Human renin biosynthesis and secretion in normal and ischemic kidneys
(prorenin/Golgi/protein processing/protein secretion/multiple pathways of secretion)

RICHARD E. PRATT*, JANE E. CARLETON*, JEROME P. RICHIE†, CHRISTOPH HEUSSER§, AND VICTOR J. DZAU**

*Molecular and Cellular Vascular Research Laboratory, Division of Vascular Medicine and Atherosclerosis, Department of Medicine, and †Department of Surgery, Brigham and Women’s Hospital, 75 Francis Street, Boston, MA 02115; and §Ciba Geigy Limited, CH-4002, Basle, Switzerland

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 proteogranules 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 was normal portions of a kidney removed radically for renal cell carcinoma.

Bioaffinity 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
and intracellularly secreted. Prorenin is constitutively located in tissues with low levels. Cleaved prorenin is seen clearly in the kidneys, as 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 NaDodSO4/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-filled 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 predominant 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 [35S]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 [3H]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 half-life 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
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. 2. Microradiosequencing of prorenin. Human kidney samples were labeled with [35S]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 [35S]methionine peak at cycle 16 predicts the amino-terminal residue to be leucine. This was confirmed in a separate experiment using [3H]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.

Fig. 4. Schematic representation of the biosynthesis and secretion of human renin.
These investigators based such demonstrations in the plasma terminal antibodies reported previously. Our data processed (truncated) differential major the intermediate forms of alterations in during in involves the prosegment may explain the dominance prorenin. denatured Taugner prorenin antibody to molecule kidney tissue incubation, we (data not labeling of the molecular mass tumor (19, 20), results renin during may be due to observed the initial obtained molecular mass of renin renin forms that a small 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.