Is Renin Secreted by Exocytotic Mechanism Through Mature Renin Granules from Juxtaglomerular Cells?

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Mature renin granules were isolated by the combination of discontinuous and continuous Percoll density gradient centrifugation. Stored renin in the renin granules was found to consist of isoelectrically seven different forms. The seven different isoelectric points (pl) were 5.6, 5.35, 5.2, 5.0, 4.8, 4.6 and 4.4. Approximately 70% of the stored renin as the total enzymatic activities from all isoelectric peaks was found in a peak which pl corresponded to be 5.35. Renin secreted from isolated glomeruli was also focused into seven peaks possessing identical values. However, the distribution pattern of renin peaks was quite different from that of stored renin. In the secreted renin, peaks of 5.35 (pl) and 5.2 (pl) showed high renin activity and each had approximately 30% of released renin as the total recovered. These results indicate multiple forms of renin are stored and secreted by rat kidney. As the distribution pattern of enzymatic activities in renin peaks between stored renin and secreted renin are different, it is probable that renin may not secreted through mature renin granules by exocytotic mechanism.

Since the pioneering work of Dr. Palade who proposed a model of intracellular pathway of secretory protein, much evidence has been accumulated supporting his model not only in secretory proteins but also in secretory substances including neurotransmitters. It is generally supposed that renin synthesized in rough endoplasmic reticulum is translated into Golgi, prototype of renin granules and then, released by exocytotic mechanism after the renin is stored in mature renin granules. In electron-microscopic studies, some investigators have found that in the pathological state, renin may be secreted by channel-like invagination of plasma membrane as an unusual form of exocytosis. However no one has reported the exocytotic process of renin in normal state. Recently we isolated mature renin granules from rat kidney cortex. In isolated fractions, mitochondria and microsome were not detectable by biochemical or electron microscopic studies. Renin in the granules was found to have isoelectrically seven different points. If renin is secreted through renin granules by exocytosis, secreted renin should have similar characteristics to stored renin. Therefore, in the present study, we examined whether renin secretion from isolated glomeruli is through renin granules by estimating identification of the biochemical characteristics between granule renin and secreted renin.

Key Words:
Renin
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MATERIALS AND METHODS
Isolation of mature renin granules
Male Sprague-Dawley rats weighing 180–220g

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were anesthetized with sodium pentobarbital (5 mg/100g body wt, ip). After an abdominal incision was made to expose the kidneys, they were removed and the kidney cortex was sliced. The cortex was gently homogenized with 0.45M sucrose (1:7, wt/vol) in order not to destruct renin granules but to disrupt cells. After centrifugation of the homogenate at 500 xg for 10 minutes, mature renin granules in the supernatant (original homogenate) were isolated by the combination of discontinuous and continuous Percoll density gradient centrifugation. Briefly, 4 ml of the original homogenate was placed on a discontinuous Percoll density gradient which consisted of three layers of 1.07 g/ml, 1.11 g/ml and 1.15 g/ml. After centrifugation, interphase between 1.11 g/ml and 1.15 g/ml was fractionated. The fractionated sample was further centrifuged at 20,000 xg for 90 minutes using angle rotor. Renin granules were obtained in fraction which corresponded to a density 1.138 g/ml. Renin granules were lysed by mixing the solution with 0.1 volume of 0.1M phosphate buffer, pH 7.0, containing Triton X-100. After centrifugation at 100,000 xg for 120 minutes, the supernatant was used as stored renin. The specific renin activity was 0.46 µg AI/h/µg protein.

Renin release from isolated glomeruli

Isolation of glomeruli was carried out by the sieving method and the glomeruli were incubated for renin release experiment as described previously. Isolated glomeruli were washed with a modified Krebs-Ringer solution (140 mM NaCl, 5 mM KCl, 0.8 mM MgSO4, 2 mM CaCl2, 10 mM sodium acetate, 2 mM sodium phosphate monobase, 10 mM glucose and N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pH 7.2) containing 0.1% bovine serum albumin which had been gassed with 95% O2-5% CO2. After preincubation for 10 minutes at 37°C under the atmosphere, the solution was decanted. After three washes, the fresh solution was added to a tube containing the glomeruli and incubated for 1 h at 37°C under the atmosphere. An aliquot of the solution was pipetted after the 30 minute incubation.

Isoelectric focusing

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Isoelectric focusing was performed in a rod of preformed 5% acrylamide gel, pH range 4–6.5 containing Pharmalyte (Pharmacia). The following electrode solutions were used: 0.01M glutamic acid at the cathode and 0.01M histidine at the anode. One hundred microliter of sample was applied to the gel and run for 10h at 4°C with 500 volts. The pH gradient was evaluated at 25°C from gel slices in 20% glycerol.

**Renin activity**

Renin activity was measured by the rate of formation of angiotensin I from rat angiotensinogen as described previously. Angiotensin I produced was assayed by radioimmunoassay according to the method of Haber et al.

**RESULTS AND DISCUSSION**

Figure 1 shows a representative isoelectric pattern of renin activity obtained after electofocusing of stored renin. The renin routinely focused into 7 peaks with isoelectric points of 5.6 (peak I), 5.35 (peak II), 5.2 (peak III), 5.0 (peak IV), 4.8 (peak V), 4.6 (peak VI) and 4.4 (peak VII). Similar results could be repeated reproducibly in four separate experiments. Approximately 70% of the stored renin in the total recovered from all peaks was found in peak II. If this stored renin is secreted by exocytotic mechanism, the released renin should have similar biochemical characteristics of stored renin. Therefore, renin secretion was observed using isolated glomeruli. We chose this material for the secretion experiment to avoid artifact by attack of protease to released renin. Figure 2 shows time course of the amount of renin secreted in the medium. Renin was secreted linearly with time up to 1h. Lactic dehydrogenase activity was not detectable even after 1h incubation. These results suggest that the renin secretion did not reflect leakage of renin from non-viable cells. Figure 3 shows a representative isoelectric pattern of renin activity obtained after electofocusing of the released renin. This renin was also focused into seven peaks possessing identical values. However, the distribution pattern of renin peaks after isoelectric focusing was quite different from that of stored renin. In the secreted renin, peaks II and III showed high renin activity and each had approximately 30% of the total recovered. There are two possible explanations for the difference of distribution pattern between stored renin and released renin. First, secreted renin may not originate from mature renin granules but from other subcellular components. Recently the presence of more than one pathway of renin secretion has been proposed. Pratt et al. suggest the existence of a pathway in which renin is not stored in mature renin granules, but directly secreted, using labelled renin-chasing method in mouse submaxillary gland. We also found the pathway in normal rat kidney and furthermore we suggested that isoproterenol-stimulated renin secretion was originated from mature renin granules (unpublished work). These results support the theory that renin secretion in basal state originates not from mature renin granules but from other compartments of juxtaglomerular cells. Second, secreted renin may originate from mature renin.
granules. Renin in the medium might have been attacked by protease released in the medium even though care was taken to prevent the effect of the non-specific protease. To examine this possibility, stored renin was added to the incubated medium and then after 1h incubation, we measured the isoelectric distribution pattern of the mixed sample. The incubated medium had no influence on the distribution pattern of the stored renin. This result excludes the possibility of artifact by protease in the medium. For the secreted renin originated from mature renin granules, it is possible that renin was secreted in the medium after renin had been released into cytosol from mature renin granules. In this case, renin may be secreted after the renin was enzymatically modified during cytosol. Funakawa et al. showed that renin is released from renin granules. It is known that acetylcholine in cytosol directly participates in the release mechanism. However, in electronmicroscopic studies using specific renin antibody, renin was localized mainly in renin granules in juxtaglomerular cells. Figure 4 shows scheme of a putative intracellular pathway of renin. Further studies are necessary to determine the intracellular pathway for a better understanding of intracellular control of renin secretion.

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