Preliminary Studies on a Rat Kidney Phospholipase A₂ Activating a Renin Preinhibitor

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Summary: A phospholipase A₂ activating a phospholipid renin preinhibitor into a lysophospholipid renin inhibitor has been isolated from rat kidney and partially characterized.

Key words: Phospholipid renin preinhibitor – Phospholipase A₂ – Rat Kidney

A phospholipid capable of inhibiting the renin-angiotensinogen reaction has been isolated from porcine, canine, and human kidney, and canine and human plasma (6, 7, 8). Although the chemical nature of the phospholipid has not been fully clarified, it has been demonstrated that its inhibitory action depends upon a previous transformation into a lysophospholipid (inhibitor) by one or more specific phospholipases (8, 2, 1).

We had previously reported that crude human kidney homogenate, unlike human and rat plasma, is capable of transforming preinhibitor into inhibitor (3). In this paper, we provide evidence for the existence of a phospholipase in rat kidney and describe some of its properties.

MATERIAL AND METHODS

Preparation of kidney homogenate

Male Sprague-Dawley rat were used. Renal tissue was washed repeatedly with 0.24 M sucrose at 4 C, minced with a razor blade, and then homogenized in a Potter-Thomas homogenizer with the roto in position 2.

Preparation of phospholipid renin preinhibitor

Phospholipid renin preinhibitor was isolated from hog kidney according to Sen et al. (2), and then dissolved in phosphate buffer, pH 7.4, 0.01 M.

Determination of protein concentration

Protein concentration in the kidney homogenate was determined according to Lowry et al., employing serum bovine albumin as a standard (5).
Enzyme assay

Enzymatic hydrolysis of the phospholipid renin preinhibitor was followed by continuous titration of the fatty acids released at constant pH (7.4), at 40°C with a Radiometer pH-Stat apparatus (TTT-2 Titrator). The assay system was standardized to 3 ml total volume, and contained a constant quantity of buffer, while the amount of substrate, Ca++, and enzyme was varied. Titration with 0.04 M NaOH was carried out in a N2 atmosphere. The titration values of substrate controls (without enzyme) and enzyme controls (without substrate) were subtracted from those of the whole system.

Specific activity of the enzyme was expressed in nano equiv. NaOH, consumed per min., per mg protein.

RESULTS AND DISCUSSION

Fig. 1 illustrates calcium dependence of enzyme activity. Maximum activity was obtained when 8 micromoles Ca++ were added to the incubation medium. Further addition of Ca++, up to 15 micromoles, had no effect. The reaction rate obtained when Ca++ was not added was not zero, possibly indicating that traces of calcium were present in the enzyme preparation. This suggestion is supported by the finding that phospholipase activity was completely suppressed when a chelating agent (EDTA, 10 µ moles) was added to the incubation medium.

Fig. 2 shows phospholipase activity as a function of time. As can be seen, the release of fatty acid increased up to about 2 hrs. After this period, enzyme activity reached a plateau.

A lag period of about 20 minutes was observed, and even ultrasonication of the homogenate did not modify either the reaction rate or the time course.