Active and Inactive Kallikrein Released by Kidney Slices from Normotensive and Hypertensive Rats

Héctor Nolly, M.D., and M. Cristina Lama, B.S.

SUMMARY  The relative rates of release of active and inactive kallikrein were investigated in the rat kidney by studying in vitro kidney slices. The slices were kept in Warburg flasks in a Krebs-Ringer solution for 15 up to 60 minutes, and their kallikrein content and the kallikrein-like activity released by them were measured at different times. Renal kallikrein concentration from two-kidney, one clip hypertensive rats was less than in the sham-operated controls ($p < 0.01$). Active kallikrein released into the bathing medium was $4.7 \pm 0.3 \text{ng/mg/hr}$ in normotensive rats ($n = 10$) and $3.3 \pm 0.3$ in two-kidney hypertensive animals ($n = 12$); the difference between both groups was statistically significant ($p < 0.01$). Inactive kallikrein is between 60% and 70% of the total kallikrein released by kidneys of normal and hypertensive rats. Thus, under the conditions of these experiments, the kidney slices were able to release a kallikrein-like enzyme in an active and an inactive form. The rate of active kallikrein released from slices of hypertensive animals was lower than in the normotensive controls. (Hypertension 3 (suppl II): II-35-II-38, 1981)

KEY WORDS  •  kallikrein release • prekallikrein activation • kininogenase • renal enzymes • kinins

RECENT reports suggest the presence of an inactive form of kallikrein in perfusates and urine of isolated rat kidneys and in the urine of normotensive patients.1•4 This form of kallikrein is inactive at physiological pH but is activated by acid pH or by treatment with trypsin. Whether inactive kallikrein represents a true proenzyme or kallikrein bound to an inhibitor remains unclear. No data are available, however, relating to the release of active and inactive kallikrein in experimental animals.

The present studies were carried out to examine the release of active and inactive kallikrein from rat renal cortex using a technique of measuring the kallikrein concentration in rat kidney slices in vitro and the kallikrein-like activity released into the incubation liquid.

Materials and Methods

Male Wistar rats (180–220 g), maintained on a regular diet, were killed by a blow on the head, and both kidneys were rapidly removed. Sections of renal cortex, approximately 0.3 mm thick, were sliced with a razor blade and washed with a modified Krebs-Ringer solution. Incubation vessels contained approximately 100 mg tissue. The incubation technique was a modification of a method described previously.4 The slices were incubated in 3 ml of the Krebs-Ringer solution for 60 minutes in a Warburg apparatus at 37°C in an atmosphere of 95% O$_2$ and 5% CO$_2$. In all experiments, the slices were preincubated for 15 minutes, and then the incubation medium was discarded. The millimolar (mM) composition of the modified Krebs-Ringer solution was: NaCl, 140; KCl, 4; MgSO$_4$, 0.6; NaH$_2$PO$_4$, 1.2; CaCl$_2$, 2.5; dextran, 0.1; and glucose, 8. The pH of the solution was 7.45, and the osmolarity was 350 mOsm/liter. In several control experiments, oxygen consumption during the incubation period was measured; it was linear up to 120 minutes of incubation, indicating that the tissue was viable during this period of time.

Kidney slices of the following experimental groups of rats were used: 1) two-kidney, sham-clipped normotensive rats; and 2) two-kidney, one clip hypertensive rats. The animals were killed 36 days after clipping. Kallikrein substrate was prepared from titrated dog plasma which had been incubated for 3 hours at 56°-58°C. Ammonium sulfate was then added, and the fraction that precipitated between 34% and 50% saturation was saved. This fraction was dialyzed against tap water, distilled water, and then lyophilized.
To measure kallikrein concentration in kidney slices, kallikrein was solubilized with deoxycholic acid using a modification of the method of Nustad. For this, 100 mg of renal cortex were homogenized with 1 ml of 0.5% sodium deoxycholic acid (pH 8.5). The mixture was incubated for 30 minutes at 4°C, centrifuged, dialyzed, and the supernatant saved for kallikrein assay. The kallikrein activity was determined by incubating 0.005 ml of the homogenate with 2000 ng of the substrate preparation, and 0.3 ml of 0.3 M sodium ethylenediaminetetraacetic acid (EDTA) to inhibit kininases. The volume was adjusted to 2.5 ml with 0.1M phosphate buffer (pH 8.5). Samples were incubated at 37°C for 30 minutes, and the reaction was stopped by placing the samples in a boiling water bath for 5 minutes. Kallikrein concentration was expressed as nanograms of bradykinin • mg⁻¹ • hr⁻¹.

To measure the kallikrein-like activity released into the incubation medium at the end of each experiment, the liquid that was bathing the slices was removed, centrifuged at 2000 g, and the clear supernatant used to determine the active and inactive kallikrein-like enzymes. To activate kallikrein, one aliquot of 0.5 ml of the medium was incubated with trypsin (1 μg) for 20 minutes at pH 8.5 and 37°C, and after that purified ovomucoid trypsin-inhibitor (10 μg) was added to stop the reaction. To measure the kallikrein-like activity 0.40 ml of the incubation medium was incubated with enough excess substrate and phosphate buffer (0.1 M) in the presence of peptidase inhibitors to measure kallikrein activity in kidney tissue. The rate of kallikrein release was expressed as the amount of kallikrein released into the medium per milligram per hour. The kinins formed were assayed in the isolated cat jejunum and the blood pressure of the anesthetized rat. Active kallikrein was determined in the absence of trypsin. Total kallikrein was estimated after trypsin treatment, and inactive kallikrein was obtained by the difference between total (active plus inactive) and active kallikrein. Recovery of bradykinin was 78% ± 4%. All results are expressed as mean ± sem. To evaluate statistical significance, Student's t tests were used. The marker enzymes, acid phosphatase and glucose-6-phosphatase, were assayed in the kidney tissue before and after incubation and in the liquid bathing them. The substrates were p-nitrophenyl phosphate and glucose-6-phosphate respectively.

Results

The average kallikrein activity in rat kidney slices (n = 22) before and after incubation for 1 hour in Krebs-Ringer's solution was 37.4 ± 4.1 ng Bk/mg/hr and 36.9 ± 3.8 ng Bk/mg/hr respectively.

The active kallikrein released from the slices into the incubation medium was measured after different periods of time; figure 1 illustrates the amount of kallikrein released at 15, 30, 45, and 60 minutes of incubation. Kallikrein was released at a rate of 4.8 ± 0.5 ng Bk/mg/hr. The released kallikrein, expressed as a percentage of the kallikrein content of slices, was about 10% during the first hour of incubation. The release of kallikrein was compared with that of acid phosphatase, glucose-6-phosphatase, and protein. In figure 2, the amounts of kallikrein, protein, acid phosphatase, and glucose-6-phosphatase released were expressed as a percentage of the content of the slices. The percentage of kallikrein released in every period was much higher than the rates of release of acid phosphatase, glucose-6-phosphatase, and protein.
RELEASE OF ACTIVE AND INACTIVE KALLIKREIN/Nolly and Lama

Total kallikrein released from renal slices during different periods of incubation is shown in figure 3. Inactive kallikrein released after 1 hour of incubation was about 60% to 70% of the total kallikrein activity. The kininogenase activity generated by trypsin in samples of the incubation medium was completely inhibited by aprotinin and unaffected by soy bean and ovomucoid trypsin inhibitors (table 1).

Systolic blood pressure measured 36 days after clipping or sham operation in unanesthetized animals by tail plethysmography was 220 ± 16 and 120 ± 10 mm Hg respectively. The renal kallikrein concentration in kidney slices of normotensive rats (n = 10) was 39.6 ± 2.4 ng/mg/hr in the right kidney and 35.4 ± 3.0 ng/mg/hr in the left one; in the two-kidney hypertensives (n = 12) it was 21.6 ± 1.2 (right) and 20.4 ± 1.2 (left). Therefore, the renal kallikrein concentration in hypertensive animals was lower than in their controls, with the difference between both groups being statistically significant (p < 0.01).

Kidney slices from a group (n = 12) of two-kidney, one clip hypertensive rats released less active kallikrein than a group (n = 10) of normotensive sham-operated rats, as shown in figure 4. The difference between both groups was statistically significant (p < 0.01). However, there was no difference in total kallikrein activity between both groups of animals (p < 0.3).

Discussion

The present study uses a procedure developed to measure the amount of renal kallikrein-like enzyme released from kidney slices into an incubation medium. This preparation appears to be free of kininogen or kinins since these substances were not detected in multiple bioassay determinations. The released kallikrein-like enzyme showed the same pH optimum and the same pattern of inhibition by trasylool and by trypsin-inhibitors as that of rat kidney and rat urinary kallikreins.

Our results have confirmed previous reports of the presence of both active and inactive forms of kallikreins in urine and perfusates of isolated rat kidneys. We have found that the inactive form of kallikrein when submitted to trypsin treatment increases its kallikrein activity in about 60%. Inactive kallikrein may represent a storage form of the enzyme.

This study demonstrates that both renal kallikrein content in kidney slices and kallikrein-like activity released into the incubation medium is much lower in two-kidney, one clip hypertensive animals relative to values in sham-operated controls. Also, the excretion of urinary kallikrein has been reported to be reduced in this model of hypertensive animals. The significance of these findings with regard to the genesis of hypertension or to renal function is uncertain. Further

### Table 1. Identification of the Kininogenase Activity Released from Kidney Slices

<table>
<thead>
<tr>
<th>Kininogenase activity inhibited by</th>
<th>Active kallikrein released to the incubation medium</th>
<th>Total kallikrein released into the incubation medium</th>
<th>Glandular kallikrein</th>
<th>Plasma kallikrein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aprotinin:</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Soy bean trypsin inhibitor (SBTI)</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Ovomucoid trypsin inhibitor (OMTI)</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>yes</td>
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</tbody>
</table>
physiological and biochemical studies will be required to clarify these issues. Thus, these preparations of rat kidney slices could be applied to studies of renal kallikrein synthesis and release and appear to be useful for the study of the effects of various drugs and biochemicals on the activity of renal kallikrein.

Acknowledgments
The assistance of Peter Schneider and Dr. Luis Maria Rinaldim in the preparation of the manuscript is gratefully acknowledged.

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doi: 10.1161/01.HYP.3.6_Pt_2.II-35

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