Urinary Kallikrein Excretion in Hypertensive Man

RELATIONSHIPS TO SODIUM INTAKE AND SODIUM-RETAINING STEROIDS

Harry S. Margolius, David Horwitz, John J. Pisano, and Harry R. Kelser

ABSTRACT

Urinary kallikrein excretion was measured by a radiochemical esterolytic assay in patients with essential hypertension or primary aldosteronism. Patients with essential hypertension excreted significantly less \( (P < 0.001) \) kallikrein than did normal subjects when they were allowed an ad libitum sodium intake or given 259 mEq sodium/day. When sodium intake was changed from ad libitum to 9 mEq/day, kallikrein excretion increased in the majority of patients with essential hypertension, but it remained significantly less \( (P < 0.001) \) than that in normal subjects; however, aldosterone excretion was similar in both groups. Fludrocortisone, 0.5 mg/day for 10 days, increased kallikrein excretion in three patients with essential hypertension. In patients with primary aldosteronism, mean kallikrein excretion was sevenfold higher \( (P < 0.001) \) than that in patients with essential hypertension; kallikrein excretion remained unchanged when dietary sodium was altered, but it was decreased by treatment with spironolactone. Mean kallikrein excretion in patients with primary aldosteronism was also significantly higher \( (P < 0.001) \) than that in a normotensive control population. The results show that kallikrein excretion reflects the effective level of circulating sodium-retaining steroid in patients with primary aldosteronism but suggest that it is relatively unresponsive to endogenous sodium-retaining steroid in patients with essential hypertension. The data raise the possibility that the kallikrein-kinin system is of pathogenetic significance in human hypertensive disease.

KEY WORDS

spironolactone
fludrocortisone
essential hypertension
low dietary sodium
primary aldosteronism
high dietary sodium

A relationship between the kallikrein-kinin system and hypertensive disease was first suggested by Elliot and Nuzum (1) 40 years ago when they found that urinary kallikrein excretion, measured by a bioassay, was subnormal in patients with essential hypertension. That finding has been confirmed recently in our laboratory (2-4). Moreover, we have found that patients with primary aldosteronism have supranormal levels of urinary kallikrein excretion (2-4). However, none of these studies considered the factors other than blood pressure—the level of dietary sodium intake and the level of activity of sodium-retaining steroids (5, 6)—that are now known to influence kallikrein excretion.

The purpose of the present study was therefore to reexamine kallikrein excretion in patients with essential hypertension under controlled sodium intakes and in response to fludrocortisone. In addition, kallikrein excretion was reexamined in patients with primary aldosteronism under controlled sodium intakes and in response to spironolactone.

Methods

PATIENTS AND CONTROL SUBJECTS

The principal subjects of this investigation were 11 patients with essential hypertension (8 black and 3 white men 34-52 years old, mean 45 years) and 3 patients with primary aldosteronism (3 black men 49-62 years old). The patients with essential hypertension were subjected to a complete hypertensive evaluation including rapid-sequence intravenous pyelography and, as indicated, renal arteriography or other tests to exclude known causes of hypertension. The patients with primary aldosteronism had persistent hypokalemia, low plasma renin activity, and supranormal aldosterone excretion rates; they had been treated successfully with spironolactone. However, no specific anatomic diagnoses had been made. No patient had congestive heart failure or malignant hypertension. All patients had blood urea nitrogen concentrations and creatinine clearances within the normal range.

The patients discontinued antihypertensive and diuretic drugs and began consuming sodium chloride ad libitum 2-3 weeks before admission to the Clinical Center. Patients who became normotensive after admission (diastolic pressures below 90 mm Hg on more than one occasion during the first 4 days of hospitalization)
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were eliminated from the protocols. Morning blood pressure levels on the fourth day of hospitalization ranged from 120/98 to 170/110 mm Hg while the patients were supine and from 130/90 to 160/130 mm Hg while they were standing.

In addition, urine collections were obtained from 28 other patients with essential hypertension and 9 other patients with primary aldosteronism. In the latter group, the anatomic diagnosis, if any (determined by surgical exploration), the aldosterone excretion rate, and the dietary sodium intake at the time of urine collection were also ascertained.

Data from hypertensive patients were compared with those from 7 group 1 normal subjects (19-29 years old, mean 22 years) and 6 group 2 normal subjects (41-55 years old, mean 47 years) studied previously (5) and with those from 44 additional normal subjects on ad libitum sodium intakes (urinary sodium excretion greater than 80 mEq/day). Informed consent was obtained from all subjects.

PROTOCOLS

Effects of Sodium Intake.—Nine patients with essential hypertension (seven black and two white men) were allowed an ad libitum diet for 5 days, followed by a diet containing 9 mEq sodium/day. After 8 days, 250 mEq of sodium as salt in a shaker was added to their diet for 7-9 days. All of the subjects were given furosemide (40 mg/day, orally) for the first 3 days when their sodium intake was restricted to 9 mEq/day to accelerate sodium loss. Three patients with primary aldosteronism were studied under the same conditions.

Effects of Fludrocortisone.—Three patients with essential hypertension (one white and two black men) were given a diet containing 109 mEq sodium/day and 100 mEq potassium/day for 22 days. After a 6-day control period, each subject took fludrocortisone (Florinef) tablets (0.5 mg, orally) each morning for 10 days. Following discontinuation of spironolactone administration the diet continued for 7 more days.

Twenty-four-hour urine collections and all other measurements were obtained as described previously (5). Data were analyzed for significance with Student's t-test.

Results

Effects of Sodium Intake: Essential Hypertension.—Kallikrein excretion, measured by the radiochemical esterolytic assay, averaged 5.0 ± 0.8 (SE) esterase units (E.U.)/day on the fourth day of ad libitum sodium intake in the hypertensive pa-

1 Urine from patients with primary aldosteronism was kindly supplied by Dr. John Luetscher, Stanford University, Dr. John Laragh, Columbia University, and Dr. Frederic Bartter of the National Institutes of Health.

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tients. This value is significantly less ($P < 0.001$) than the levels in either group 1 (younger) or group 2 (older) normotensive subjects; it is also significantly less ($P < 0.001$) than the mean kallikrein excretion for groups 1 and 2 combined (Fig. 1). The mean kallikrein excretion of the hypertensive patients increased to 9.4 ± 1.8 E.U./day on the seventh day of the diet containing 9 mEq sodium/day, a value significantly higher ($P < 0.001$) than that during ad libitum sodium intake. However, three of the nine patients showed little or no increase in kallikrein excretion. On the diet containing 259 mEq sodium/day, kallikrein excretion gradually decreased over 7-9 days to values which were not significantly different from those observed during ad libitum sodium intake. Kallikrein excretion throughout the entire protocol was significantly lower ($P < 0.001$) in hypertensive patients than it was in either group 1 or group 2 normal subjects (Fig. 1).

Plasma renin activity in patients with essential hypertension on day 7 of the diet containing 9 mEq sodium/day (12.2 ± 2.2 ng/ml hour$^{-1}$) was significantly lower ($P < 0.001$) than that in the comparably aged group 2 normal subjects (17.4 ± 1.3 ng/ml hour$^{-1}$) (Table 1). However, aldosterone excretion in the hypertensive patients on day 7 of the diet...
TABLE 1

Urinary Sodium Excretion, Plasma Renin Activity, Aldosterone Excretion Rate, and Urinary Kallikrein Excretion in Patients with Essential Hypertension Compared with Values in Normal Subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Ad libitum Na intake</th>
<th>9 mEq Na/day</th>
<th>259 mEq Na/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U_{\text{Na}} (mEq/day)</td>
<td>PRA (ng/ml hour (^{-1}))</td>
<td>Aldo (\mu g/day)</td>
</tr>
<tr>
<td>Essential hypertensives (9)</td>
<td>170 ± 13</td>
<td>2.5 ± 0.4</td>
<td>9.4 ± 1.5</td>
</tr>
<tr>
<td>Group 1 Normals (7)</td>
<td>154 ± 19</td>
<td>2.2 ± 0.6</td>
<td>8.7 ± 2.2</td>
</tr>
<tr>
<td>Group 2 Normals (6)</td>
<td>105 ± 13†</td>
<td>5.1 ± 1.7</td>
<td>12.9 ± 0.9‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. U_{\text{Na}} urinary sodium excretion, PRA plasma renin activity, Aldo = aldosterone excretion rate, and Kallikrein (E.U./day) = esterase units in a 24-hour collection of urine. Number of subjects studied is given in parentheses.

* P < 0.001 compared with mean of patient data.
† P < 0.005 compared with mean of patient data.
‡ P < 0.05 compared with mean of patient data.

TABLE 2

Urinary Sodium Excretion, Plasma Renin Activity, Aldosterone Excretion Rate, and Urinary Kallikrein Excretion in Patients with Primary Aldosteronism or Essential Hypertension

<table>
<thead>
<tr>
<th>Patients</th>
<th>Ad libitum Na intake</th>
<th>9 mEq Na/day</th>
<th>259 mEq Na/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U_{\text{Na}} (mEq/day)</td>
<td>PRA (ng/ml hour (^{-1}))</td>
<td>Aldo (\mu g/day)</td>
</tr>
<tr>
<td>Primary aldosteronism (3)</td>
<td>113 ± 18</td>
<td>1.2 ± 0.1</td>
<td>34.3 ± 10.8</td>
</tr>
<tr>
<td>Essential hypertension (9)</td>
<td>170 ± 13</td>
<td>2.5 ± 0.4</td>
<td>9.4 ± 1.5</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.025</td>
<td>&lt; 0.005</td>
<td>&lt; 0.025</td>
</tr>
</tbody>
</table>

All values are means ± SE. U_{\text{Na}} urinary sodium excretion, PRA plasma renin activity, Aldo = aldosterone excretion rate, and E.U. = esterase unit.
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Effect of spironolactone on urinary kallikrein excretion, urinary sodium excretion (U\textsubscript{Na}V), weight (Wt.), and blood pressure in a patient with primary aldosteronism. E.U. = esterase unit.

containing 9 mEq sodium/day (42.7 ± 11.4 µg/day) was similar to that in group 2 normal subjects (41.4 ± 3.1 µg/day) (Table 1). Thus, differences in kallikrein excretion between patients with essential hypertension and comparably aged normal subjects are associated with different levels of plasma renin activity but with similar levels of aldosterone excretion. Mean urinary sodium excretion in patients with essential hypertension was similar to that in comparably aged normal subjects when sodium intake was either 9 or 259 mEq/day (Table 1).

**TABLE 3**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Control (0.5 mg/day)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.H.</td>
<td>53</td>
<td>9.6</td>
<td>10.1</td>
</tr>
<tr>
<td>C.N.</td>
<td>44</td>
<td>3.4</td>
<td>4.5</td>
</tr>
<tr>
<td>N.S.</td>
<td>42</td>
<td>8.4</td>
<td>8.9</td>
</tr>
<tr>
<td><strong>MEAN</strong></td>
<td>7.1</td>
<td>17.8</td>
<td>7.8</td>
</tr>
<tr>
<td>± 8E</td>
<td>1.9</td>
<td>5.5</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Kallikrein (E.U./day) = esterase units in a 24-hour collection of urine.

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**FIGURE 3**

Kallikrein excretion in normal subjects and patients with either essential hypertension or primary aldosteronism. E.U. = esterase unit, Ad lib Na\textsuperscript{+} = ad libitum sodium intake, and U\textsubscript{Na}V = sodium excretion.

Effects of Sodium Intake: Primary Aldosteronism.—Mean kallikrein excretion in three patients with primary aldosteronism was not changed by either 7 days on a diet containing 9 mEq sodium/day or 9 days on a diet containing 259 mEq sodium/day and was significantly higher (P < 0.05) than that in patients with essential hypertension studied under the same conditions (Table 2). In patients with primary aldosteronism, plasma renin activity was lower (P < 0.005) and aldosterone excretion was higher (P < 0.025) than they were in patients with essential hypertension under the same conditions (Table 2). In nine other patients with primary aldosteronism, kallikrein excretion averaged 22.7 ± 4.7 E.U./day when sodium intake was 9 mEq/day and 28.7 ± 4.6 E.U./day when sodium intake was high (259-300 mEq/day); these values are significantly higher (P < 0.001) than those in patients with essential hypertension.
Effects of Fludrocortisone.—In three patients with essential hypertension on a sodium intake of 109 mEq/day, kallikrein excretion averaged 7.1 ± 1.9 E.U./day (days 5 and 6), increased with fludrocortisone treatment to 17.8 ± 5.5 E.U./day (days 9 and 10), and decreased to 7.8 ± 1.7 E.U./day (days 5 and 6) after fludrocortisone treatment had been stopped (Table 3).

Effects of Spironolactone.—In a single subject with primary aldosteronism on a sodium intake of 109 mEq/day, kallikrein excretion decreased from 22.3 E.U./day on the last day of the control period to 6.1 E.U./day after 8 days of spironolactone (400 mg/day) treatment. Kallikrein excretion increased to 22.9 E.U./day 7 days after administration of spironolactone had been stopped (Fig. 2). Kallikrein excretion in this subject ranged from 18.9 to 32.0 E.U./day during the 23 additional days of study of the effects of altered sodium intake (Table 2).

Summary of Urinary Kallikrein Excretion in Normal and Hypertensive Subjects.—In 57 normal subjects, kallikrein excretion averaged 8.5 ± 0.6 E.U./day (range 2.4 to 19.6 E.U./day), whereas it averaged 3.8 ± 0.4 E.U./day (range 0.2 to 10.2 E.U./day) in 39 patients with essential hypertension (P < 0.001) (Fig. 3). Kallikrein excretion averaged 26.4 ± 3.6 E.U./day (range 9.3 to 44.8 E.U./day) in 10 patients with primary aldosteronism, a value significantly higher (P < 0.001) than that in either normal subjects or patients with essential hypertension (Fig. 3). All of these subjects were excreting more than 80 mEq sodium/day when these determinations were made.

Discussion

Patients with essential hypertension excreted significantly less kallikrein than did either younger or older normal subjects over a range of dietary sodium intakes from 9 to 259 mEq/day. Patients with essential hypertension also showed significantly smaller increases in kallikrein excretion in response to a diet containing 9 mEq sodium/day than did normal subjects. The relationship of this subnormal kallikrein response to the subnormal renin response to a low-sodium diet is unclear. We have shown that kallikrein excretion is controlled primarily by the effective level of circulating sodium-retaining steroid (5). Therefore, two possible explanations for the low and less-responsive kallikrein excretion in patients with essential hypertension are: (1) these patients have subnormal levels of circulating sodium-retaining steroid or (2) the factors which control kallikrein excretion in these patients are hyporesponsive to sodium-retaining steroids. Aldosterone excretion was the same in patients with essential hypertension and the age-matched normal subjects on the diets containing 9 mEq sodium/day and 259 mEq sodium/day (Table 3). Moreover, it has been reported that sodium-retaining steroid activity is either unchanged (7) or increased (8) in patients with benign essential hypertension. From this fact one would expect kallikrein excretion to be normal or supranormal in patients with essential hypertension, but clearly it is not. Therefore, the first possibility seems unlikely. Kallikrein excretion increased in three patients with essential hypertension in response to treatment with fludrocortisone. However, it remains to be determined if the response to exogenously administered sodium-retaining steroid in patients with essential hypertension is different from that of appropriately matched normal subjects. Therefore, we cannot explain the lower kallikrein excretion in patients with essential hypertension, but it is not due to differences in dietary sodium intake and apparently not the result of differences in sodium-retaining steroid activity. Thus, it may reflect a more fundamental difference between patients with essential hypertension and normal subjects and perhaps be of pathogenetic significance.

Patients with primary aldosteronism excreted significantly more kallikrein than did normal subjects, and kallikrein excretion was unaffected by dietary sodium intake over a range from 9 to 259 mEq/day. In addition, in a single patient with primary aldosteronism, kallikrein excretion was reduced by administration of spironolactone. In this latter instance, the response was equivalent to the decrease in kallikrein excretion produced by administration of spironolactone to normal subjects on a diet containing 9 mEq sodium/day (5). Collectively, the data suggest that the supranormal excretion of kallikrein in patients with primary aldosteronism is due to their increased sodium-retaining steroid activity.

The mean level of kallikrein excretion in patients with primary aldosteronism was seven times higher than that in patients with essential hypertension. Only 1 of 12 patients with primary aldosteronism had a level of urinary kallikrein excretion within the range of values found in patients with essential hypertension. Thus, measurement of kallikrein excretion may be useful in evaluating adrenal-renal interactions in hypertensive patients.

The excretion of kallikrein is regulated by sodium-retaining steroid activity. The excretion of
subnormal amounts of kallikrein by patients with essential hypertension may represent a defect in this vasodilator system, and a complete understanding of this abnormality may provide additional insights into the pathogenesis of essential hypertension.

References


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