ABSTRACT

Basal 24 hour urinary kallikrein excretion of 20 patients with uncomplicated essential hypertension did not differ significantly from that of 18 normotensive age-matched control subjects. 4 of the 20 hypertensive patients, however, had low kallikrein excretion. Furosemide (40 mg i.v.) caused an increase of urinary kallikrein excretion in the normotensive subjects and in most of the patients with essential hypertension. The stimulating effect of furosemide was less pronounced or even absent in 7 hypertensives. No circadian rhythm of urinary kallikrein excretion was observed. There were weak correlations between 24 hour kallikrein excretion and urinary volume (r=0.59; p<0.05), and potassium excretion (r=0.51; p<0.05) in the normotensives. In the hypertensives correlations were found between 24 hour kallikrein excretion and potassium excretion (r=0.51; p<0.05), aldosterone excretion (r=0.57; p<0.01), and creatinine clearance (r=0.59; p<0.01). Our findings do not support the concept that the renal kallikrein-kinin system might play a primary role in the pathogenesis of essential hypertension.
INTRODUCTION

Kallikrein detected in the urine is probably formed in and secreted by the kidney (13). A possible role of the intrarenal kallikrein-kinin system in renal handling of water and electrolytes has been discussed (1, 2, 14). There seems to be a close interrelation between the kallikrein-kinin system and the renin-angiotensin-aldosterone system as well as the renal prostaglandins (2). However, the physiological role of the renal kallikrein-kinin system is still far from being clear.

A subnormal urinary kallikrein (U.Kall.) excretion in patients with essential hypertension (EH) had already been described more than 40 years ago (4, 20). The interest in this subject was revived by the studies of Margolius et al. (12) who reconfirmed the older findings and proposed a possible role of the renal kallikrein-kinin system in the pathogenesis of hypertension.

The aim of this study was to examine the possible interrelation between the U.Kall. excretion and the urinary water and electrolyte excretion, as well as with the renin-angiotensin-aldosterone system in normotensive subjects (NS) and in patients with EH.

PATIENTS AND METHODS

A total of 18 male NS and 20 male patients with mild (WHO grade I, n=13) or moderate EH (WHO grade II, n=7) between 19 and 50 years of age were included in the study. The mean age of the NS was 33 ± 2.1 (SEM)
and of the patients with EH 35 ± 2.1 years. Subjects were considered to be hypertensive when diastolic blood pressure was above 90 mm Hg at measurements on three different days. Secondary causes of hypertension had been excluded in all cases by appropriate methods.

Average blood pressure in the NS was 117 ± 1.9/75 ± 1.7 (systolic/diastolic) mm Hg and in the patients with EH 161 ± 5.0/106 ± 2.1 mm Hg. Antihypertensive treatment had been withdrawn for at least two weeks, diuretic agents had been withdrawn for at least four weeks prior to the study. 10 patients with EH had not been treated with antihypertensive drugs or diuretics before.

Studies were performed under outpatient conditions without dietary restrictions. However, nicotine, alcohol, and caffeine were not allowed 12 hours before and during the studies. Urine was collected for 24 hours in three 8 hour periods (period I: 7 a.m. - 3 p.m.; period II: 3 p.m. - 11 p.m.; period III: 11 p.m. - 7 a.m.). In 3 NS and 5 patients with EH urine was collected in a single 24 hour period only. On the following morning, after a 30 min resting period, blood samples were taken from the fasting individuals for estimation of plasma renin activity (PRA) and creatinine. Thereafter, 40 mg of furosemide (Lasix<sup>R</sup>) were given intravenously and urine was collected for another 30 min period. At the end of the collection period blood was taken again for estimation of PRA.

In the urine samples of each collection period kallikrein, creatinine, sodium, potassium, and aldosterone were measured. U.Kall. was estimated by four different methods:
1. The **amidolytic activity** of U.Kall. was estimated using the synthetic chromogenic substrate D-valyl-L-leucyl-L-arginine-p-nitroanilide (S 2266; Kabi) as suggested by Kabi company (Stockholm). The amidolytic activity of U.Kall. measured by this method is equal to the fraction of the total amidolytic activity in urine which is inhibited by addition of aprotinin (Trasylol\textsuperscript{R}). To make sure that kallikrein activity was totally inhibited, 25 U/ml of the kallikrein inhibitor aprotinin were added to the sample's blank, which is 2.5 times the amount suggested by Kabi. The amidolytic activity of U.Kall. was estimated in non-dialyzed as well as in dialyzed urine samples.

2. The **esterolytic activity** of U.Kall. was measured using the synthetic substrate acetyl-L-phenylalanyl-L-arginine-ethylester (APAE) as proposed by Fiedler et al. (5). This method allows measurements of U.Kall. in dialyzed urine only. Values of U.Kall. activity of assay 1 and 2 are expressed in units of substrate splitting activity (U/l= \textmu m mole/min/l).

3. **Bioassay:** The blood pressure lowering effect of U.Kall. was tested in mongrel dogs (weight: 8-10 kg) anesthetized with sodium pentobarbitone (Nembutal\textsuperscript{R} 40 mg/kg) following intravenous injections of dialyzed urines. Blood pressure was measured intraarterially in the hindlimb.

4. U.Kall was also estimated by a direct radioimmunoassay (RIA) (10) in non-dialyzed and in dialyzed urines.
Correlation between the amidolytic (S 2266) and the esterolytic (APAE) activity of urinary kallikrein (U.Kall.) in urines of normotensive and hypertensive subjects. For further explanation see text.

Standard curves for bioassay and direct RIA were obtained using a purified human urinary kallikrein preparation (7). The U.Kall. values of these assays are expressed in ng/ml.

Aliquots of the urine samples were dialyzed against distilled water at +4°C for 24 hours and non-dialyzed samples (assay 1 und 4) were stored at +4°C for 24 hours. Thereafter samples were either analyzed immediately or frozen at -20°C.
Measurements of the amidolytic and the esterolytic activity of the urine samples were performed within 48 hours after urine collection had been completed. Preliminary experiments in our laboratory had shown that in non-dialyzed urines the amidolytic activity may decrease by as much as 75% after only 4 weeks of storage at -20°C. The bioassay was performed within one week, and the radioimmunological determinations were done within two months.

Sodium, potassium, and creatinine were measured by standard methods. Urinary aldosterone was determined by RIA. PRA was estimated as previously described (3).

Statistical evaluations of the results were performed by Student's t-test, analysis of variance and linear regression analysis. Data are given as means ± SEM.

RESULTS

Comparison of Different Methods for Urinary Kallikrein Determination: Comparing the different methods used for estimation of U.Kall. in NS and patients with EH the following correlations were obtained.

1. S 2266 (y) vs. APAE (x) in dialyzed urines (n=123):
   \[ y = 0.0168 x + 0.0051; \quad r = 0.98 \quad p < 0.001 \] (Fig.1)
2. Bioassay (y) vs. S 2266 (x) in dialyzed urines (n=71):
   \[ y = 0.44 x + 0.76; \quad r = 0.80 \quad p < 0.01 \]
3. RIA (y) vs. S 2266 (x) in non-dialyzed urines (n=22):
   \[ y = 1.18 x - 2.25; \quad r = 0.95 \quad p < 0.001 \]
4. RIA (y) vs. S 2266 (x) in dialyzed urines (n=22):
   \[ y = 0.88 x - 1.14; \quad r = 0.91 \quad p < 0.001 \]
5. RIA (y) in non-dialyzed vs. RIA (x) in dialyzed urines (n=22):
\[ y = 0.96 \times + 3.17; \quad r = 0.95 \quad p < 0.001 \]

The U.Kall data presented below as well as in Fig. 2-4 and in Table 1 have been obtained by using S 2266 as substrate.

**Basal Urinary Kallikrein Excretion (Fig.2):** 24 hour kallikrein excretion as measured by the amidolytic activity of U.Kall. in the NS and patients with EH is demonstrated in Fig.2. No significant differences in the U.Kall excretion were found between the two groups. Individual values varied considerably in both groups. In the NS the range of U.Kall in non-dialyzed urines was from 347 to 1568 mU/24 h; in the patients with EH U.Kall ranged from 145 to 2089 mU/24 h. Dialysis of urines led to a consistent increment of U.Kall in all subjects with one exception in the NS.

Mean increase of U.Kall was 20 % in the NS, and 28 % in the patients with EH. U.Kall excretion of 4 patients with EH was lower than that found in the NS. 2 of these 4 patients had established hypertension (WHO grade II).

**Urinary Kallikrein Excretion and Plasma Renin Activity following Furosemide (Fig. 3, Table 1):** Furosemide caused a marked increase of U.Kall excretion in the following 30 min in both groups (NS: \( p < 0.001 \); EH: \( p < 0.01 \)) compared to the mean basal excretion per 30 min. The mean increase of U.Kall excretion was similar in both groups (Table 1). In 7 patients with EH, however, a smaller increase or even a decrease of U.Kall excretion was observed when compared to the NS. 3 of these 7 patients had established EH (WHO grade II).
### TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>U.Kall. (S 2266)</th>
<th>PRA ng/ml/h</th>
<th>Volume ml/period</th>
<th>Sodium meq/period</th>
<th>Potassium meq/period</th>
<th>$C_{Cr}$ ml/min/1.73m²</th>
<th>Aldosterone µg/period</th>
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<tr>
<td></td>
<td>non-dialysed</td>
<td>dialysed</td>
<td></td>
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<tr>
<td><strong>period I</strong></td>
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<tr>
<td>7 a.m. - 3 p.m.</td>
<td>NS 282±37</td>
<td>350±34***</td>
<td>427±55</td>
<td>56±8</td>
<td>35.9±3.1</td>
<td>115±9</td>
<td>5.9±1.1</td>
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<td></td>
<td>EH 317±55</td>
<td>401±60***</td>
<td>466±42</td>
<td>66±6</td>
<td>31.5±2.0</td>
<td>120±9</td>
<td>6.2±0.7</td>
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<tr>
<td><strong>period II</strong></td>
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<tr>
<td>3 p.m. - 11 p.m.</td>
<td>NS 283±32</td>
<td>321±32*</td>
<td>558±74</td>
<td>75±6</td>
<td>29.0±3.6</td>
<td>107±6</td>
<td>3.6±0.5</td>
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<tr>
<td></td>
<td>EH 271±44</td>
<td>329±50***</td>
<td>522±68</td>
<td>80±9</td>
<td>29.4±3.1</td>
<td>119±7</td>
<td>5.3±0.6</td>
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<td><strong>period III</strong></td>
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<tr>
<td>11 p.m. - 7 a.m.</td>
<td>NS 257±38</td>
<td>315±39**</td>
<td>431±66</td>
<td>52±6</td>
<td>14.8±1.8</td>
<td>99±4</td>
<td>3.0±0.5</td>
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<tr>
<td></td>
<td>EH 264±38</td>
<td>347±44***</td>
<td>433±55</td>
<td>65±9</td>
<td>15.3±1.2</td>
<td>102±5</td>
<td>3.6±0.5</td>
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<tr>
<td><strong>period I+II+III</strong></td>
<td>NS 822±97</td>
<td>986±96***</td>
<td>1416±156</td>
<td>184±16</td>
<td>79.6±6.8</td>
<td>107±5</td>
<td>12.5±2.0</td>
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<tr>
<td>7 a.m. - 7 a.m.</td>
<td>EH 850±129</td>
<td>1072±146***</td>
<td>1421±138</td>
<td>211±20</td>
<td>76.2±4.5</td>
<td>113±5</td>
<td>15.1±1.5</td>
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<td><strong>control</strong></td>
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<tr>
<td>30 min</td>
<td>NS 17.6±1.8</td>
<td>2.3±0.2</td>
<td>30±3</td>
<td>3.7±0.3</td>
<td>1.6±0.1</td>
<td>105±4</td>
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<tr>
<td></td>
<td>EH 22.7±2.6</td>
<td>2.4±0.4</td>
<td>30±3</td>
<td>4.4±0.4</td>
<td>1.6±0.1</td>
<td>117±5</td>
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<tr>
<td><strong>furosemide</strong></td>
<td></td>
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<tr>
<td>30 min</td>
<td>NS 37.2±3.7</td>
<td>6.3±0.7</td>
<td>523±18</td>
<td>67.0±2.3</td>
<td>8.7±0.4</td>
<td>106±9</td>
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<td></td>
<td>EH 36.6±4.0</td>
<td>5.6±1.2</td>
<td>487±21</td>
<td>63.2±2.8</td>
<td>7.8±0.3</td>
<td>101±5</td>
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</table>

*p<0.05; **p<0.01; ***p<0.001

Urinary kallikrein, volume, sodium, potassium, aldosterone excretion, creatinine clearance, and plasma renin activity in normotensive subjects (NS) and patients with essential hypertension (EH) under basal conditions (NS: n = 15, except aldosterone excretion: n = 14; EH: n = 15) and after furosemide (40 mg i.v.) (NS: n = 15; EH: n = 20).

U.Kall. = urinary kallikrein; PRA = plasma renin activity; $C_{Cr}$ = creatinine clearance; control: mean per 30 min of basal 24 h excretion.

Mean values ± SEM.
24 hour urinary kallikrein (U.Kall.) excretion in normotensive and hypertensive subjects. The amidolytic activity of U.Kall. was measured using the synthetic substrate S 2266. Note the consistent increase (except one in the normotensive group) of U.Kall. following dialysis of urine samples.

During recumbency and after furosemide stimulation mean values of PRA did not differ significantly in both groups, whereas the percent rise of PRA was lower (p<0.05) in the patients with EH compared to the NS. No correlation was found either between absolute values or percent changes of PRA and U.Kall. excretion before and after furosemide administration.
Plasma renin activity (PRA) and urinary kallikrein (U.Kall.) excretion (dialyzed urines; substrate S 2266) in normotensive and hypertensive subjects under basal conditions and 30 min after furosemide (40 mg i.v.) administration.
Urinary Kallikrein Excretion, Urine Volume, Sodium, Potassium, Aldosterone Excretion and Creatinine Clearance in Periods I, II, III and following Furosemide (Fig.4, Table 1): There were no significant differences of U. Kall. excretion, urine volume, sodium, potassium, aldosterone excretion, and creatinine clearance between NS and patients with EH in each period. Comparing the three periods only the potassium excretion (period I, II vs. III; p < 0.05) and the aldosterone excretion (period I vs. III; p < 0.05) proved to be different in both groups as was shown by analysis of variance.

Values of urine volume, sodium, potassium excretion, and creatinine clearance following the administration of furosemide were similar in both groups (Table 1).

In the NS positive correlations were found between total U.Kall./24 h (non-dialyzed urines) and urine volume/24 h (r=0.59; p < 0.05), U.Kall./24 h (non-dialyzed urines) and potassium excretion/24 h (r=0.51; p < 0.05); in patients with EH between U.Kall./24 h (dialyzed urines) and potassium excretion (r=0.51; p < 0.05), U.Kall./24 h (dialyzed urines) and aldosterone excretion/24 h (r=0.57; p < 0.01), U.Kall./24 h (dialyzed urines) and mean creatinine clearance/24 h (r=0.59; p < 0.01). However, no correlations could be revealed between U.Kall. and urinary excretion of the parameters mentioned above in periods I, II or III and following furosemide administration in either group.

DISCUSSION

Comparison of the different kallikrein assays demonstrates that the synthetic substrates used are ap-
Urinary kallikrein (U.Kall.), (non-dialyzed urines; substrate S 2266), urine volume (UV), sodium (U_NaV), aldosterone, potassium excretion (U_KV), and creatinine clearance (C_Kr) in period I, II, and III in normotensive and hypertensive subjects. For further explanation see text. (Aldosterone excretion in normotensives: n=14)
propriate for the estimation of urinary kallikrein. Dialysis of urine samples resulted in an increase of the amidolytic activity. However, urinary kallikrein did not differ when the direct RIA was applied. The increase of kallikrein activity following dialysis could be due to the removal of inhibitory substances which might be present in the untreated urine. It seems possible that a specific kallikrein inhibitor as found in renal tubules in rats (6) is excreted into the urine and partially removed by dialysis.

In the present study we did not find a difference in the mean basal kallikrein excretion between the patients with uncomplicated EH and age-matched normotensive control subjects. Only 4 out of 20 patients with EH showed low kallikrein excretion. A normal kallikrein excretion has also been reported by Lawton and Fitz (8) who compared younger male patients with mild EH with age-matched control subjects. Other authors reported upon a normal kallikrein excretion in labile EH, and a decreased kallikrein excretion in established EH (15, 18). The latter findings are compatible with the assumption that subnormal kallikrein excretion could be secondary to the hypertensive process. This interpretation is supported by data of Holland et al. (personal communication) who found kallikrein excretion to be normal in patients with EH and normal renal function (creatinine clearance > 80 ml/min), but decreased in patients with EH and reduced renal function (creatinine clearance 40 - 80 ml/min). The positive correlation between kallikrein excretion and creatinine clearance we observed in the patients with EH would be consistent with Holland's data.

Furosemide is known to stimulate urinary kalli-
kallikrein excretion. In patients with EH, however, a reduced stimulating effect of furosemide on urinary kallikrein excretion has been reported (17, 19). We observed a diminished stimulation of kallikrein excretion only in a minority of patients with EH. As demonstrated in rats, increments of urinary kallikrein excretion following furosemide administration are due to an increased synthesis rate in the kidney (16). Therefore, our findings could indicate a reduced synthesis of kallikrein in the kidneys following furosemide stimulation in a proportion of patients with EH.

Levy et al. (9) reported on a day-night rhythm of kallikrein excretion in white patients with EH, but not in black patients with EH or in normal control subjects. In this study no circadian rhythm of urinary kallikrein excretion was observed. There was, however, a similar circadian rhythm of potassium and aldosterone excretion in both groups (Fig. 4). Neither between kallikrein and potassium excretion nor between kallikrein and aldosterone excretion a correlation could be demonstrated in any 8 hour period. There was, however, a positive correlation between the kallikrein excretion/24 h and the potassium excretion/24 h (in NS and EH), and also between the kallikrein excretion/24 h and the aldosterone excretion/24 h (in EH only). The latter finding in the patients with EH is compatible with the hypothesis that aldosterone is an important factor in the long-term control of kallikrein formation (11).

In conclusion, we found low kallikrein excretion only in a minority of patients with mild or moderate EH. Possibly, the reduced kallikrein excretion found in
a proportion of patients with EH is secondary to the hypertensive process and not related to its origin. However, since racial differences in kallikrein excretion are known to exist (9), low kallikrein excretion could also be due to a genetic determinant.

Thus, patients with low kallikrein excretion might not represent a homogenous group, and at least in some of the patients a genetic component might be responsible for the low kallikrein excretion.

ACKNOWLEDGMENTS

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REFERENCES