Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 7837–7840, November 1987
Biochemistry

Human renin biosynthesis and secretion in normal and ischemic kidneys

(prorenin/Golgi/protein processing/protein secretion/multiple pathways of secretion)

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Communicated by Irvine H. Page, July 16, 1987 (received for review April 16, 1987)

ABSTRACT **The pathway of renin biosynthesis and secretion in normal and ischemic human kidneys has been investigated by pulse-labeling experiments. The results indicate that in normal human kidney, preprorenin is rapidly processed to 47-kDa prorenin. Microradiosequencing showed that this molecule was generated by cleavage between Gly-23 and Leu-24, yielding a 43-amino acid proregion. Analysis of prorenin secreted by the kidney tissue yielded an identical sequence, indicating that prorenin is secreted without any further proteolysis. An examination of the kinetics of processing and secretion suggested that a majority of the newly synthesized prorenin is quickly secreted, while only a small fraction is processed intracellularly to the mature renin. The differences in secretion kinetics between prorenin and mature renin and the selective inhibition of prorenin secretion by monensin suggest that they are secreted independently via two pathways: a constitutive pathway probably from the Golgi or protogranules that rapidly release prorenin and a regulated pathway that secretes mature renin from the mature granules. A comparison of the kinetics of processing between normal and ischemic tissues suggests that renal ischemia leads to an overall increase in the rate of processing of prorenin to mature renin. In addition, prolonged biosynthetic labeling of renin in the ischemic kidney yielded two smaller molecular weight immunoreactive forms suggestive of renin fragments that may be degradative products. These fragments were not detected in normal kidney tissue labeled for similar lengths of time.**

Renin is an aspartyl proteinase synthesized primarily in the kidney and secreted into the plasma. It is the rate-limiting enzyme in the biochemical cascade that generates the potent vasoconstrictor angiotensin II (1). The molecular cloning of the cDNA corresponding to human renin mRNA has provided the amino acid sequence of preprorenin (2). However, many questions concerning the biosynthetic processing of human renin remain unanswered. For instance, analysis of the sequence of the preproregion suggests three possible cleavage sites that could result in prosegments of 43, 46, or 48 amino acids (3). Cell-free translation of human kidney mRNA in the presence of dog pancreatic microsomes results in a single prorenin containing the 43-amino acid prosegment (4). It remains to be determined if this is the only cleavage site for the native authentic processing in the human kidney.

The details of intracellular processing and secretion of human kidney prorenin have not been reported. It is unclear if prorenin and mature renin are cosecreted by the same pathway or are separately compartmentalized and independently secreted. It has long been assumed that prorenin is secreted and accounts for the inactive renin found in human plasma. Hirose *et al.* (4) proposed that the inactive renin in human plasma was a partially processed (i.e., "truncated") prorenin. This postulate has not been confirmed by the direct structural analysis of the secreted prorenin. It is also unclear from their results if the processing of human prorenin to the intermediate form is an intracellular or extracellular event. If it is an intracellular event, is this an obligatory step for secretion of prorenin or is the full-length prorenin also secreted? Finally, it is not known whether renal ischemia influenced renin processing and the pathway of secretion. With these questions in mind, we undertook an examination of renin biosynthesis and secretion in normal and ischemic human kidneys.

MATERIALS AND METHODS

We performed pulse-labeling experiments on four separate fresh kidney samples obtained during nephrectomy. Three of the kidneys were removed for advanced renal ischemia, whereas the fourth sample represented normal portions of a kidney removed radically for renal cell carcinoma.

Biosynthetic Labeling of Human Kidney. All preincubations and incubations were performed in 20-ml vials gassed with 95% O_2/5% CO_2 containing approximately 200 mg (wet weight) of tissue in 1.5 ml of RPMI 1640 medium (GIBCO) saturated with 95% O_2/5% CO_2. Renal cortex, preincubated in medium lacking methionine for 30 min at 37°C was incubated with fresh medium containing [^{35}S]methionine (>1000 Ci/mmol, 0.5–1.0 mCi/ml New England Nuclear; 1 Ci = 37 GBq) at 37°C for various times as described (5). Chase experiments were performed by incubating the radiolabeled tissue in medium containing unlabeled methionine. Labeling of tissue to generate renin for radiosequencing was performed in a similar manner except that the [^{35}S]methionine was increased to 2 mCi/ml. In a separate experiment, labeling was also performed with [^{3}H]leucine (140 Ci/mmol, 2–3 mCi/ml, New England Nuclear).

In some experiments, the effect of monensin (6–8) was also determined. Tissue was exposed to 0.1 μM monensin during the preincubation and for the 2-hr labeling.

Immunoaffinity Chromatography. Following incubation, the tissue was sonicated in 0.1 M Tris·HCl, pH 7.4/0.1% Triton X-100/0.1 mM phenylmethylsulfonyl fluoride/0.25 mM ethylenediaminetetraacetic acid/0.25 mM sodium tetrathionate. The sonicate and media (supplemented with the same levels of Triton X-100 and inhibitors) were rocked at 4°C overnight with an immunoaffinity column [monoclonal antibody R-3-47-10, which is specific for human renin (9), bound to Sepharose (Pharmacia) (10)] or with a control column (bovine serum albumin coupled to Sepharose). After a washing with 20 mM phosphate buffer containing 0.3 M

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NaCl, 0.125% Triton X-100, and 0.01% $NaDodSO_4$, the renins were eluted by incubation at 100°C in the presence of $NaDodSO_4$ sample buffer (11) containing 2-mercaptoethanol, were analyzed by $NaDodSO_4$/PAGE (11), and were detected by fluorography with EN^{3}HANCE (New England Nuclear). Where indicated, the fluorograms were quantitated by densitometry (LKB, Paramus, NJ).

Radiosequencing. The radiolabeled renins, isolated as above, were eluted from the antibody-Sepharose by heating to 56°C for 15 min. After $NaDodSO_4$/PAGE, the gel was dried and the renin forms were located by autoradiography. The appropriate regions of the gel were cut, the gel was rehydrated, and the proteins were electroeluted as described (12). Automated Edman degradation was performed with an Applied Biosystems gas-phase sequencer, and the radioactive material eluted at each cycle was quantitated by using a LKB 1212 liquid scintillation counter with the sample mixed with Aquasol (New England Nuclear). Each fraction was assayed for radioactivity for 20 min.

RESULTS

Continuous labeling of the ischemic and normal human kidneys resulted in the appearance in both the tissue and medium of predominantly two forms of renin with molecular masses of 47 and 41 kDa (Fig. 1). The 47-kDa protein was prorenin because its molecular mass was comparable to that reported by Hirose *et al.* (4) for prorenin generated from mRNA translated in oocytes and by Fritz *et al.* (13) and Hobart *et al.* (14) for the molecular mass of prorenin synthesized in cultured mammalian cells transfected with the human renin cDNA and gene, respectively. Additional proof that this 47-kDa protein was prorenin was obtained by microradiosequencing (Fig. 2). Sequential Edman degradation of the 47-kDa prorenin isolated from tissue labeled for 6 hr with [^{35}S]methionine yielded a radioactive peak at cycle 16. When analysis was performed on the prorenin isolated from medium, a profile identical to tissue prorenin was observed (Fig. 2). Since the proregion contains three methionine residues, the possible N termini are Leu-24, Lys-37, or Arg-49 (2). Analysis of the 47-kDa tissue and medium prorenin labeled with [^{3}H]leucine yielded a radioactive peak at cycle 1 (data not shown). Taken together, these data are consistent with the removal of the presequence by cleavage between Gly-23 and Leu-24, resulting in a proregion of 43 amino acids (Fig. 2) and a prorenin with a molecular mass of 47 kDa. This indicates that the majority, if not all, of the prorenin secreted by human kidney tissue is released intact without any further proteolytic processing. Qualitatively, these observations were identical for both normal and ischemic kidneys.

The results of the continuous labeling experiment suggested that the prorenin is secreted constitutively. This is more clearly seen in the pulse–chase experiments in which the tissues were labeled for 1.5, 3, or 6 hr, followed by a 1-hr chase. Examination of the medium and tissue samples (Fig. 1 *Middle*) revealed that prorenin is either secreted rapidly or is cleaved intracellularly by a slower process to mature renin. Indeed, after the 1-hr chase, prorenin was barely detected, if at all, in these tissues. This is in sharp contrast to the continuous labelings (Fig. 1 *Top*) where radiolabeled prorenin constituted a large fraction of the total radiolabeled renins. In tissues chased for 1 hr, mature renin could be detected but at low levels. However, in all three experiments, prorenin was readily seen in the medium. Thus, it appears that the majority of prorenin has a short halflife in the tissues (<1 hr) and is rapidly secreted. A small fraction of the prorenin is processed intracellularly to mature renin.

A comparison of renin processing and secretion by normal and ischemic kidney tissues was performed. The result (Fig. 1 *Bottom*) showed that, qualitatively, the processing and secretion in normal kidney tissue is similar to that seen in Fig. 1 *Top* for the ischemic kidney. However, the rate of processing appeared to be considerably slower. Densitometric analysis showed that for the same period of labeling, the ratio of prorenin to mature renin was 2- to 3-fold greater in normal kidney as compared to the ischemic kidney (5.5 ± 0.4 vs. 2.1 ± 0.5, respectively; $n = 2$).

To gain more insight into the secretory pathway of renin, we performed pulse-labeling in the presence of the carboxylic

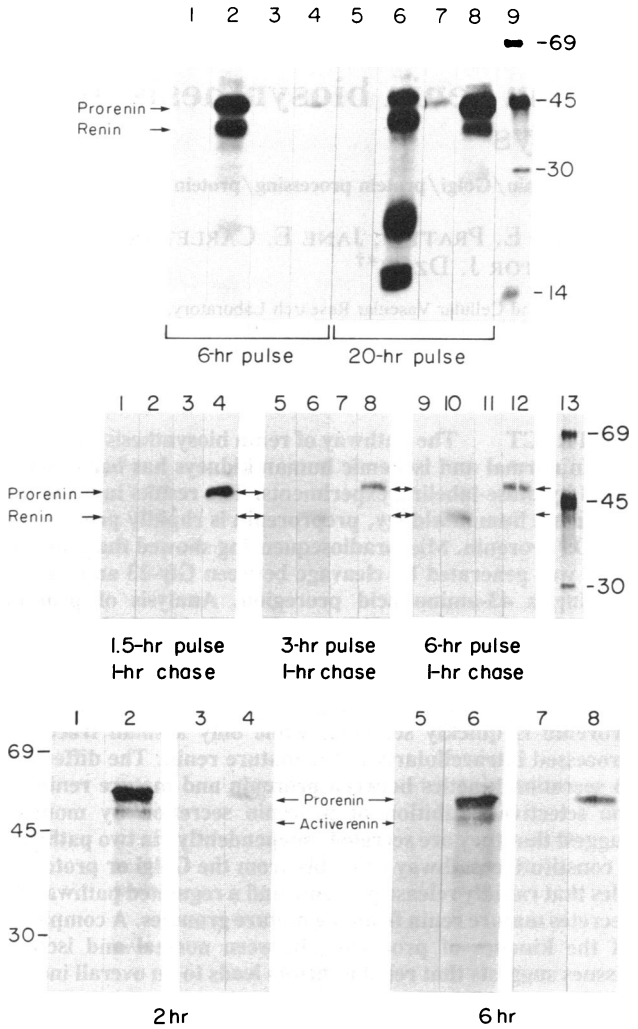

FIG. 1. (*Top*) Continuous labeling of an ischemic kidney. Samples from tissue homogenates (lanes 1, 2, 5, and 6) and medium (lanes 3, 4, 7, and 8) were incubated with antirenin Sepharose (even-numbered lanes) or a control bovine serum albumin-conjugated Sepharose (odd-numbered lanes). The eluates were analyzed by $NaDodSO_4$/PAGE. Radiolabeled molecular size standards are shown in lane 9 in kDa. (*Middle*) Pulse–chain labeling of an ischemic kidney. Tissue from the same kidney used in *Top* was labeled with [^{35}S]methionine for 1.5, 3, or 6 hr at which time the medium was replaced with medium containing unlabeled methionine and was incubated for an additional hour. The tissue (lanes 1, 2, 5, 6, 9, and 10) and medium (3, 4, 7, 8, 11, and 12) samples were incubated with antirenin antibody-conjugated Sepharose (even-numbered lanes) or control bovine serum albumin-conjugated Sepharose (odd-numbered lanes). Radiolabeled molecular size standards are shown in lane 13 in kDa. (*Bottom*) Pulse labeling of a normal human kidney. Samples from tissue homogenates (lanes 1, 2, 5, and 6) and medium (lanes 3, 4, 7, and 8) were incubated with antirenin antibody-conjugated Sepharose (even-numbered lanes) or a bovine serum albumin-conjugated Sepharose (odd-numbered lanes). The eluates were analyzed. Molecular mass standards are shown in kDa.

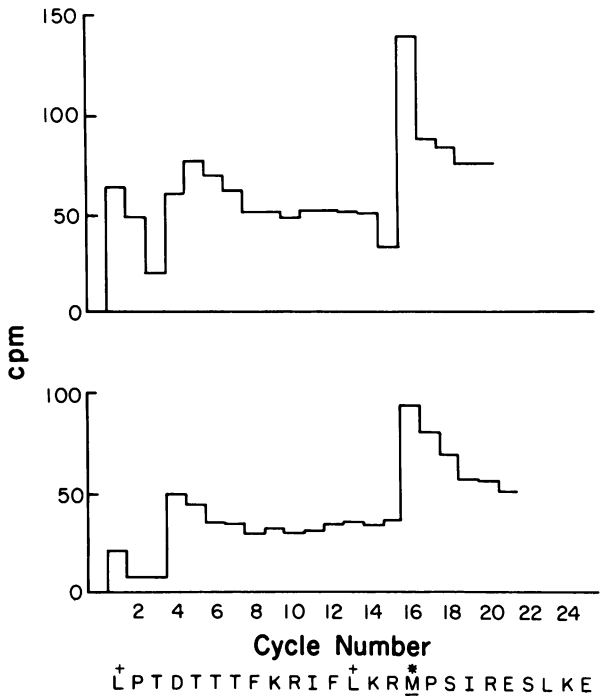

FIG. 2. Microradiosequencing of prorenin. Human kidney samples were labeled with [^{35}S]methionine. The prorenin was isolated from the tissue (*Upper*) and medium (*Lower*) and was sequenced by a gas-phase sequenator. The radioactivity of the material eluted at each cycle is plotted. For reference, the sequence of the first 25 amino acids of prorenin is displayed along the bottom. The detection of the [^{35}S]methionine peak at cycle 16 predicts the amino-terminal residue to be leucine. This was confirmed in a separate experiment using [^{3}H]leucine in which leucine was detected at cycle 1 (data not shown).

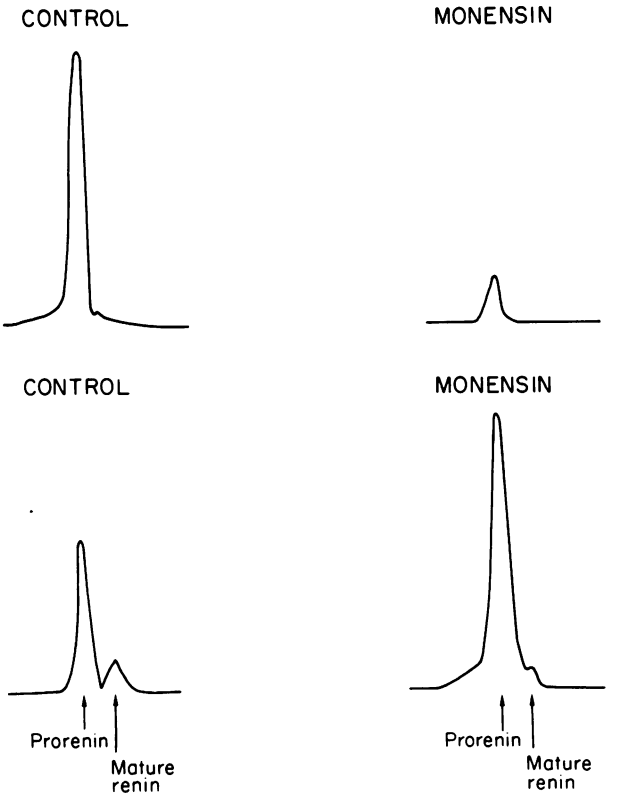

FIG. 3. Densitometric analysis of radiolabeled renin in medium (*Upper*) and tissue (*Lower*) synthesized in the presence (*Right*) or absence (*Left*) of monensin. The ordinate shows the density of the bands, and the abscissa shows the direction of migration in the gel (from left to right). Note that monensin reduced the secretion of prorenin and increased the amount of tissue prorenin.

ionophore monensin. Monensin (6–8), which disrupts the transport of proteins through the Golgi, invariably led to an inhibition of the secretion of radiolabeled prorenin from normal and ischemic tissue. As an example, Fig. 3 shows the renin forms synthesized and secreted in the normal kidney in the presence or absence of monensin. Monensin led to an increase in tissue prorenin and a decrease in the secretion of prorenin into the medium.

We also observed the presence of two small molecular forms of renin (23.4 and 18.6 kDa) in the ischemic kidney (Fig. 1 *Top*) but not in the normal kidney (Fig. 1 *Bottom*). Each represented 5–10% of the tissue renins after 6 hr of labeling and 25% of the total tissue renin after 20 hr of labeling. Analysis of the renins secreted after the 20-hr labeling revealed low levels (<1% of medium renins) of these smaller forms, suggesting that these are primarily intracellular forms.

DISCUSSION

To our knowledge, detailed examination of the biosynthetic processing of renin in the human kidney has not been reported previously. Our data taken together with those of others (4, 15) provide the following scheme for renin biosynthesis in the human kidney (Fig. 4). Translation of renin mRNA yields preprorenin. Cotranslational removal of the 23-amino acid signal peptide sequence occurs during the transfer of preprorenin into the cisterne of the rough endoplasmic reticulum. At some time point during the transfer from the rough endoplasmic reticulum to the Golgi, the processing of prorenin to mature renin begins. Prorenin has two major fates. It is rapidly secreted by a constitutive pathway directly from the Golgi or protogranule. Prorenin also may be packaged into immature granules, where it is further processed to the active 41-kDa renin during condensation and maturation of the secretory granules. In normal human kidney, the latter process is relatively slow. However, in ischemic kidneys prorenin conversion to active renin is accelerated.

Our data demonstrate that human kidney secretes renin by two cellular pathways. Prorenin is secreted principally by the constitutive pathway, while mature renin is secreted from the secretory granules. Although these secretory pathways have been proposed by other investigators based on studies of human chorionic cells (16) and juxtaglomerular tumor cells

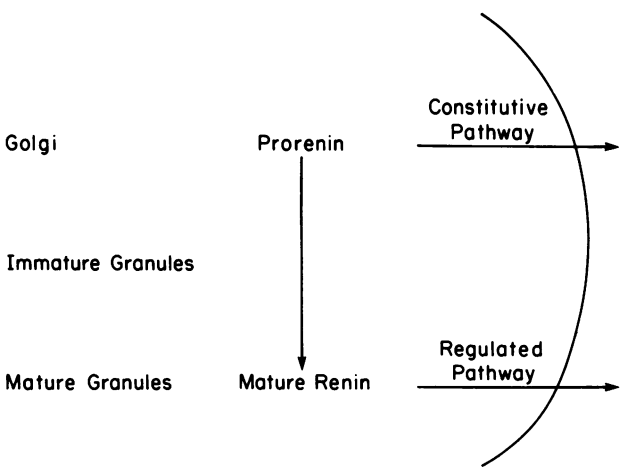

FIG. 4. Schematic representation of the biosynthesis and secretion of human renin.

(17), such demonstrations in the human kidney have not been reported previously. Our data also suggest that prorenin is secreted by the kidney in the intact form and not as a partially processed (truncated) form as proposed by Hirose *et al.* (4). These investigators based their conclusions on the inability of antibodies generated to peptides corresponding to the amino-terminal region and midregion of the prosegment to bind plasma inactive renin. Recently, Taugner *et al.* (15) could not detect intracellular staining with the same amino-terminal region-directed antisera. This observation is perplexing since our biosynthetic studies demonstrated that intact prorenin is the major renin precursor form. Instead, these investigators observed differential staining of the proto- and intermediate granules with the midregion- and carboxyl-terminal region-directed antibodies. Based on these observations, they proposed that prorenin was rapidly and completely converted to intermediate forms of renin via several sequential cleavages. Our data do not support either hypothesis. Rather, based on the homogeneity of the tissue and medium prorenin bands on the fluorograms and on the microradiosequencing data, we propose that a single cleavage is responsible for the conversion of prorenin to mature renin. We speculate that the apparent discrepancy between our data and that of Taugner *et al.* (15) and Hirose *et al.* (4) could be explained by alterations in the tertiary structure of the prorenin molecule during the maturation process of the secretory granules. This process involves packaging, condensation, and concentration of granular contents and may result in a progressive folding of the prorenin molecule. We have shown (18) that a monoclonal antibody raised to the amino terminus of the prorenin did not bind the native molecule but would bind denatured prorenin. This observation suggests that the amino terminus of native prorenin may not be on the surface of the molecule and, hence, is not available for antibody binding. This may explain the failure of amino-terminal-directed antibody to stain human kidney and the differential staining by antibodies to the midregion and carboxyl region of the prosegment in the proto vs. mature granules as reported by Taugner *et al.* (15). Although the data in this paper cannot exclude low concentrations of an intermediate prorenin form, they clearly demonstrate the dominance of the intact prorenin molecule as the secretory product and as the immediate precursor to mature renin.

We have observed that a small amount of an intermediate form of renin (43 kDa) could be detected in the medium of an ischemic kidney tissue after a prolonged pulse experiment (data not shown). Since this was only detected after prolonged incubation, we believe that this represented an extracellular degradative process and was not an intracellular obligatory step of renin processing.

Two small immunoreactive renin forms observed in the labeling of the ischemic kidney probably represent the putative renin fragments described by Galen *et al.* (19) and Do *et al.* (20) obtained from human cadaver kidney purification. The sum of the molecular masses of these two forms equals that of the mature renin. We believe that these fragments represent the initial degradative products of intracellular renin metabolism, which may be accelerated during renal ischemia. It has been argued (21) that these smaller molecular mass forms were due to nonspecific proteolysis of native renin during the purification procedures. Our labeling results raise the possibility that the formation of this protein may be due to an intracellular process. However, since these fragments were detected only in the ischemic kidney (Fig. 1) or during the isolation of renin from cadaver kidneys or renin tumor (19, 20), the possibility exists that these fragments are not authentic renin forms found in normal "healthy" tissue. Indeed, in the normal kidney (Fig. 1 *Bottom*), we were unable to detect these subunits despite prolonged labeling.

In summary, studies on human kidney renin biosynthesis indicate that prorenin is secreted by a constitutive pathway. Prorenin also can be processed to mature renin, which is secreted by a regulated pathway. In the ischemic kidney, the processing of prorenin to mature renin is facilitated. Furthermore, smaller molecular mass forms of renin can be detected in the ischemic kidneys. The formation of these fragments may be the result of intracellular renin degradation, which may be greatly enhanced during ischemia.

We thank Dr. Lawrence Duffy for performing the protein sequencing. We thank Ms. Donna MacDonald for providing expert secretarial assistance. This work was supported by National Institutes of Health Grants HL35610, HL35792, HL19259, and HL35252 and by the National Institutes of Health Specialized Center of Research in Hypertension Grant HL36568. V.J.D. is an Established Investigator of the American Heart Association.

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