Isolation of Renal Membranes That Contain Kallikrein, Angiotensin I-Converting Enzyme (Kininase II), and Angiotensinase

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**Title:** Isolation of Renal Membranes That Contain Kallikrein, Angiotensin I-Converting Enzyme (Kinase II), and Angiotensinase.

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20. Abstract

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ISOLATION OF RENAL MEMBRANES THAT CONTAIN KALLIKREIN, ANGIOTENSIN I-CONVERTING ENZYME (KININASE II), AND ANGIOTENSINASE


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SUMMARY

Fractions highly enriched in plasma membrane, endoplasmic reticulum, or brush border were prepared from rat kidney cortex. Kallikrein was concentrated in the plasma membrane fraction, but not in the brush border fraction. Angiotensin I-converting enzyme (kininase II) and angiotensinase were localized in the brush border membrane. It is suggested that kallikrein in the urine may originate from plasma membrane other than the brush border proximal tubules and the conversion of angiotensin I and the inactivation of bradykinin and angiotensin II may occur on the lumen membrane of the proximal tubular cells.

INTRODUCTION

Because kinins and angiotensins are involved in regulation of blood pressure, we are studying the renal enzymes that mediate the genesis and degradation of these vasoactive polypeptides. Kidney contains kallikrein (Nustad, 1970), angiotensin I-converting enzyme or kininase II (Erdős and Yang, 1967; Oshima et al., 1974; Erdős, 1975), and angiotensinase (Erdős, 1971). Although the isoenzymes of urinary kallikrein were isolated in very pure form (Nustad & Pierce, 1974), less is known about kallikrein in renal tissue. Changes in kallikrein excretion have been correlated with changes in sodium excretion and with certain hypertensive states (Marin-Grez et al., 1972; Croxatto et al., 1973; Margolius et al., 1974; Geller et al., 1975; Mills and Ward, 1975). Because the kinins possess natriuretic, diuretic, and hypotensive
properties (Webster and Gilmore, 1964; Nasjletti et al., 1975), it is thought that they are important physiologically.

As a prelude to an investigation of the biosynthesis, fate, and physiological alterations of these enzymes, we studied the subcellular distribution of kallikrein, angiotensin I-converting enzyme (kininase II) and angiotensinase in rat kidney. Kallikrein had previously been reported to reside in the lysosomal fraction (Carvalho and Diniz, 1966), or in another study in the microsomal fraction (Nustad, 1970) of kidney homogenates.

METHODS

Unanesthetized male Sprague-Dawley rats (250 to 350 g) were decapitated, and the kidneys then perfused through the aorta until free of blood with a solution of 0.25 M sucrose and 5 mM NaHCO₃, pH 7.5. Renal cortex from three to six rats (about 10 g) was dissected free, weighed, and minced.

Cells of the minced tissue were disrupted by nitrogen cavitation (Dowben et al., 1968), and a microsomal fraction prepared by differential centrifugation. Highly enriched fractions of plasma membrane (PM) and endoplasmic reticulum (ER) were obtained from the microsomal fraction by a modification (Birckbichler et al., 1973) of the method of Wallach and Kamat (1966) using sedimentation to equilibrium in a discontinuous gradient of Dextran 110.

Large fragments of brush border membranes from the proximal convoluted tubular cells were prepared by the method of Wilfong and Neville (1970). The cells were disrupted by gentle homogeni-
zation in hypotonic medium and harvested in a low speed sediment - 500 X g for 20 minutes.

Transmission and scanning electron micrographs of the various preparations were obtained as described elsewhere (Ward et al., 1976), and used to identify the membranes and to monitor the purity of the preparations.

Renal kallikrein was assayed by incubating samples (50 to 100 µl) with dog plasma kininogen substrate in the presence of the kininase inhibitors nonapeptide SQ 20881, EDTA, and o-phenanthroline at 37°C. The amount of kinin generated was determined on an isolated rat uterus (1 unit of kallikrein activity is the amount of enzyme that forms the equivalent of 1 ng bradykinin in 1 min) as described by Ward et al., (1975). The esterase activity of kallikrein was assayed using α-N-tosyl-L-arginine[3H]methyl ester as substrate (Beaven et al., 1971).

Renal kallikrein was assayed by incubating samples of renal tissues (20 µl), bradykinin (200 ng in 200 µl) and 0.1 M Tris, pH 7.4, 0.2 M NaCl (200 µl) at 37°C and following the inactivation of the peptide on the isolated rat uterus (1 unit of kininase activity is the amount of enzyme that inactivates the equivalent of 1 µg of bradykinin in 1 min).

Renal angiotensin I-converting enzyme (kininase II) (Yang et al., 1971) was assayed by incubating samples of renal tissues (500 µl) with 1 mM hippurylglycylglycine. The amount of glycylglycine released was assayed in a Beckman 121C amino acid analyzer.

The activities of various marker enzymes were also deter-
mained. Alkaline phosphatase was assayed with p-nitrophenylphosphate substrate (Linhardt and Walter, 1965). Glucose-6-phosphatase was assayed in the presence of 4 mM EDTA and 2 mM KF (Harper, 1965; Hübscher and West, 1965). Membrane ATPase was assayed by the method of Post and Sen (1967). Inorganic phosphate was determined by the method of Fiske and SubbaRow (1925). Protein was determined by the method of Lowry et al., (1951).

RESULTS

The results of six experiments are summarized in Table 1. The PM-enriched fraction collected at the interface of the two densities in the discontinuous gradient showed high activities of PM marker enzymes, particularly membrane ATPase activity and low activities of ER marker enzymes, particularly glucose-6-phosphatase. The ER-enriched fraction which was pelleted contained high activities of ER marker enzymes and low activities of PM marker enzymes.

The PM-enriched fraction contained more than 10% of total kallikrein activity present in the crude homogenate. As determined by bioassay, the specific activity of kallikrein in the PM-enriched fraction was three times higher than in the microsomal fraction, five times higher than in the ER-enriched fraction and 28-fold higher than in the crude homogenate. Similar enrichment of kallikrein activity was found by measuring the esterase assay with α-N-tosyl-L-arginine[3H]methyl ester.

The specific kininase activity was not higher in the PM-en-
riched than in ER-enriched fractions. It should be noted that the potent kininase inhibitor EDTA was used in the preparation of the membrane fractions; it is possible that the enzyme activity may be depressed and the apparent distribution of kininase is misleading after even transient exposure to EDTA.

Brush border-enriched fractions, studied in five experiments, were found to contain high activities of the brush border marker enzyme alkaline phosphatase. The relative specific activity of kallikrein in the brush border preparations was very low (0.2) while those of angiotensin I-converting enzyme (kininase II) and angiotensinase were high, the specific activities being enriched about 10- and 9-fold respectively.

DISCUSSION

It has been found in the past that the microsomal fraction of the homogenates of renal cortex contains enzymes that liberate or inactivate vasoactive peptides including kininase and angiotensin I-converting enzyme (Erdős and Yang, 1967; Yang et al., 1970; Erdős, 1975), kallikrein (Nustad, 1970), and renin (Wilson et al., 1976). Considering the extensive washing procedures employed in preparing the highly enriched membrane fractions used in the studies reported here, the enrichment of kallikrein, renal kininase, angiotensin I-converting enzyme, and angiotensinase in some of these fractions indicate that these enzymes are integral membrane proteins.

Kallikrein was most highly enriched in plasma membrane fractions but not in brush border preparations. Some kallikrein is found in ER fractions which is consistent with the possibility that
it is synthesized by membrane-bound polysomes. In contrast to kallikrein, renal kininase, angiotensin I-converting enzyme, and angiotensinase are present in high concentrations in brush border preparations. This selective distribution of these enzymes may play an important role in their physiological function.

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**TABLE 1**

**FRACTIONATION OF MICROSOMES FROM RENAL CORTEX**

<table>
<thead>
<tr>
<th></th>
<th>Plasma Membrane Fraction</th>
<th></th>
<th>Endoplasmic Reticulum Fraction</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specific Activity*</td>
<td>Relative Specific Activity**</td>
<td>Specific Activity*</td>
<td>Relative Specific Activity**</td>
</tr>
<tr>
<td>Glucose-6-Phosphatase</td>
<td>0.06 ± 0.01</td>
<td>1.1</td>
<td>0.43 ± 0.01</td>
<td>8.5</td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>0.64 ± 0.13</td>
<td>6.5</td>
<td>0.35 ± 0.09</td>
<td>3.6</td>
</tr>
<tr>
<td>Membrane ATPase</td>
<td>1.40 ± 0.42</td>
<td>3.5</td>
<td>0.60 ± 0.09</td>
<td>1.5</td>
</tr>
<tr>
<td>Kallikrein</td>
<td>28.0 ± 5.1</td>
<td>28.0</td>
<td>5.6 ± 1.1</td>
<td>5.6</td>
</tr>
<tr>
<td>Kininase</td>
<td>1.17 ± 0.11</td>
<td>3.5</td>
<td>1.01 ± 3.34</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* For units see Ward et al., 1975.

** Specific activity relative to crude homogenate.


