients with normal-renin and low-renin hypertension and patients with primary aldosteronism. Mean values ± SD are shown by horizontal lines.

**TABLE 1.** Mean values for age, blood pressure, plasma creatinine concentration, urinary sodium, potassium and kallikrein excretion in control subjects, in patients with normal-renin and low-renin hypertension and in patients with primary aldosteronism. Results are expressed as mean values ± SEM. Significance of difference from control subjects: *P < 0.001.

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (years)</th>
<th>Blood pressure (mmHg)</th>
<th>Plasma creatinine (µmol/l)</th>
<th>Urinary sodium (mmol/24 h)</th>
<th>Urinary potassium (mmol/24 h)</th>
<th>PRA (pmol h⁻¹ ml⁻¹)</th>
<th>Urinary kallikrein (units/24 h)</th>
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<td></td>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
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<tr>
<td>Control subjects</td>
<td>40</td>
<td>41 ± 2</td>
<td>124 ± 2</td>
<td>78 ± 1</td>
<td>80 ± 2</td>
<td>134 ± 7</td>
<td>45 ± 3</td>
</tr>
<tr>
<td>Essential hypertension, normal PRA</td>
<td>56</td>
<td>43 ± 2</td>
<td>183 ± 3</td>
<td>116 ± 2</td>
<td>80 ± 2</td>
<td>121 ± 8</td>
<td>40 ± 2</td>
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<tr>
<td>Essential hypertension, low PRA</td>
<td>18</td>
<td>53 ± 2*</td>
<td>194 ± 7</td>
<td>115 ± 3</td>
<td>77 ± 3</td>
<td>118 ± 13</td>
<td>39 ± 3</td>
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<tr>
<td>Primary aldosteronism</td>
<td>6</td>
<td>42 ± 2</td>
<td>186 ± 2</td>
<td>119 ± 4</td>
<td>84 ± 5</td>
<td>102 ± 23</td>
<td>40 ± 6</td>
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et al., 1974b). Thus urinary kallikrein excretion is increased in chronic aldosteronism, both primary and secondary, and is not related to PRA. In these cases, changes in extracellular fluid volume and mineralocorticoid hormone concentrations are very consistent, whereas in renin subgroups of patients with essential hypertension changes in extracellular fluid volume, if present, are much more subtle.

Kallikrein excretion was found to be suppressed in both normal- and low-renin essential hypertension, as compared with normotensive control subjects. It is possible that an abnormality of the so-called pressure natriuretic mechanism (Lowenstein, Beranbaum, Chasis & Baldwin, 1970; Brown, Lever, Robertson & Schalekamp, 1974), not directly related to mineralocorticoids and renin, is somehow linked with the kallikrein–kinin system.

Low urinary excretion of kallikrein seems to be one of the biochemical markers of essential hypertension. This phenomenon might be linked with the stage of the disease, but the underlying mechanism is unknown.

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