Human Urinary and Plasma Kinins

Relationship to Sodium-Retaining Steroids and Plasma Renin Activity

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SUMMARY Sodium-retaining steroids increase urinary kallikrein but their effects on urinary kinins and plasma bradykinin are not known. Thirty-six normal subjects were studied during several different manipulations of dietary sodium and potassium or the administration of fludrocortisone or adrenocorticotropic hormone (ACTH). Urinary kallikrein and aldosterone excretion changed pari passu over a 30-fold range for kallikrein and an 80-fold range for aldosterone. Urinary kinin excretion was invariant. Plasma, bradykinin, on the other hand, responded to the same stimuli as plasma renin activity and not primarily to the level of sodium-retaining steroid. These studies show that: (1) urinary kallikrein is dependent on the level of aldosterone over a wide range of excretion values; (2) urinary kallikrein determines neither the level of urinary kinins nor the level of plasma kinins; (3) urinary kinins are independent of the level of sodium-retaining steroid; and (4) there is a strong correlation between plasma bradykinin and renin activity but not between plasma bradykinin and sodium-retaining steroid activity. We suggest that: (1) urinary kallikrein is an index of sodium-retaining steroid activity and may participate in the antinatriuretic and kaliuretic effects of these hormones; (2) plasma bradykinin is highly correlated with plasma renin activity because both responded to changes in extracellular fluid volume and not because angiotensin-converting enzyme controls both systems in an interrelated fashion; and (3) plasma bradykinin may act physiologically to antagonize angiotensin II and may contribute to maintenance of normal blood pressure in hyperreninemic states.

BRADYKININ, the most potent vasodilator peptide in man, is generated by a limited proteolytic system analogous to the renin-angiotensin system. Kallikrein is the proteolytic enzyme which generates bradykinin from a precursor globulin called kininogen. The kallikrein-kinin system found in glands such as the kidney is clearly different from that found in plasma (Pisano, 1975; Jacobsen, 1966; Pierce and Webster, 1961). Associated with its vasodilatory properties, bradykinin infused into the renal artery causes natriuresis and diuresis (Stein et al., 1972; Willis et al., 1969). This effect, however, appears to be hemodynamically mediated (Willis et al., 1969) and may not relate to the physiological role of the kallikrein-kinin system in the kidney.

Despite several reports that implicate plasma bradykinin as a vasodilator in several disease states and other studies that imply a role for the renal kallikrein-kinin system in salt and water metabolism (Mills et al., 1976), little is known about the renal and cardiovascular actions of endogenous kinins and the physiological and hormonal factors that may influence their formation, metabolism, or excretion.

Although the renal kallikrein-kinin system has been implicated in salt homeostasis, no one has measured kinin excretion in response to manipulations of dietary sodium and potassium intake.

Also, although it is known that urinary kallikrein is increased by sodium-retaining hormones (Margolius et al., 1974), the effects of these hormones on urinary and plasma kinin are not known. A positive relationship between plasma bradykinin and renin activity has been shown (Wong et al., 1975) but not clearly differentiated from a similar relationship that exists between plasma bradykinin and the level of sodium-retaining steroid (Wong et al., 1975) until the recent study by Mersey et al. (1977).

To answer these questions, we studied the renin-angiotensin and kallikrein-kinin systems, comprehensively, in 36 normal subjects who were fed various intakes of sodium and potassium and who were given fludrocortisone or ACTH.
HUMAN URINARY AND PLASMA KININS/Vinci et al.

Methods

Clinical Methods

Thirty-six normal Caucasian women, ranging in age from 19 to 59 years (25 ± 12 years is mean ± SD) participated in one or more of the following studies:

Diets with Variable Sodium and 100 mEq Potassium

Seventeen subjects were fed a basic constant diet containing 9 mEq of sodium/day and 70 mEq of potassium/day supplemented with 30 mEq of KCl/day. As indicated, this basic diet also was supplemented with either 100 (29 subjects) or 250 (8 subjects) mEq of NaCl/day. Observations were made after 5 days on any specified diet.

Diets with Variable Potassium and 109 mEq Sodium

Five subjects were fed a basic constant diet containing 9 mEq of sodium/day and 25 mEq of potassium/day for 14 days. On days 6–11, they received 0.5 mg of fludrocortisone orally daily in divided doses. Observations were made after 5 days on any specified diet.

Fludrocortisone Administration

Nine subjects were fed a constant diet with 109 mEq of sodium/day and 100 mEq of potassium/day for 14 days. On days 6–11, they received 0.5 mg of fludrocortisone orally daily in divided doses. Observations were made daily throughout the study.

Corticotropin Infusion

Eleven subjects were fed a constant diet with 9 mEq of sodium/day and 100 mEq of potassium/day for 11 days. On the 8th and 9th days they received a 48-hour infusion of cortrosyn (40 units intravenously every 12 hours). Observations were made daily for the 3 days before, the 2 days of, and the 2 days after the infusion.

It should be emphasized that fluid intake was 2500 ml/day in all patients throughout all phases of all studies. Mean blood pressure (subjects supine) was 101/66 ± 5/5 (mean ± SD) mm Hg.

All subjects were hospitalized at the Clinical Center, National Institutes of Health, during the study. The protocols for these studies were approved by the Clinical Research Committee of the NIH, and all subjects gave informed consent in writing.

Biochemical Methods

Venous blood was obtained from the median basilic vein for the determination of plasma renin activity (Menard and Catt, 1972), plasma aldosterone concentration (Ito et al., 1972), plasma bradykinin (Vinci et al., 1978), and plasma prekallikrein (Imanari et al., 1976) between 8:00 a.m. and 9:00 a.m. when the subjects had been recumbent overnight and again when the subjects had been upright for 3 hours. Urine was collected daily in 24-hour periods as previously described (Vinci et al., 1978); samples were taken for measurement of (Na⁺), (K⁺), aldosterone, kallikrein, and kinins. Urinary aldosterone was determined by radioimmunoassay (Langam et al., 1974). Plasma renin activity and aldosterone concentration and urinary aldosterone were assayed by Hazelton Laboratories, Inc., Virginia, under a special contract.

Urinary Kallikrein

Samples were assayed in duplicate by a modification (Vinci et al., 1978) of the radiochemical esterolytic method of Beaven et al. (1971). Values are expressed as TAME (N-tosyl-L-arginine-³H-methyl ester) esterase units (TU) excreted per 24 hours. One esterase unit is defined as the amount of kallikrein which hydrolyzes 1 μmol of TAME/min at pH 8.0 and 30°C as measured in a titrametric assay.

Prekallikrein

Samples were obtained and processed as previously described (Vinci et al., 1978). Prekallikrein was determined in duplicate by a radiochemical assay (Imaneri et al., 1976) against the same kallikrein standard used in the assay for urinary kallikrein. Values are expressed in TU/ml plasma.

Plasma Bradykinin and Urinary Kinins

Whole blood and urine were collected and processed as previously described (Vinci et al., 1978; Talamo et al., 1969; Wintroub et al., 1973). Both urinary kinin (bradykinin and lysyl-bradykinin) and plasma bradykinin were determined by the same radioimmunoassay as recently reported (Vinci et al., 1978). Values for urinary kinin are expressed as μg/24 hr and those for plasma bradykinin (corrected for recovery of 45 nCi bradykinin triacetate [2-prolyl-3,4-³H(N)]), specific activity, 50 Ci/mmol) as ng/ml.

Statistical Methods

Values are reported as mean ± SEM unless stated otherwise. Student's t-test was used to determine statistical significance, and P = 0.05 was considered the upper limit of significance. Lines of regression, which are depicted graphically, and correlation coefficients that relate any two variables under more than one condition are derived from the regression line and correlation coefficients between these two variables for each subject. The mean slope, intercept, and correlation coefficient, where n is the number of subjects, is then computed (Rao, 1965). The selection of subjects (Figs. 1 and 2) is based on the statistical requirement for at least
Effects of Na Intake on the Kallikrein-Kinin System (Table 1, Fig. 1)

Normal (mean ± SEM) values for urinary kallikrein and kinin excretion, plasma prekallikrein, and plasma bradykinin are tabulated in Table 1 for subjects fed 9, 109, or 259 mEq of sodium/day and constant potassium (100 mEq/day). Mean kallikrein excretion of subjects fed 9 mEq of sodium/day was significantly (P < 0.001) higher than that of subjects who consumed 109 and 259 mEq of sodium/day. Regardless of sodium intake, the level of kallikrein excretion, or the aldosterone excretion rate in each group, the excretion of urinary kinins was similar.

Mean plasma bradykinin (subjects recumbent) of subjects fed 9 mEq of sodium/day (5.4 ± 0.7 ng/ml) was significantly (P < 0.001) higher than that of subjects who consumed 109 and 259 mEq of sodium/day (3.3 ± 0.3 ng/ml and 2.9 ± 0.3 ng/ml, respectively). It should be noted that these values when uncorrected for recovery (3.1 ± 0.4, 1.9 ± 0.2 and 1.7 ± 0.2 ng/ml, respectively) are similar to uncorrected values reported by others (Mersey et al., 1977; Talamo et al., 1969; Wintroub et al., 1973) who use this method of Talamo et al. or a modification of it. Mean plasma bradykinin was increased after the subjects had been upright for 3 hours; 54% (P < 0.005) in sodium-depleted subjects, 36% (P < 0.02) in subjects fed 109 mEq of sodium/day, and 38% (not significant) in subjects fed 259 mEq of sodium/day. No significant difference in mean plasma prekallikrein (subject recumbent) was observed between subjects consuming 109 or 259 mEq of sodium/day and those in the sodium-depleted group.

Intersubject correlations between basal levels of plasma renin activity and plasma bradykinin were significant in the 17 sodium-depleted recumbent subjects as well as in the 29 sodium-replete recumbent and upright subjects (Table 2). Levels of plasma aldosterone and plasma bradykinin, as well as aldosterone and kallikrein excretion, on the other hand, correlated poorly. Intra-subject correlations between plasma bradykinin and plasma renin activity were also significant [mean correlation coefficient (r) ± SEM was 0.88 ± 0.7, P < 0.001] in each of 11 subjects who were fed both diets containing 109 and 9 mEq of sodium/day (Fig. 1).
Effects of Sodium Intake on the Kallikrein-Kinin System

P (9 vs. 109)

High salt intake, weight increased significantly (P < 0.001). (These data are based on the 15 days of the study.) After consumption of 9 mEq of sodium/day for 9 days, mean sodium excretion was 3 ± 1 mEq/day, aldosterone excretion was 16.3 ± 1.8 μg/day, kallikrein excretion was 19.5 ± 2.4 IU/day, and mean kinin excretion was 12.4 ± 1.9 IU/day.

There were significant correlations between kinin excretion and urinary kallikrein (r = −0.19 ± 0.20, P > 0.40), aldosterone excretion (r = −0.19 + 29, P > 0.70), or sodium excretion (r = 0.26 ± 0.21, P > 0.25).

Effects of Potassium Intake on the Kallikrein-Kinin System

The study of five subjects fed potassium, 185 and 259 mEq of sodium/day and 100 mEq of potassium/day, is summarized in Figure 3. After 7 days of high potassium intake, mean (days 5–7) urinary potassium and sodium excretion were 156 ± 11 mEq/day and 83 ± 8 mEq/day, respectively; mean aldosterone excretion was 18.0 ± 3.1 μg/day and urinary kallikrein and kinin excretions were 14.7 ± 1.9 TU/day and 11.4 ± 3.0 μg/day.

Table 1 Effects of Sodium Intake on the Kallikrein-Kinin System

<table>
<thead>
<tr>
<th>Dietary</th>
<th>Na (mEq/day)</th>
<th>K (mEq/day)</th>
<th>n</th>
<th>UNaV (mEq/day)</th>
<th>UKV (μg/day)</th>
<th>AER (μg/day)</th>
<th>UKaV (μg/day)</th>
<th>UKV (μg/day)</th>
<th>PRAR (ng/ml per hr)</th>
<th>PRAU (ng/ml per hr)</th>
<th>PACR (ng/100 ml)</th>
<th>PACU (ng/100 ml)</th>
<th>PBKR (ng/ml)</th>
<th>PBKU (ng/ml)</th>
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<tr>
<td>9 100</td>
<td>17</td>
<td>1 93</td>
<td>56.3 12.5</td>
<td>3.8 8.8</td>
<td>39.9 165</td>
<td>5.4 8.3 1.38</td>
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<tr>
<td>P (9 vs. 109) (mEq Na/day)</td>
<td>0.001</td>
<td>NS</td>
<td>0.001</td>
<td>0.001</td>
<td>NS</td>
<td>0.02</td>
<td>0.005</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>NS</td>
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<td>109 100</td>
<td>29</td>
<td>94 98</td>
<td>12.4 8.7</td>
<td>13.4 5.2</td>
<td>5.4 44.4</td>
<td>3.3 4.5</td>
<td>1.2</td>
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<tr>
<td>P (109 vs. 259) (mEq Na/day)</td>
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<td>NS</td>
<td>0.06</td>
<td>0.06</td>
<td>NS</td>
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<td>NS</td>
<td>NS</td>
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<td>259 100</td>
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<td>241</td>
<td>101</td>
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<td>2.9</td>
<td>4.0</td>
<td>13.3</td>
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Table 2 Correlation between PRA, PAC, and PBK

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<th>PRAR</th>
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<th>PACR</th>
<th>PACU</th>
<th>PBKR</th>
<th>PBKU</th>
</tr>
</thead>
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<td>PRAR r</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<td>&lt;0.001</td>
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<tr>
<td>P</td>
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<td>0.56</td>
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<td>0.26</td>
</tr>
<tr>
<td>PRAU r</td>
<td>0.70</td>
<td>0.028</td>
<td>0.43</td>
<td>0.384</td>
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</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>PACR r</td>
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<td>0.02</td>
<td>0.88</td>
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<td>0.49</td>
</tr>
<tr>
<td>P</td>
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<td>0.98</td>
<td>&lt;0.001</td>
<td>0.215</td>
<td>0.073</td>
</tr>
<tr>
<td>PACU r</td>
<td>0.14</td>
<td>0.10</td>
<td>−0.02</td>
<td>0.38</td>
<td>0.45</td>
</tr>
<tr>
<td>P</td>
<td>0.54</td>
<td>0.64</td>
<td>0.93</td>
<td>0.181</td>
<td>0.105</td>
</tr>
<tr>
<td>PBKR r</td>
<td>0.66</td>
<td>0.45</td>
<td>−0.13</td>
<td>−0.02</td>
<td>0.185</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.002</td>
<td>0.04</td>
<td>0.64</td>
<td>0.95</td>
<td>0.185</td>
</tr>
<tr>
<td>PBKU r</td>
<td>0.57</td>
<td>0.38</td>
<td>−0.18</td>
<td>0.08</td>
<td>0.070</td>
</tr>
<tr>
<td>P</td>
<td>0.008</td>
<td>0.09</td>
<td>0.50</td>
<td>0.75</td>
<td>&lt;0.001</td>
</tr>
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</table>

Corrections (r) between plasma renin activity (PRA), plasma aldosterone concentration (PAC) and plasma bradykinin (PBK) in 29 recumbent (R) and upright (U) subjects fed 109 mEq of sodium/day and 100 mEq of potassium/day and in 17 recumbent (R) and upright (U) subjects fed 9 mEq of sodium/day and 100 mEq of potassium/day.
**Effects of Dietary Potassium Intake on Urinary Excretion**

Figure 3 shows the effect of dietary potassium intake on (mean ± SEM) urinary potassium (UKV), sodium (UNaV), kallikrein (UKaV), and kinin (UKiV) excretion and aldos- 
terone excretion rate (AER) in five subjects who were fed diets containing 185 mEq of potassium and 109 mEq of sodium/day for 7 days and 25 mEq of potassium and 
109 mEq of sodium/day for the next 8 days. * = P < 0.05. Values for plasma renin activity (PRA), plasma aldosterone concentration (PAC), and plasma bradyk 
inin (PBK) [when subjects were recumbent (R) and upright (U)] and for prekallikrein (pKa) (subjects recumbent) on the last day of each diet are listed in Table 2.

respectively. By the third day of low potassium intake, urinary potassium equaled dietary intake (28 ± 0.7 mEq/day), and aldosterone excretion had decreased to 4.0 ± 1.0 μg/day. Both values remained unchanged for the rest of the study. Urinary kalli 
krein excretion decreased in a stepwise fashion during low potassium intake, but urinary kinin excretion was unchanged. Serum potassium decreased from 3.9 ± 0.1 mEq/liter to 3.4 ± 0.1 mEq/liter (P < 0.02), but there was no change in weight.

After 8 days of potassium restriction (Table 3), plasma renin activity (mean percentage change) was reduced by 83 ± 3% (P < 0.001) when subjects were recumbent and by 68 ± 7% (P = 0.002) when subjects were upright. Plasma bradykinin was reduced by 35 ± 11% (P = 0.05) when subjects were upright but was unchanged when they were recum 

bent. Plasma prekallikrein (subject recumbent) was unchanged.

Urinary potassium and aldosterone excretion were highly correlated in each subject (r was 0.77 ± 0.06, P < 0.001), as was urinary kallikrein with aldosterone excretion and plasma renin activity in recumbent and upright subjects (Table 4). There were no significant correlations between urinary kallikrein and kinin excretion or between urinary kinin and aldosterone excretion, as well as between plasma renin activity and plasma bradykinin.

**Effects of Fludrocortisone on the Kallikrein-Kinin System**

Figure 4 summarizes the study of nine subjects who received 0.5 mg of fludrocortisone daily for 6 days while they were fed constant diets with 109 mEq of sodium/day and 100 mEq of potassium/day. Fludrocortisone caused a significant (P < 0.01) re 
duction in urinary sodium excretion, a significant (P = 0.01) increase in potassium excretion, and a mean increase of 1.2 ± 0.2 kg (P = 0.002) in weight. By the sixth day, “escape” from the sodium-retaining properties of fludrocortisone had occurred. During fludrocortisone administration, kallikrein excretion increased in a stepwise fashion, yet kinin excretion was unchanged. As expected, aldosterone was suppressed by fludrocortisone.

Steroid administration decreased plasma renin activity (subjects recumbent or upright) significantly and progressively and also decreased plasma bradykinin 20% (not significant) when subjects were recumbent and 38% (P < 0.02) when subjects were upright. Plasma prekallikrein, subject recumbent (not shown) was not changed (control value of 1.11 ± 0.05 TU/ml).

When fludrocortisone was withdrawn, there was a marked natriuresis, (from a mean of 53 ± 6 mEq/day during fludrocortisone to 239 ± 27 mEq/day on day 13), a concomitant return of weight and urinary kallikrein to control values, and a significant decrease in potassium excretion, all of which are consistent with a decrease in the level of sodium-retaining activity. Aldosterone, which was suppressed by exogenous fludrocortisone, was still suppressed by day 14. The fact that aldosterone and kallikrein excretion does not correlate in the study reflected the fact that aldosterone excretion was not indicative of the level of sodium-retaining steroid activity provided by fludrocortisone. Despite marked changes in urinary kallikrein excre 

<table>
<thead>
<tr>
<th>Table 3 Effect of Potassium Intake on the Kallikrein-Kinin System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary potassium (mEq/day)</td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>185</td>
</tr>
<tr>
<td>25</td>
</tr>
<tr>
<td>P</td>
</tr>
</tbody>
</table>

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TABLE 4 Correlation between PRA, PBK, UKAV, UKiV, AER, and UKV in Five Subjects

<table>
<thead>
<tr>
<th></th>
<th>AER</th>
<th>UKV</th>
<th>UKiV</th>
<th>PRA</th>
<th>PRAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>UKAV r</td>
<td>0.57 ± 0.11</td>
<td>0.40 ± 16</td>
<td>0.08 ± 0.20</td>
<td>0.85 ± 0.06</td>
<td>0.80 ± 0.08</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>UKiV r</td>
<td>0.17 ± 18</td>
<td>-0.16 ± 19</td>
<td>0.04 ± 27</td>
<td>0.02 ± 36</td>
<td>0.08 ± 33</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>PBKR r</td>
<td>0.16 ± 0.30</td>
<td>0.04 ± 27</td>
<td>-0.22 ± 0.25</td>
<td>-0.05 ± 32</td>
<td>-0.02 ± 35</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>PBKU r</td>
<td>0.01 ± 0.26</td>
<td>0.07 ± 31</td>
<td>-0.03 ± 0.11</td>
<td>0.27 ± 28</td>
<td>0.24 ± 0.24</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
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</table>

Mean correlations (r) between plasma renin activity (PRA), plasma bradykinin (PBK), urinary kallikrein (UKAV), kinin (UKiV), aldosterone (AER), and potassium excretion (UKV) in five subjects fed 185 mEq of potassium/day and 25 mEq of potassium/day during constant sodium consumption (109 mEq/day). These data are based on the 15 days of the study for each patient represented in Figure 3.

Figure 4: Effect of fludrocortisone, 0.5 mg/day, on (mean ± SEM) urinary sodium (UNaV), potassium (UKV), kallikrein (UKaV), and kinin (UKiV) excretion, aldosterone excretion rate (AER), plasma renin activity (PRA) (subjects recumbent (●) and upright (○)), plasma bradykinin concentration (PBK) (subjects recumbent (●) and upright (○)), and weight (WT as change in weight) in nine subjects who were fed 109 mEq of sodium and 100 mEq of potassium/day. * = P < 0.05.

Figure 5: Effect of ACTH on the Kallikrein-Kinin System

The study of 11 subjects fed a diet with 9 mEq of sodium/day and 100 mEq of potassium/day and infused with ACTH for 48 hours is summarized in Figure 5. All subjects were sodium-depleted (mean sodium excretion was 3.8 ± 1.4 mEq/day) and showed a high rate of aldosterone (63 ± 9 µg/day) and kallikrein (20.5 ± 1.9 TU/day) excretion. ACTH resulted in a dramatic and abrupt increase in aldosterone excretion on the first (P = 0.002) and second (P = 0.02) days of infusion with an associated reduction in sodium excretion but no change in body weight (not shown). Potassium excretion increased significantly (P = 0.001) on the first day of infusion, but was significantly reduced from control on the second day (P < 0.001). Kallikrein excretion increased significantly on the first (P = 0.007) and second (P = 0.001) days of ACTH administration, but despite this supramaximal stimulus, urinary kinin excretion was unchanged.

On the second day of ACTH administration, plasma renin activity increased significantly when the subject was recumbent (P = 0.02) and upright (P = 0.004) for 3 hours, but plasma bradykinin (patient upright) was not changed. When ACTH was withdrawn, the excretion of aldosterone and potassium decreased to levels below control, whereas sodium excretion increased on the first 2 days of observation. Urinary kallikrein was still elevated the day after ACTH withdrawal at a time when aldosterone was significantly below control, indicating a reverse trend to the correlation of aldosterone with kallikrein. Again, kinin excretion was unchanged.

Figure 6 demonstrates the highly significant correlation (r was 0.92 ± 0.03, P < 0.001) between urinary kallikrein and aldosterone excretion for the 11 subjects who participated in at least three studies. There were no correlations between kinin excretion and urinary kallikrein or aldosterone excretion, but urinary kinin excretion was not significantly affected.
FIGURE 5 The effect of an infusion of ACTH (Corticotropin, 40 units every 12 hours for 48 hours) on (mean ± SEM) urinary sodium (UNaV), potassium (UKV), kallikrein (UKaV), and kinin (UKiV) excretion, aldosterone excretion rate (AER), plasma renin activity (PRA) [subjects recumbent (□) and upright (○)], and plasma bradykinin (PBK) (subjects upright) in 11 subjects fed diets containing 9 mEq of sodium and 100 mEq of potassium/day. * = P < 0.05.

FIGURE 6 Highly significant correlation between urinary kallikrein excretion (UKaV) and aldosterone excretion rate (AER) for 11 subjects during any three of the following dietary intakes or studies: when subjects were fed diets containing 9 or 10 mEq of sodium and 100 mEq of potassium/day; 25 or 185 mEq of potassium and 10 mEq of sodium/day; and when given ACTH while being fed 9 mEq of sodium and 100 mEq of potassium/day. Each point represents the mean of at least 2 days in a study when a subject had reached hormonal equilibrium. Calculations are described in the Methods section.

Discussion

In this study of primary and secondary excesses of sodium-retaining steroids in normal women, urinary kallikrein was positively correlated with the level of sodium-retaining steroid but plasma and urinary kinins were not. Levels of plasma bradykinin increased in response to sodium depletion and upright posture and were positively correlated with the level of plasma renin activity. Urinary kinin excretion was unresponsive to changes in the level of sodium-retaining steroid or plasma renin activity.

The kallikrein-kinin system exists in glands (e.g., exocrine glands, kidneys) and plasma as separate systems (Pisano, 1975; Jacobsen, 1966; Pierce, 1961; Vinci et al., 1978). In plasma, prekallikrein activators generate active kallikrein from the precursor pre-kallikrein which probably is synthesized in the liver (Colman, 1974). Kallikrein generates bradykinin from the kininogens, but plasma proteins rapidly inhibit plasma kallikrein, and plasma and tissue kininases rapidly destroy bradykinin.

Nevertheless, in normal subjects, upright posture (Streeter et al., 1972; Wong et al., 1975; Vinci et al., 1978) and sodium depletion (Wong et al., 1975; Vinci et al., 1978) increased, and saline infusions (Wong et al., 1975) decreased the levels of plasma bradykinin and plasma renin activity in a parallel fashion. In patients with Bartter's syndrome (Vinci et al., 1978), plasma renin and bradykinin were elevated in the basal state and were decreased by prostaglandin synthetase inhibitors. The importance of these correlations is that they further support the concept that bradykinin may modulate, via the prostaglandins, the pressor responsiveness to angiotensin II (Aiken and Vane, 1973).

Urinary kallikrein is a glandular kallikrein that is synthesized by the kidney (Nustad et al., 1975) in cells of the distal tubule (Orstavik et al., 1976; Ward et al., 1975) is released into the urine, and dependent, in large part, on the level of sodium-retaining steroids (Margolius et al., 1974; Vinci et al., 1978). It generates lysyl-bradykinin from kininogen, probably in distal segments of the nephron (Ward et al., 1975), and lysyl-bradykinin is converted to bradykinin by an amino-peptidase (Pierce and Webster, 1961). Both kinins, which exist in human urine, are not filtered plasma kinins, because bradykinin infused into the renal artery is poorly recovered in urine (Nasjletti et al., 1975), and 85% of the brady-
kinin injected into the rat proximal tubule is hydrolyzed and reabsorbed by proximal tubule cells (Carone et al., 1976). Instead, it is likely that kinins are generated within the distal tubule where kallikrein is located. The fact that virtually all bradykinin infused into the rat distal tubule is fully recovered (Carone et al., 1976) indicates the absence of both degradation and net reabsorption of kinins in distal tubular fluid. Despite the fact that several studies imply that urinary kinins are involved in salt and water metabolism because bradykinin causes natriuresis and diuresis when infused into the renal artery (Willis et al., 1969; Mills et al., 1976), the results of these studies may not relate to the physiological role of this system, since these effects are hemodynamically mediated by the infusion of pharmacological doses of bradykinin into the renal vasculature. Indeed, neither the physiological role of endogenous urinary kinins nor any of the factors that may influence their formation, metabolism, or excretion in normal men are known. In subjects with Bartter's syndrome who exhibited the only known abnormality in kinin excretion (Vinci et al., 1978), the low kinin excretion appeared to be mediated by the level of renal prostaglandin E and not by the level of urinary kallikrein.

This study confirmed the observation that urinary kallikrein is dependent on aldosterone and other sodium-retaining steroids and extended the original observation (Margolius et al., 1974) to encompass an 80-fold range in aldosterone excretion and a 30-fold range in kallikrein excretion (Fig. 6). Although this relationship between kallikrein and sodium-retaining steroids appears certain and has also been reported in primary aldosteronism (Margolius et al., 1974) and Bartter's syndrome (Vinci et al., 1978), the physiological basis for it remains undefined. Indeed, other factors besides the level of sodium-retaining steroid may influence kallikrein excretion, as demonstrated by the temporally disparate effects of ACTH on kallikrein and aldosterone excretion (Fig. 5). Although the latter phenomenon may merely reflect differences in the duration of the response of aldosterone to ACTH and of kallikrein to aldosterone, based on this data it is also possible that ACTH may influence kallikrein excretion either directly or indirectly via stimulation of other adrenal hormones.

The fact that kinin excretion was invariant in this study despite a 30-fold change in kallikrein excretion demonstrates that urinary kallikrein does not control kinin excretion in normal subjects. As unexpected as this result might seem, it is also true of patients with Bartter's syndrome who have been shown to excrete abnormally low kinins vis-à-vis elevated kallikrein excretion (Vinci et al., 1978). When patients with this syndrome were treated with prostaglandin synthetase inhibitors—which suppressed the abnormally high excretion of prostaglandin E2 (Vinci et al., 1978) and caused marked sodium retention, weight gain, and potassium retention—kallikrein excretion decreased while kinin excretion increased. Thus, in subjects with this syndrome, the low kinin excretion was mediated by prostaglandins and not kallikrein. In the present study, just as kinin excretion did not change in the presence of immunoreactive prostaglandin E excretion did not change either (unpublished observations). Thus it is possible that alterations in kinin excretion depend, in part, on the same factors that alter the excretion of prostaglandin E.

The biochemical mechanisms by which kinin excretion was invariant as kallikrein excretion changed, in this study, may involve factors that altered substrate availability, the activity of kallikrein on the luminal membrane, or the fate of formed kinins.

Because kallikrein and kinin excretion were unrelated in these studies, one might argue that our determination of kinin excretion is unrelated to levels of kinin generated in the distal tubule. In urine collected in acid and peptatin, total urinary kinins (lysyl-bradykinin and bradykinin) as measured by this radioimmunoassay were highly correlated with values obtained by bioassay (Hial et al., 1976; Vinci et al., 1978). Kininogen, which exists in human urine (Hial et al., 1976), did not interfere with this assay, since chromatography of urine with QAE-Sephadex and IRC-50 (which effectively removes kininogen) does not affect the level of kinin measured (unpublished observations). Thus, the assay accurately reflects levels of kinin in voided urine. Whether the latter also reflects levels of kinins generated in the kidney is unknown and difficult to prove. Studies in rats have indicated the absence of both degradation and net reabsorption of kinins injected into the distal tubule (Carone et al., 1976), and preliminary unpublished observations with voided human urine indicate that kinnase activity in human urine is also low. The fact that kininogen and kallikrein coexist in voided human urine (Hial et al., 1976) suggests that (1) the measurement of urinary kininogen may have largely reflected an immunologically active but biochemically inert fragment of kininogen, (2) the activity of kallikrein is reduced in the normal acidity of urine, or (3) urine may contain kallikrein inhibitors.

This study confirms the observation that plasma bradykinin increased in response to upright posture and sodium depletion and was highly correlated with the response in plasma renin activity (Streeten et al., 1972; Wong et al., 1975). We have excluded the possibility that these correlated responses were due to secondary increases in the level of sodium-retaining steroid as fludrocortisone decreased plasma bradykinin. During the preparation of this manuscript, Mersey et al. (1977) had reported that acute changes in angiotensin II and potassium also did not affect plasma bradykinin.

The effect of potassium restriction in this study was to lower both plasma renin activity (subject upright) and plasma bradykinin (subject upright).
The fact that renin activity decreased during potassium deprivation is not surprising despite reports to the contrary (Brunner et al., 1970). The influence of potassium on plasma renin activity may be altered by concomitant sodium intake and retention during potassium depletion but also by shifts of intracellular fluid to the extracellular space as demonstrated in studies of similar duration on subjects taking the same diets (Bartter, 1963) as in the present study.

Although changes in plasma bradykinin paralleled changes in plasma renin activity after the above manipulations, this was not true when ACTH was given to sodium-depleted normal subjects. The ACTH-induced increase in plasma renin activity, however, was related to the effects of ACTH-stimulated cortisol [which increased from 6.5 ± 0.5 (4 p.m.) to 47.0 ± 4.3 μg/dl (P < 0.001)] on renin substrate concentration [which increased from 0.91 ± 0.06 to 1.49 ± 0.09 μg/ml, P < 0.001 (Kraffoff, 1973)]. In all studies except the latter, the changes in both plasma bradykinin and plasma renin activity appear related to changes in extracellular fluid volume: upright posture and sodium depletion decreased extracellular fluid volume and increased both renin activity and plasma bradykinin, whereas potassium restriction, saline infusion (Wong et al., 1975) and sodium-retaining steroids increased the extracellular fluid volume and decreased both renin activity and plasma bradykinin. In support of this observation is the fact that prostaglandin inhibition decreased plasma renin activity in sodium-depleted normal subjects but had no effect on either plasma bradykinin or weight (Vinci et al., 1978). In patients with Bartter's syndrome, however, prostaglandin inhibition caused marked sodium retention, weight gain, a fall in plasma renin activity, and a marked decrease in plasma bradykinin. Thus, it is likely that plasma bradykinin and plasma renin activity are highly correlated because both respond to changes in extracellular fluid volume.

The biochemical mechanism by which plasma bradykinin changed in these studies is unknown. If the increase in plasma bradykinin had been caused by an increase in plasma kallikrein, then the levels of prekallikrein should have been decreased when plasma bradykinin was elevated. (The level of prekallikrein falls when it is converted by Hageman factor into free kallikrein.) On the contrary, in our study of subjects fed high- and low-sodium diets, the level of prekallikrein increased as sodium consumption decreased, and this increase appeared to reflect hemococoncentration. Thus, from the data, the elevations in plasma bradykinin cannot be linked to an increased production of this hormone. It should be emphasized, however, that the plasma prekallikrein pool is in great excess compared to the pool of free plasma kallikrein. Thus, a several-fold change in free kallikrein may result in such a minute alteration in prekallikrein that it is undetectable by current methodology.

It is unlikely that renal kallikrein was released into the circulation and generated bradykinin because renal kallikrein, as reflected by the excretion of kallikrein (Nustad et al., 1975), was markedly increased by the administration of fludrocortisone and ACTH but plasma bradykinin was not increased. Furthermore, glandular kallikreins normally are not found in human plasma (Pisano, 1975).

Alternatively, bradykinin levels may have been elevated because rates of bradykinin destruction were decreased. Although there are several enzymes that destroy bradykinin (Erdos, 1971), peptidyl dipeptide hydrolase (commonly known as angiotensin-converting enzyme or kininase II) not only hydrolyzes bradykinin but also hydrolyzes angiotensin I to angiotensin II (Ilgic et al., 1972). This pivotal enzyme generates the potent pressor peptide, angiotensin II, and inactivates the potent depressor peptide, bradykinin. Because the latter function is inhibited in vitro by angiotensin I (Alabaster and Bakhle, 1973), when renin levels are high, the elevation of angiotensin I may decrease the rate of bradykinin destruction. Although this is a tempting hypothesis proposed by Wong et al. (1975) and recently reiterated and supported by Mersey et al. (1977), it ignores the contribution made by other kininases (Erdos, 1971). Also, this theory does not accommodate well the fact that the level of plasma renin activity and plasma bradykinin are not always related, as during ACTH when levels of angiotensin I were undoubtedly high, and as during indomethacin (Vinci et al., 1978) which decreased renin but not bradykinin in normals. Furthermore, although Mersey et al. (1977) have shown a transitory increase in plasma bradykinin in normals 5 minutes after inhibition of converting enzyme with SQ 20881, the level of bradykinin returned to control after 10 minutes despite evidence of continued inhibition of the converting enzyme. With doses of SQ 20881 that were 35 and 100 times that of the latter study, plasma bradykinin was unchanged despite unequivocal inhibition of converting enzyme in hypertensive subjects (Vinci et al., 1977). Thus it is unlikely that this mechanism accounts for the changes in plasma bradykinin observed in the present study.

Finally, this study makes several contributions to our knowledge of the kallikrein-kinin system, a system which may be of importance in the regulation of blood pressure. It confirms the relationship of urinary kallikrein to sodium-retaining steroids under such an extremely wide range of excretion values that urinary kallikrein is a reliable index of sodium-retaining steroid activity. This study further suggests that urinary kallikrein may be influenced by other hormones such as ACTH, and demonstrates that urinary kinins are independent of either sodium-retaining steroids or kallikrein. The data show that plasma bradykinin and plasma renin activity are highly correlated, probably because
both are responsive to changes in extracellular fluid volume rather than because angiotensin-converting enzyme controls both systems in an interrelated fashion. The close relationship between kallikrein and sodium-retaining steroids suggests that kallikrein may participate in the antinatriuretic and/or kaliuretic effects of aldosterone and other sodium-retaining steroids. The changes of plasma bradykinin and renin activity further implies that this potent vasodilator, which stimulates the pressor action of angiotensin II and contribute to the maintenance of normal blood pressure when levels of angiotensin II are high.

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