SUMMARY To determine whether maneuvers known to modify immunoreactive urinary kallikrein excretion (iUKK) also alter the concentration of immunoreactive glandular kallikrein (iGKK) in plasma, we measured iGKK in the plasma and urine of rats before, at 1 week, and at 3 weeks after induction of two-kidney, one clip hypertension, low sodium intake, and DOCA-salt hypertension. Glandular kallikrein in plasma and urine was measured by radioimmunoassay. Clipping of a renal artery decreased iUKK from 11.7 ± 0.5 μg/24 hr/100 g body weight (BW) to 7.8 ± 0.5 and 8.2 ± 0.5 at 1 and 3 weeks after surgery without significantly changing iGKK in plasma. The level of iGKK in the plasma did not correlate significantly with iUKK in the clipped group. Low sodium intake significantly increased iUKK, which rose from 6.6 ± 0.3 μg/24 hr/100 g BW to 9.6 ± 0.5 and 13.9 ± 0.7 after 1 and 3 weeks. In addition, low sodium intake appeared to increase iGKK in plasma, and a significant positive correlation was observed between iUKK and iGKK in plasma in the group on low sodium diet (r = 0.65, p < 0.01). DOCA-salt treatment increased iUKK significantly from 10.4 ± 0.6 μg/24 hr/100 g BW to 17.1 ± 1.4 and 22.6 ± 2.3 at 1 and 3 weeks after. The iGKK in plasma increased from 13.8 ± 0.5 to 15.4 ± 0.7 ng/ml (p < 0.05) at 1 week after the DOCA-salt treatment began, but it returned to pretreatment levels 3 weeks later (14.5 ± 0.7 ng/ml, n.s.). There was no significant correlation between iUKK and iGKK in the plasma of the DOCA-salt-treated group. We conclude that the concentration of glandular kallikrein in plasma does not necessarily parallel changes in urinary kallikrein excretion, indicating that urinary kallikrein excretion cannot be taken as an indicator of the activity of the renal kallikrein-kinin system in the systemic circulation.

KEY WORDS • kidney • release • hypertension • low-sodium • deoxycorticosterone acetate

U RINARY kallikrein, a serine protease synthesized by the kidney and excreted in the urine, catalyzes the formation of kinins, which are vasoactive peptides. The renal kallikrein-kinin system has been the focus of considerable attention because of its possible involvement in the regulation of renal function and in the pathogenesis of hypertension. Renal kallikrein is a plasma membrane-bound enzyme present in the tubular cells of the distal nephron from where it is released into the tubular fluid. Besides being released into the urine, kallikrein enters the interstitial compartment and appears in the renal lymph in vivo and in the venous effluent from the isolated perfused kidney. However, we have recently demonstrated that the concentration of glandular kallikrein in renal venous blood is significantly lower than in arterial blood. We and others have also found that bilateral nephrectomy increases the levels of kallikrein in plasma. These data suggest that the kidney participates in the regulation of the levels of glandular kallikrein by removing the enzyme from plasma as it passes through the renal circulation. Thus, the contribution of the kidney to circulating levels of glandular kallikrein in vivo is not well understood.

The aim of the present study was to determine whether changes induced in the urinary kallikrein excretion rate would affect the level of glandular kallikrein in peripheral plasma. Thus, we studied glandular kallikrein in the plasma and urine of rats in three different sets of experimental conditions: two-kidney, one clip hypertension; low sodium intake; and DOCA-salt hypertension. We chose these experimental conditions because urinary kallikrein excretion is below normal in two-kidney, one clip hypertension; low sodium intake; and DOCA-salt hypertension. We observed that the concentration of glandular kallikrein in peripheral plasma is significantly lower than in arterial plasma. We and others have also found that bilateral nephrectomy increases the levels of kallikrein in plasma. These data suggest that the kidney participates in the regulation of the levels of glandular kallikrein by removing the enzyme from plasma as it passes through the renal circulation. Thus, the contribution of the kidney to circulating levels of glandular kallikrein in vivo is not well understood.

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Material and Methods

Heparin (Liquaemin Sodium) was obtained from Organon Inc., W. Orange, New Jersey; DOCA (deoxy cortisol acetate) from Sigma Chemical Company, St. Louis, Missouri; silicone rubber (3110 RTV) from Dow Corning Company, Midland, Michigan; Sephadex G15 from Pharmacia Fine Chemicals, Piscataway, New Jersey; and standard (Rodent Laboratory Chow No. 5001) and sodium-deficient (0.05% sodium) diets from Ralston Purina Company, St. Louis, Missouri.

Experimental Groups

Male Sprague-Dawley rats weighing 200–250 g were used in these experiments. All surgical procedures and blood withdrawals were performed under ether anesthesia.

Two-Kidney, One Clip Hypertension

Hypertension was induced in 22 rats by placing a U-shaped silver clip with an internal gap of 0.23 mm around the left renal artery. The contralateral kidney was left untouched. In nine sham-clipped rats, a clip with an internal gap of 0.40 mm was placed around the left renal artery. The animals were placed into metabolic cages and received a standard laboratory diet and tap water ad libitum.

Low Sodium Intake

Twenty rats, singly housed in metabolic cages, received a standard laboratory diet and tap water ad libitum for 7 days. Ten rats were then fed a sodium-deficient diet and distilled water to drink, while the remaining 10 rats were kept on the standard diet with tap water.

DOCA-Salt Hypertension

Rats in this group were separated into three subgroups. In the first subgroup, consisting of 18 rats, one strip of DOCA-silicone rubber (100 mg of DOCA per kg body weight) was inserted subcutaneously. Strips of DOCA-silicone were prepared by mixing DOCA with silicone rubber in a ratio 1:3. These rats received 1% sodium chloride as drinking fluid (DOCA-salt rats). In the second and third subgroups, consisting of nine rats each, strips of silicone without DOCA were implanted. The rats of the second subgroup received 1% sodium chloride (saline controls) and the rats of the third subgroup tap water (water control) as drinking fluid. All rats were placed into metabolic cages and were fed the same standard laboratory diet.

Immunoreactive Glandular Kallikrein in Plasma and Urine

Before and at the end of the first and third-week of treatment, urine was collected for a 24-hour period and stored at -20°C until assayed for kallikrein. Plasma was separated by centrifugation and kept at -20°C until its use. Immunoreactive glandular kallikrein in the plasma and urine was determined by radioimmunoassay (RIA) as previously described and expressed in ng/ml of plasma and μg/24 hr/100 g body weight, respectively. Before kallikrein determination, urine was gel-filtered on a Sephadex G15 bed to eliminate salt and urea, which might have interfered with the RIA.

Statistical Methods

Data are expressed as the arithmetic means ± the standard error of the mean (means ± SEM). To determine the within-group significance, we used a paired t test with an experiment α-error of 5%, using the Bonferroni method. Comparison between groups was done using the Student t test or the Scheffe’s method for multiple comparison analysis where appropriate. To establish the existence of correlation between the different variables studied, linear regression analysis was used. In all cases, the probability of 0.05 was used as the criterion of significance.

<table>
<thead>
<tr>
<th>Systolic BP (mmHg)</th>
<th>129 ± 29</th>
<th>127 ± 14</th>
<th>122 ± 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary Kallikrein Excretion (μg/24 hr/100 g bw)</td>
<td>12 ± 12</td>
<td>11 ± 11</td>
<td>11 ± 11</td>
</tr>
<tr>
<td>1 Glandular Kallikrein in Plasma (ng/ml)</td>
<td>20 ± 20</td>
<td>10 ± 10</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

**Figure 1.** Systolic blood pressure, urinary kallikrein excretion, and glandular kallikrein in plasma in rats before, at 1, and at 3 weeks after clipping of the left renal artery or sham operation. Glandular kallikrein concentration in the urine and plasma was measured by radioimmunoassay. Asterisks (*) indicate a significant difference between groups. Daggers (†) indicate a significant difference from the control period. ***, †† = p < 0.01; ***, ††† = p < 0.001; † = immunoreactive.
Results

Two-Kidney, One Clip Hypertension

In the experimental group, systolic blood pressure rose from 129 ± 4 to 143 ± 4 and 165 ± 8 mm Hg at 1 and 3 weeks after clipping. Throughout the 3-week period, there was no significant change in the blood pressure of the sham-operated group (fig. 1). At 1 week after clipping, urinary kallikrein excretion of the experimental group was significantly lower than that of the sham group and remained low through the third week (fig. 1, upper panel). No changes in the level of immunoreactive glandular kallikrein in plasma were observed after clipping (fig. 1, lower panel). Glandular kallikrein in plasma did not correlate significantly with urinary kallikrein excretion or blood pressure in the experimental group (r = 0.17 and −0.12).

Low Sodium Intake

The effects of chronic low sodium intake on urinary kallikrein excretion and on the concentration of glandular kallikrein in plasma are shown in figure 2. Urinary kallikrein excretion increased 45% and 110% after 1 and 3 weeks on a low sodium diet, respectively. This increase was unrelated to blood pressure, which did not change significantly throughout the study (fig. 2). Glandular kallikrein in plasma rose significantly from 9.5 ± 0.4 to 12.5 ± 0.5 and 13.9 ± 0.4 ng/ml after 1 and 3 weeks of low sodium intake (fig. 2, lower panel). During the control period, the concentration of glandular kallikrein in the plasma of the low sodium group was significantly lower than that of the control group. In the control group, at 1 and 3 weeks there was a small but significant increase in the level of glandular kallikrein in plasma. A statistically significant positive correlation was observed between urinary kallikrein excretion and the level of glandular kallikrein in the plasma in the group that received the sodium-deficient diet (fig. 3). No significant correlation was observed in the control group between these two parameters (r = −0.089).

DOCA-Salt Hypertension

At 3 weeks after DOCA pellet implantation, systolic blood pressure rose from 122 ± 3 to 149 ± 4 mm Hg (fig. 4). Only 8 of 18 rats treated with DOCA and saline had a systolic blood pressure above 150 mm Hg at 3 weeks after the treatment. Urinary kallikrein excretion increased significantly in the group receiving DOCA, from 10.4 ± 0.6 to 17.1 ± 1.4 and 22.6 ± 2.3 µg/24 hr/100 g BW after 1 and 3 weeks of treat-
ment. Urinary kallikrein excretion of the DOCA-treated group was significantly higher than that of both water and saline controls. A small but significant ($p = 0.02$) decrease in the excretion of urinary kallikrein occurred in the control group receiving water after 1 week. Urinary kallikrein excretion did not change in the saline control group during the experiment. The concentration of glandular kallikrein in plasma rose from $13.8 \pm 0.5$ to $15.4 \pm 0.7$ ng/ml ($p = 0.008$) after 1 week of DOCA treatment, and fell toward baseline after the third week of treatment, when urinary kallikrein excretion was highest. Glandular kallikrein in plasma did not correlate significantly with urinary kallikrein excretion or blood pressure in the experimental group treated with DOCA and saline ($r = 0.16$ and $-0.17$). Glandular kallikrein in the plasma of the DOCA-treated group was significantly higher than in the saline control group at 1 and 3 weeks after the treatment, but it did not differ from that of the control group on water. Three weeks after treatment, glandular kallikrein in the plasma of the saline control group decreased below the level found before the high sodium intake ($p = 0.007$).

**Discussion**

Renal kallikrein is located at the level of the distal tubule where it passes into the urine. Several investigators have proposed that the kidney also releases kallikrein into the circulation. This hypothesis was based on experiments demonstrating the presence of renal kallikrein both in urine and venous effluent from isolated rat kidneys perfused with a kallikrein-free medium. More recently, other investigators have documented that the basolateral membrane of the rat kidney contains an active kallikrein and a prekallikrein. From the basolateral membrane, these kallikreins may be released into the renal lymph and/or renal venous effluent. Because the kidney may release kallikrein into both urine and blood, it has often been assumed that the excretion of urinary kallikrein is an index of the activity of renal kallikrein in the interstitial and vascular compartments. For example, urinary kallikrein excretion is diminished in renovascular hypertension, and the subnormal production of this vasodilator system is thought to be involved in the pathogenesis of this type of hypertension. If urinary kallikrein excretion reflects the activity of the system in the peripheral circulation, changes in urinary kallikrein excretion should be expected to be paralleled by changes in the plasma concentration of immunoreactive glandular kallikrein. In the present study, we investigated whether maneuvers known to alter urinary kallikrein excretion also affect the levels of glandular kallikrein in plasma.

Decreased urinary kallikrein excretion has been reported in rats with two-kidney, one clip hypertension. The present study is in agreement with these reports and extends these observations by demonstrating that rats with this type of renovascular hypertension have normal levels of immunoreactive glandular kallikrein in plasma. This finding indicates that if renal kallikrein plays a role in the pathogenesis of the hypertension, its contribution must be through a local mechanism in the kidney and not through a systemic effect, since immunoreactive glandular kallikrein in plasma did not change.

Our present data also confirm previous observations that urinary kallikrein excretion increases after chronic sodium restriction. During the control period, the low sodium group showed a significant low level of IGKK in plasma as compared to the control group. We have no explanation for this. Nevertheless, we found that low sodium intake appears to increase the concentration of glandular kallikrein in plasma, and a significant positive correlation was observed between glandular kallikrein in plasma and urinary kallikrein excretion. However, the possibility that the elevated glandular kallikrein in plasma during low sodium intake derives entirely from the kidney appears remote. It is possible that low sodium intake, through an increase in sympathetic activity, increases the release of kallikrein into the circulation from the submandibular gland.

After DOCA administration, the excretion of kallikrein in urine increased markedly. However, only a small increase or no change in the plasma levels of
glandular kallikrein was observed in this situation. High salt intake alone, on the other hand, decreased the concentration of glandular kallikrein in plasma after 3 weeks, without affecting the excretion of kallikrein in urine.

In summary, the present study demonstrates that in rats with two-kidney, one clip hypertension, the levels of circulating glandular kallikrein are within the normal range despite the fact that their urinary kallikrein excretion is subnormal. Chronic sodium restriction increased both urinary kallikrein excretion and glandular kallikrein levels in plasma. DOCA-salt treatment induced a marked increase in the excretion of kallikrein in the urine accompanied by either a small increase or no change in the level of glandular kallikrein in plasma. We conclude that the concentration of glandular kallikrein in plasma does not necessarily parallel changes in urinary kallikrein excretion, indicating that urinary kallikrein excretion cannot be taken as an indicator of the activity of the renal kallikrein-kinin system in the systemic circulation.

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Hypertension. 1983;5:V153
doi: 10.1161/01.HYP.5.6_Pt_3.V153

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