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S A Atlas, … , M C Ruddy, M Aurell

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Research Article

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Characterization of Inactive Renin ("Prorenin") from Renin-secreting Tumors of Nonrenal Origin

Similarity to Inactive Renin from Kidney and Normal Plasma

Steven A. Atlas, Thomas E. Hesson, Jean E. Sealey, Bhanumas Dharmgrongartama, and John H. Laragh
Hypertension Center and Department of Medicine, Cornell University Medical College and The New York Hospital, New York 10021

Michael C. Ruddy
Department of Medicine, University of Medicine and Dentistry of New Jersey-Rutgers Medical School, New Brunswick, New Jersey 08903

Mattias Aurell
Department of Medicine, University of Göteborg, Göteborg, Sweden

Abstract. Inactive renin comprises well over half the total renin in normal human plasma. There is a direct relationship between active and inactive renin levels in normal and hypertensive populations, but the proportion of inactive renin varies inversely with the active renin level; as much as 98% of plasma renin is inactive in patients with low renin, whereas the proportion is consistently lower (usually 20–60%) in high-renin states. Two hypertensive patients with proven renin-secreting carcinomas of non-renal origin (pancreas and ovary) had high plasma active renin (119 and 138 ng/h per ml) and the highest inactive renin levels we have ever observed (5,200 and 14,300 ng/h per ml; normal range 3–50). The proportion of inactive renin (98–99%) far exceeded that found in other patients with high active renin levels. A third hypertensive patient with a probable renin-secreting ovarian carcinoma exhibited a similar pattern. Inactive renins isolated from plasma and tumors of these patients were biochemically similar to semipurified inactive renins from normal plasma or cadaver kidney. All were bound by Cibacron Blue-agarose, were not retained by pepstatin-Sepharose, and had greater apparent molecular weights (M_r) than the corresponding active forms. Plasma and tumor inactive renins from the three patients were similar in size (M_r 52,000–54,000), whereas normal plasma inactive renin had a slightly larger M_r than that from kidney (56,000 vs. 50,000). Inactive renin from each source was activated irreversibly by trypsin and reversibly by dialysis to pH 3.3 at 4°C; the reversal process followed the kinetics of a first-order reaction in each instance. The trypsin-activated inactive renins were all identical to semipurified active renal renin in terms of pH optimum (pH 5.5–6.0) and kinetics with homologous angiotensinogen (Michaelis constants, 0.8–1.3 μM) and inhibition by pepstatin or by serial dilutions of renin-specific antibody. These results indicate that a markedly elevated plasma inactive renin level distinguishes patients with ectopic renin production from other high-renin hypertensive states. The co-production of inactive and active renin by extrarenal neoplasms provides strong presumptive evidence that inactive renin is a biosynthetic precursor of active renin. The unusually high proportion of inactive renin in plasma and tumor extracts from such patients is consistent with ineffective precursor processing by neoplastic tissue, suggesting that if activation of "prorenin" is involved in the normal regulation of active renin levels it more likely

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Dr. Atlas is the recipient of Research Career Development Award HL-00570 from the National Institutes of Health. Address all correspondence to Dr. Atlas.

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occurs in the tissue of origin (e.g., kidney) than in the circulation.

Introduction

Renin (EC 3.4.99.19) is a proteolytic enzyme that is found most abundantly in the kidney. Renal renin is secreted into the circulation, where it catalyzes the rate-limiting step in the formation of the vasopressor and aldosterone-stimulating peptide angiotensin II. More than half of the renin present in normal human plasma exists as an enzymatically inactive form. Inactive renin can be activated in vitro by acidification to pH 3.3 or by treatment with certain proteolytic enzymes (1–8). This substance has several chromatographic properties that clearly distinguish it from the active enzyme (8–13) and it also has a slightly greater molecular weight by gel filtration (8, 12–14).

The precise biochemical relationship between inactive and active renins is undefined at present. Inactive renin has several characteristics of a biosynthetic precursor of renin (8, 12, 13). Other evidence has been cited to support the view that it may be a postsynthetic modification of active renin (15–17), especially since, in renal cortical extracts from several species, renin can form a large molecular weight complex with a polypeptide binding substance (18–20). However, these complexes are enzymatically active and thus differ from the completely inactive substance found in human plasma (8). Truly inactive renal renin has been isolated from human (13, 21) and hog (22, 23) kidney and is released by human kidneys perfused ex vivo (21); this substance has biochemical and immunochemical properties that are very similar to plasma inactive renin (24).

The present studies demonstrate that the characteristics of inactive renin isolated from plasma and tissue extracts of patients with renin-secreting neoplasms of nonrenal origin are similar to those of inactive renin from normal plasma and cadaver kidney. The demonstration that, after neoplastic transformation, active and inactive renins are produced together by cells that normally do not synthesize renin increases the likelihood that inactive renin is a precursor of the active enzyme.

Methods

Patients. Of the three patients studied, two had documented extrarenal renin-secreting tumors; their detailed case histories have been described elsewhere (25–27). Both presented with severe, treatment-resistant hypertension associated with hypokalemia, hyperreninemia, and secondary hyperaldosteronism. Patient A (25) was a 56-yr-old white female who also had intermittent abdominal pain, nausea, and malaise and was found at exploratory laparotomy to have a well-differentiated, mucin-producing adenocarcinoma of the pancreas with hepatic metastases; she died unexpectedly during the postoperative period. Patient B (26, 27) was a 32-yr-old white female with a pelvic mass. Extirpation of the tumor (a poorly differentiated carcinoma, probably of epithelial origin), which was adjacent to the left ovary, initially cured the hypertensive syndrome. Hypertension and hypokalemia subsequently recurred in association with pelvic and abdominal metastases and once again responded to removal of all visible tumor. After the development of extensive unresectable metastatic disease, recurrent hypertension and hypokalemia were cured with the angiotensin-converting enzyme inhibitor captopril until she died 18 mo later from carcinomatosis (27).

A third patient (patient C) is presented as a presumptive case of renin-secreting malignancy although tumor tissue has not been obtained for confirmation. She is a 47-yr-old white female who had a resectable adenocarcinoma of the ovary with evidence of local metastases that initially responded well to chemotherapy. 3 yr later she presented with extensive abdominal metastases, at which time she had new-onset hypertension (160–220/100–110) associated with profound hypokalemia (2.5 meq/liter), hypomagnesemia (0.8 meq/liter), and marked elevations in urine aldosterone excretion and plasma renin (see Results). Hypertension was refractory to spironolactone and to large doses of propranolol and hydralazine. Her initial course was complicated by intermittent signs of small bowel obstruction, generalized convulsions, and a brief period of encephalopathy; examination of cerebrospinal fluid and computerized tomography of the brain were negative. All symptoms responded dramatically to a course of chemotherapy, which also resulted in profound decreases in plasma renin and aldosterone and correction of the hypertension and electrolyte abnormalities (see Results).

Blood and tissue samples. Blood was collected into EDTA and centrifuged at room temperature to avoid inadvertent cryoactivation of inactive renin (28). Plasma was stored frozen at −40°C until ready for use. Plasma samples were also obtained from ambulatory normal volunteers and untreated patients with essential and secondary forms of hypertension and with other conditions as indicated in Results. Tumor tissue was obtained at surgery and frozen at −40°C as soon as possible. Human kidneys were obtained at autopsy.

Materials. Bovine pancreatic trypsin was obtained from Boehringer-Mannheim GmbH (Federal Republic of Germany) and soybean trypsin inhibitor (SBTI)[1] was from Sigma Chemical Co. (St. Louis, MO). Other serine protease inhibitors were benzamidine-HCl (Eastman Chemicals, Rochester, NY), diisopropylfluorophosphate (DFP) (Aldrich Chemical Co., Milwaukee, WI) and phenylmethylsulfonyl fluoride (PMSF) (Sigma Chemical Co.). The acid protease inhibitor pepstatin A was obtained from Peninsula Laboratories (San Carlos, CA). Bovine serum albumin (BSA) (Pentex Bovine Albumin, Fraction V) used for preparation of buffers was obtained from Miles Laboratories (Elkhart, IN). Molecular weight standards for gel filtration (BSA, M_r 67,000; ovalbumin, M_r 43,000; chymotrypsinogen A, M_r 25,000; and ribonuclease A, M_r 13,700) were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). In addition, the following radiolabeled ([*methyl*-^{14}C]-methylated) proteins, obtained from New England Nuclear (Boston, MA), were used as internal standards: [*methyl*-^{14}C]-methylated-gamma globulin, M_r 150,000 (26.9 μCi/mg); -BSA, M_r 69,000 (16 μCi/mg); -ovalbumin, M_r 46,000 (12 μCi/mg); and -cytochrome *C*, M_r 12,300 (25 μCi/mg). The chromatographic media used were as follows: Sephadex G-100, regular grade (Pharmacia Fine Chemicals); Cibacron Blue-F3G-A-agarose (Affi-Gel Blue, Bio-Rad Laboratories, Richmond, CA); and pepstatin-aminohexyl-Sepharose 4B, prepared as described by Murakami et al. (29).

Purified reagents. Active renal renin was partially purified from human kidneys (sp act 0.08 Goldblatt units [GU]/mg) by the method of Haas et al. (30). Angiotensinogen (4.5 μmol/mg) was prepared from human plasma (31). Inactive renin was partially purified from human plasma (0.8×10^{-3} GU/mg) and from human renal cortex (52×10^{-3}

1. *Abbreviations used in this paper:* Ang I, angiotensin I; DFP, diisopropylfluorophosphate; GU, Goldblatt units; PMSF, phenylmethylsulfonyl fluoride; PRA, plasma renin activity; SBTI, soybean trypsin inhibitor.

GU/mg) by affinity, ion-exchange, and hydrophobic interaction chromatography (24). Rabbit antiserum to pure human renin (32, 33) was the kind gift of Drs. Pierre Corvol and Joël Ménard of INSERM, U-36, Paris. This antiserum exhibits no cross-reactivity to either human cathepsin D or porcine pepsin (33).

Preparation of tissue extracts. Tumor tissue or dissected renal cortex was minced and homogenized in a Brinkman Polytron in 5 vol of 0.05 M Tris-Cl, pH 8.0, containing 0.1 M NaCl, 10 mM benzamidine-HCl, and 3 mM Na_2EDTA (Buffer I). DFP (5 mM) was added after the first of five 30-s passes. The homogenates were centrifuged at 100,000 *g* for 60 min (Beckman Spinco Ultracentrifuge, Beckman Instruments, Inc., Fullerton, CA) and the supernatants used for further study.

Chromatographic procedures. Plasma or tissue extracts were applied directly to a column of Cibacron Blue-agarose equilibrated with Buffer I; column dimensions and flow rate varied with the sample size (see Results). After washing with at least five bed volumes of starting buffer, the retained inactive renin was eluted with Buffer I containing 1 M NaCl (21). Residual active renin in the inactive renin-containing fractions was removed by passage of the pooled fractions over a 0.8- × 9-cm column of pepstatin-Sepharose, as previously described (13, 21). Analytical gel filtration of partially purified preparations was performed on a 2- × 96-cm column of Sephadex G-100 equilibrated with 0.05 M sodium phosphate, pH 7.4, containing 0.10 M NaCl and 3 mM Na_2 EDTA (13). In addition to the standard external calibration described previously (13), [*methyl*-^{14}C]-methylated proteins (0.05 μCi each) were added to the unknown sample before chromatography to serve as internal standards; radioactive peaks were determined by counting aliquots of each fraction in 10 ml Atomlight (New England Nuclear) in a Packard Tri-Carb scintillation spectrophotometer.

Determination of active and inactive renin. Renin activity was determined as the rate of angiotensin I formation from human angiotensinogen at pH 5.7 and 37°C, in the presence of 3 mM PMSF and 3 mM Na_2EDTA. Angiotensin I (Ang I) was quantitated by radioimmunoassay (34). Endogenous angiotensinogen was used as the source of substrate for determination of activity in whole plasma, which was adjusted to pH 5.7 by direct addition of 1/10 vol of 1.24 M maleic acid (34). Chromatographic fractions or purified preparations were diluted and assayed in Buffer I containing 0.5% BSA and 0.5 μM purified angiotensinogen, adjusted to pH 5.7 with 40 μl/ml maleic acid. Samples were diluted to a final renin concentration of $1–4 \times 10^{-4}$ GU/ml and incubated for 60 min at 37°C, under which conditions the activity of semipurified human renal renin was determined to be 90 μg Ang I/h per GU. Active renin was defined as the activity in untreated samples. Inactive renin was defined as the increment in activity produced by 1 h of preincubation with trypsin before adjustment to pH 5.7. Whole plasma (pH 7.8) was incubated at −4°C with 1.5 mg/ml trypsin in the presence of 5 mM benzamidine; this modification of our previous method (35) minimizes destruction of renin by excessive trypsin in samples with low trypsin inhibitor concentrations. Chromatographic fractions or purified preparations (pH 8.0) were incubated at 25°C with 100 μg/ml trypsin in the presence of 10 mM benzamidine and 0.5% BSA (13). The reaction was stopped by adding equimolar amounts of SBTI, as well as PMSF and EDTA as above, and adjustment to pH 5.7. The values for inactive renin obtained by this method ranged between 90 and 130% of the values obtained by acid activation. Recovery of semipurified active renin incubated with trypsin under these conditions was 84–93%.

Kinetic and pH optimum studies. Semipurified active renin or trypsin-activated inactive renin preparations were diluted to between 1 and 2×10^{-4} GU/ml in 0.05 M sodium maleate, pH 5.7, containing 0.1 M NaCl, 0.5% BSA, and 0.5% lysozyme (Buffer II). Samples were incubated in duplicate with eight concentrations of angiotensinogen that provided an even distribution of reciprocal values (range 0.13–2.6 μM). Data were examined by both Lineweaver-Burk and Eadie-Hofstee analyses and regression lines determined by the method of least squares. To determine the pH optimum, enzyme samples were diluted in sodium acetate, sodium phosphate, and Tris-Cl buffers (pH 4–8 range) as previously described (13).

Pepstatin inhibition. Pepstatin was dissolved and serially diluted in methanol. The IC_{50} was determined by preincubating diluted enzyme in Buffer II with pepstatin (10^{-8}–10^{-5} M) for 15 min at 25°C. The apparent inhibitory constant was determined by kinetic analysis as described above (eight concentrations of angiotensinogen in the presence or absence of pepstatin).

Antibody inhibition. Rabbit antiserum to pure human renin, serially diluted in Buffer II, was preincubated with diluted enzyme for 1 h at 37°C. Angiotensinogen (0.5 μM), PMSF, and EDTA were then added to determine renin activity. Preincubation of enzyme at 37°C without antiserum (for controls) did not affect activity.

Results

Active and inactive renin in plasma

Active plasma renin levels in the two patients (A and B) with documented renin-secreting malignancies and in the patient with a suspected renin-secreting ovarian carcinoma (patient C) were 20- to 30-fold higher than the upper limit of the range found in normal subjects on unrestricted sodium intake (Table I). They were also somewhat higher than is typically found in patients with renovascular or malignant hypertension, although occasional patients with the latter condition exhibit values in this range.

In contrast, inactive renin levels in each of the three patients (1,250–14,000 ng angiotensin I/h per ml) were one to two orders of magnitude higher than we have encountered in any other

Table I. Active and Inactive Renins in Plasma

	Active renin	Inactive renin	% Inactive
Patient A	119	5,200	98
Patient B	138	14,300	99
Patient C	190	1,250	87
Normal subjects (n = 105)	0.5–6	3–45	70–95
Malignant or renovascular hypertensives (n = 20)	8–150	8–50	20–70

Activity expressed as nanograms Ang I per hour per milliliter. The ranges given for normal and hypertensive subjects are based on the means±2 SD. To measure the extraordinary concentrations of inactive renin in patients A, B, and C without excessive use of endogenous substrate, activated plasmas were diluted 20- to 200-fold in Buffer II and substrate was restored to its initial level by addition of semipurified angiotensinogen (31). The renin levels in patient A and in normal subjects have been published previously (25).

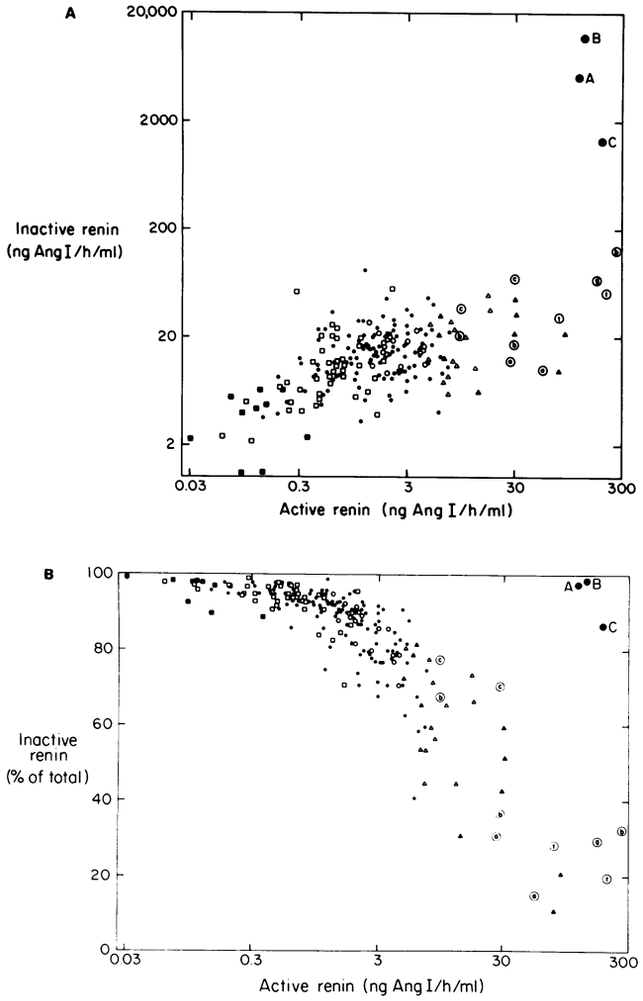


Figure 1. Relationship between plasma active renin levels and the absolute level (*A*) or proportion (*B*) of plasma inactive renin. The solid circles labeled *A*, *B*, and *C* represent the three patients with renin-secreting malignancies. Solid dots represent normal subjects; open symbols, essential hypertensive patients with low (□), normal (○), or high (△) renin-sodium profiles; solid squares, patients with aldosterone-producing adenomas; and solid triangles, patients with malignant or renovascular hypertension. Lettered symbols refer to patients with other conditions associated with hyperreninemia; *a*, adrenocortical insufficiency; *b*, Bartter's syndrome; *c*, hepatic cirrhosis; *g*, ganglioneuroma; and *t*, trophoblastic disease.

clinical condition (Table I, Fig. 1). Even with marked elevations of active renin, as in patients with malignant or renovascular hypertension or in patients with cirrhosis, adrenocortical insufficiency, Bartter's syndrome, and other nonhypertensive conditions, it is unusual for inactive renin to exceed 50 ng/h per ml (Fig. 1 *A*). In normal pregnant women (not shown) somewhat higher levels (50–200 ng/h per ml in our laboratory) are commonly observed (4, 36).

These patients also had an abnormal relationship between inactive and active renin in plasma. Among normal subjects and patients with hypertension and other conditions there is a weak direct relationship between active and inactive renin in plasma (Fig. 1 *A*) although the range of inactive renin is narrower than that of active renin. Inactive renin generally comprises between 60 and 97% of the total in normal subjects; the proportion is consistently higher in patients with suppressed plasma renin activity (PRA), and the proportion falls as active renin increases (Fig. 1 *B*). It is common for inactive renin to comprise only 20–30% of the total in patients with elevated PRA. In the three patients with tumors, inactive renin represented 87–99% of the total plasma renin, clearly outside the usual range (Fig. 1 *B*).

Effect of antineoplastic therapy on active and inactive renins

Tumor tissue from patient C was not available for analysis, so that confirmation of the diagnosis of a renin-secreting ovarian carcinoma was not possible. However, the clinical and biochemical responses to a course of chemotherapy are highly suggestive of such a diagnosis (Fig. 2). Hypertension was refractory to very large doses of propranolol and hydralazine as well as a brief course of spironolactone. Profound hypokalemia

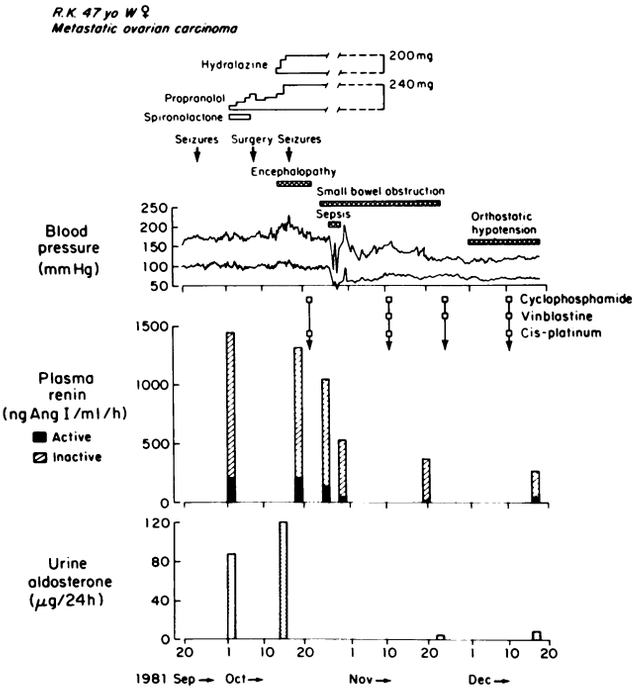


Figure 2. Clinical course of patient C, showing the effect of antineoplastic therapy on blood pressure, plasma active and inactive renins, and urine aldosterone excretion. Intravenous doses of cyclophosphamide (1,000 mg), vinblastine (9 mg) and *cis*-plastinum (100 mg) were administered on the days indicated.

(initially 2.5 meq/liter) recurred intermittently despite intensive oral and intravenous replacement and administration of spironolactone followed by triamterene (not shown). After initiation of a course of cyclophosphamide, vinblastine, and *cis*-platinum, PRA (i.e., active plasma renin) fell dramatically from the 190–210 range to 134, 36, and 17 ng Ang I/h per ml at 1, 2, and 4 wk, respectively. Inactive renin fell concurrently from the 1,000–1,250 range to 911, 482, and 345 ng/h per ml. Urine aldosterone excretion fell from 80–125 μg/d to 3–8 μg/d. Along with other symptoms, hypertension and hypokalemia gradually resolved despite the discontinuation of all antihypertensive medication.

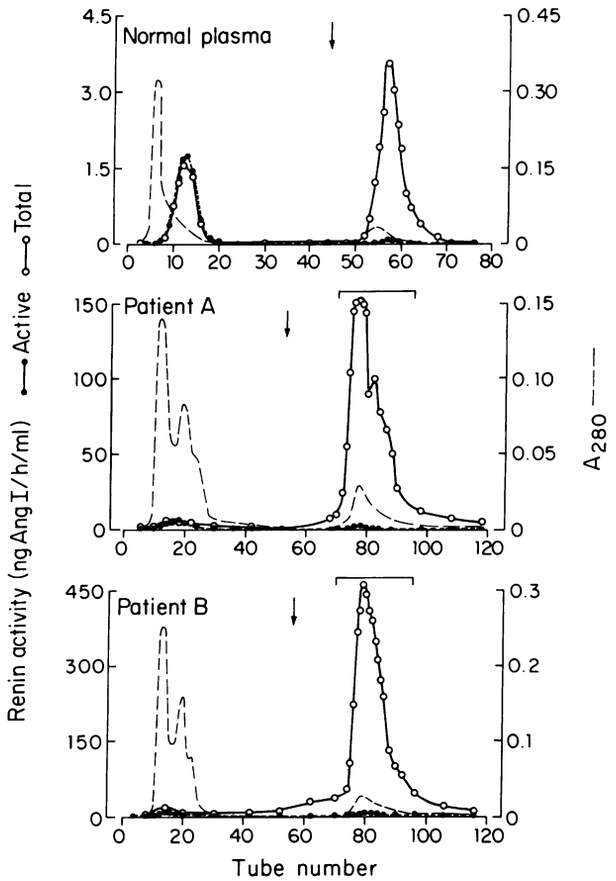


Figure 3. Elution of active and inactive renins during chromatography of plasma on Cibacron Blue-agarose. The vertical arrows indicate the point at which the elution buffer was changed to Buffer I containing 1.0 M NaCl (see Methods). Sample volumes were 10 ml for normal plasma, and 0.5 ml each for patients A and B. Chromatography was performed on a 2.5- × 20-cm column of gel (flow rate 1.0 ml/min; 10-min fractions) for normal plasma and on a 0.9- × 9-cm column (flow rate 0.125 ml/min, 5-min fractions) for samples from patients A and B. The horizontal brackets indicate the fractions that were pooled and pressure concentrated (Amicon PM-10 membrane) for further study.

Table II. Active and Inactive Renin in Tissue Extracts

	Active renin	Inactive renin	% Inactive
Tumor A	1.0–1.9	1.1–3.0	40–70
Tumor B	1.2–2.2	1.0–2.1	40–50
Normal kidney (n = 15)	0.1–0.4	0.01–0.1	5–30

Values expressed as GU per gram of tissue. The range of values found in three different portions of tumor tissue is given. The renin levels in tumor A and in normal kidney have been published previously (25).

By 8 wks, PRA had risen slightly (to 40 ng/h per ml), at which time the patient had developed orthostatic hypotension (possibly related to prolonged bed rest); however, inactive renin continued to decline (to 212 ng/h per ml).

Chromatographic isolation of plasma inactive renin

Inactive renin from the plasma of patients with renin-secreting tumors was isolated by chromatography on Cibacron Blue F3G-A-agarose (Fig. 3). Active renin from patients with and without tumors was not appreciably retained by the gel, whereas inactive renin was bound and could be eluted with buffer containing 1 M NaCl. These results suggest a similarity to normal plasma inactive renin and validate the estimate of extraordinarily high absolute and relative concentrations of inactive renin in plasma from patients with renin-secreting malignancies. Similar results were obtained with plasma from patient C (not shown).

Active and inactive renin in tumor extracts

The active renin concentration of tumor extracts from patients A and B was 5- to 20-fold higher than that of human kidney (Table II). Trypsin-activable inactive renin comprised 40–70% of the total renin in the tumor extracts, a greater proportion than we have found in normal human kidney extracted under the same conditions (Table II).

Inactive renin was isolated from tumor extracts by chromatography on Cibacron Blue-agarose (not shown). The elution pattern was identical to that shown for plasma (see Fig. 3) and previously reported for cadaver kidney (13, 21). The pooled inactive renin-containing fractions had small amounts of residual renin activity, which was removed by passage over pepstatin-aminohexyl-Sepharose. These preparations were used for further analysis.

Molecular size of tumor, plasma, and kidney renins

The apparent molecular weights of the isolated active and inactive forms of renin were determined by analytical gel filtration. The results obtained with samples from patient A are shown in Fig. 4. In both plasma and tumor, the M_r of inactive renin was slightly greater than the corresponding active form. Plasma and tumor inactive renins appeared to be of similar size in each patient, in contrast to our previous observation that renins from

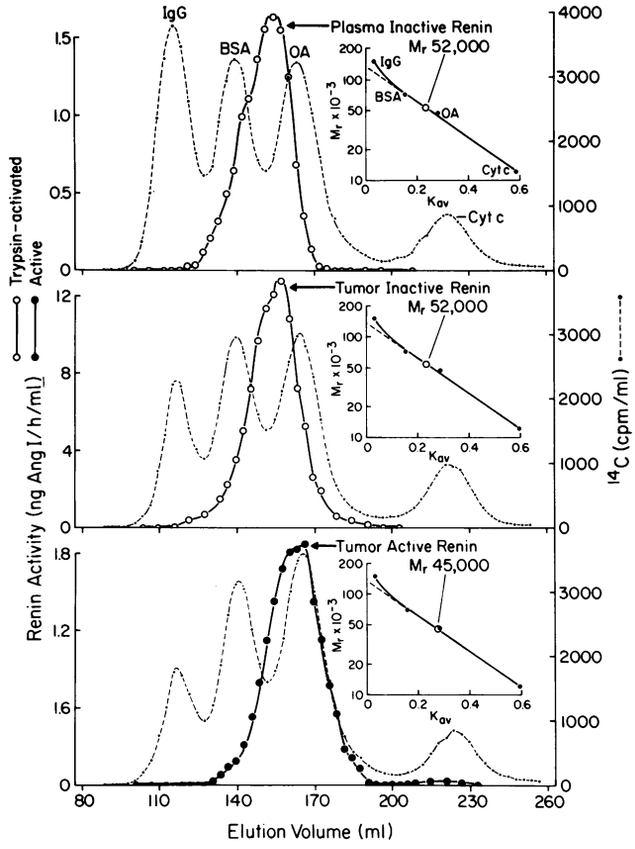


Figure 4. Gel filtration of plasma and tumor inactive renins and tumor active renin from patient A. Samples were aliquots of the pooled fractions from Cibacron Blue-agarose (see Fig. 3). The insets in each panel show the standard curve obtained from the elution volumes of radiolabeled internal standards in each experiment (IgG, gamma globulin; BSA, bovine serum albumin; OA, ovalbumin, cyt C, cytochrome *C*). Best straight lines were determined by linear regression analysis, omitting the values for IgG, since it falls outside the linear range for Sephadex G-100.

normal plasma have greater apparent molecular weights than the corresponding forms in kidney (Table III).

Reversible and irreversible activation of inactive renin

Like inactive renins from normal plasma and kidney (17, 24, 37), the tumor inactive renins were activated irreversibly by incubation with trypsin, whereas the activation produced by dialysis at 4°C to pH 3.3, while complete, was reversed in a time-dependent fashion during subsequent incubation at higher pH and temperature (Fig. 5 *A*). After complete reversal of acid activation, full activity could be restored by trypsin treatment or reacidification. Semilogarithmic transformation of the data (Fig. 5 *B*) indicate that the reversal of activation proceeded as a first-order decay process; moreover, the half-time of the reversal process was independent of the initial concentration of inactive renin (Fig. 5 *B*), strongly suggesting that reversal is a unimolecular process.

Other characteristics of activated tumor inactive renins

Reaction with homologous angiotensinogen. The rate of Ang I formation from human substrate by trypsin-activated inactive renins from tumors A and B occurred optimally between pH 5.5 and 6.0; the profiles of activity vs. pH (Fig. 6) were identical to those reported previously for active and inactive renins from normal plasma and kidney (13, 35). The kinetics of the reaction with human angiotensinogen were also indistinguishable from that of active kidney renin or activated inactive renin from normal plasma or kidney (Fig. 7), with Michaelis constants ranging from 0.8 to 1.2 μM.

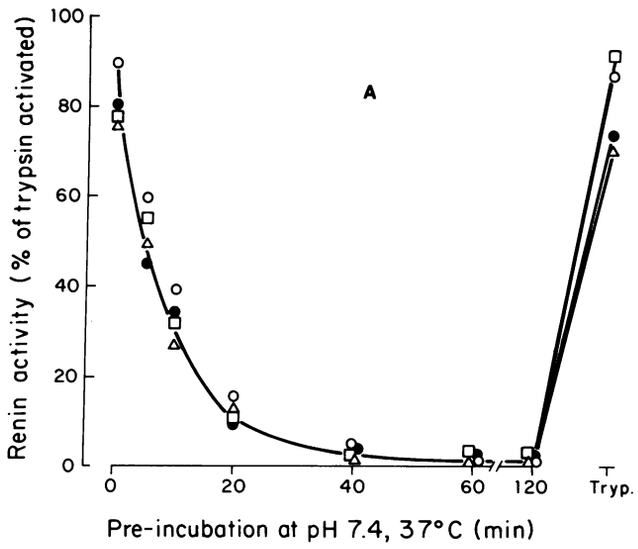
Inhibition by pepstatin. The peptidic acid protease inhibitor pepstatin produced similar inhibition of activated inactive renins from the tumors, kidney, and normal plasma and of active kidney renin (Fig. 8). The IC_{50} ranged between 2 and 4 μM. As reported previously for activated inactive renins from normal plasma and kidney (24), kinetic analysis suggested a predominantly noncompetitive type of inhibition at low pepstatin concentrations (1–3 μM), but a mixed pattern of inhibition was observed at higher concentrations.

Inhibition by antibody to active renin. The activated forms of inactive renin from both tumors, as well as from kidney and normal plasma, were all inhibited by monospecific antibodies to human active renin (Fig. 9). Antibody titration produced virtually identical inhibition of each renin preparation compared with partially purified active renin from human kidney. Identical results were obtained with active renin from the tumors (not shown).

Table III. Apparent Molecular Weights of Active and Inactive Renins

	Internal standards	External calibration*	Previously reported*
Inactive Renin			
Plasma A	52,000	50,000	—
Plasma B	54,000	53,000	—
Plasma C	53,000	52,000	—
Normal plasma	56,500	55,000	56,000±1500
Tumor A	52,000	50,000	—
Tumor B	53,000	51,500	—
Normal kidney	50,500	49,000	49,500±1000
Active Renin			
Tumor A	46,000	44,000	—
Tumor B	45,500	43,500	—
Normal kidney	40,500	39,500	39,500±500
Normal plasma	—	—	48,000±2000

* Values calculated from the external calibration curve are provided for comparison with previously reported values (13).



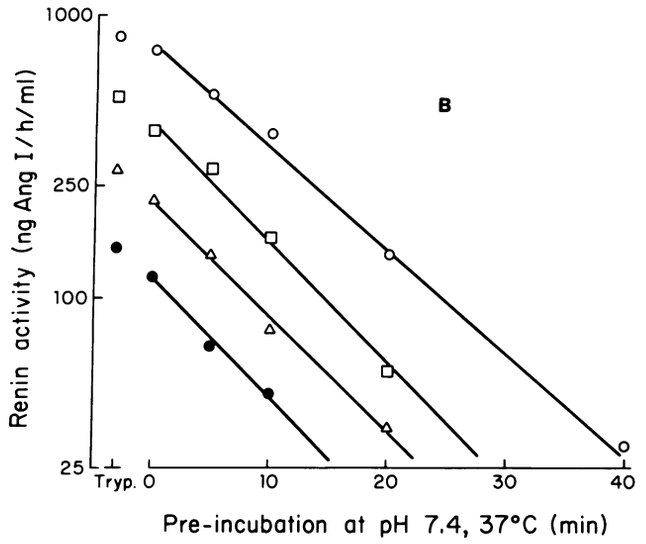


Figure 5. Reversal of acid activation of tumor inactive renins. Samples were dialyzed overnight at 4°C against 0.05 M glycine-HCl, pH 3.3, containing 0.10 M NaCl and 3 mM EDTA. Aliquots (10–40 μl) were then diluted on ice to 0.3 ml final volume with 0.10 M sodium phosphate, pH 7.4, containing NaCl, EDTA, and 0.5% BSA, and incubated for the times indicated at 37°C before adjustment to pH 5.7 and addition of angiotensinogen; renin activity was then determined during a 6-min incubation. Closed circle, inactive renin from tumor A; open symbols, three concentrations of inactive renin from tumor B: △, 10 μl; □, 20 μl; and ○, 40 μl per 300 μl reaction volume. (*A*) Activity expressed as a percentage of that obtained from an identical aliquot that was incubated with trypsin. As shown at the right, an additional aliquot was treated with trypsin after a 120-min preincubation, demonstrating that full activity could be recovered. (*B*) Semilogarithmic transformation of the data, showing the absolute levels of renin activity. Best straight lines were determined using the method of least squares.

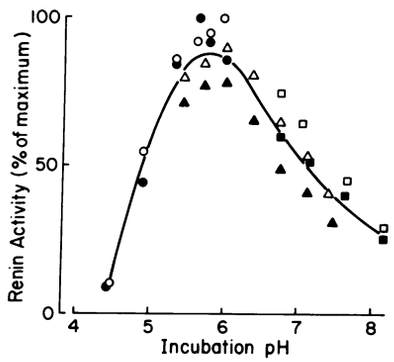


Figure 6. Effect of incubation pH on the activity of trypsin-activated inactive renins from tumor A (open symbols) and tumor B (closed symbols), using sodium acetate (circles), sodium phosphate (triangles), and Tris-Cl (squares) buffers.

Discussion

These studies have established that the inactive form of renin present in plasma and tumor extracts of patients with extrarenal renin-secreting malignancies bears striking biochemical and immunochemical similarity to inactive renin isolated from normal plasma and cadaver kidney. Inactive renin from each source

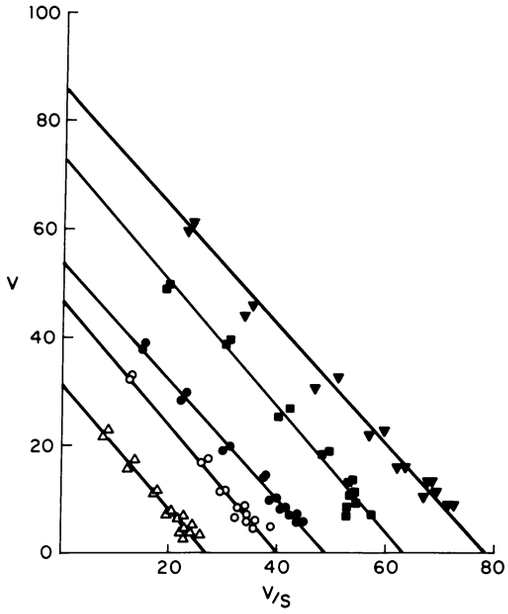


Figure 7. Eadie-Hofstee plots of the reaction between human angiotensinogen and either active renin or activated inactive renins. Solid circles refer to semipurified active renal renin. Other symbols refer to trypsin-activated inactive renins from normal plasma (△), kidney (○), tumor A (■), or tumor B (▼). V, velocity (nanograms per hour per milliliter); S, substrate concentration (micromolar).

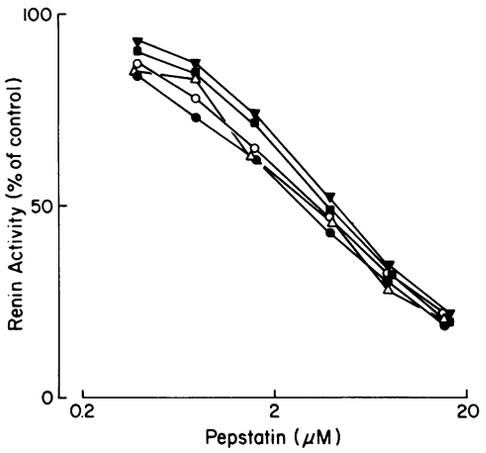


Figure 8. Inhibition of semipurified active renal renin or trypsin-activated inactive renins by pepstatin. Symbols are the same as for Fig. 7.

binds to affinity gels containing the Cibacron Blue dye as ligand, is not retained by affinity gels bearing the renin inhibitor pepstatin, and has a greater M_r than its corresponding active form. Each is activated reversibly at acid pH and is activated irreversibly by trypsin. Moreover, the product of in vitro activation closely resembles the naturally occurring active form of kidney renin in terms of pH optimum, reaction kinetics, and inhibition by pepstatin or by monospecific anti-renin antibodies.

Plasma levels of inactive renin in two patients (A and B) with proven ectopic renin production were orders of magnitude greater than we have observed in any other clinical state (Fig. 1). It was the finding of similarly elevated inactive renin levels in a third patient (C) that led us to suspect the possibility of a renin-secreting ovarian carcinoma. Although tumor tissue was not available for confirmation, the dramatic responses of active and inactive renin to a course of chemotherapy (Fig. 2) supports this diagnosis. Other explanations, for instance relief of tumor-mediated renal artery compression, might be invoked to explain this response. However, the evidence to date suggests that plasma inactive renin rarely exceeds the normal range in states of hypersecretion of renin by the ischemic kidney (Fig. 1). Given the evidence from patients A and B, it is likely that marked elevation of plasma inactive renin may be an indication of ectopic renin production. This finding may not be limited to extrarenal neoplasms. One of the earliest descriptions of inactive renin in human plasma was the report by Day and Luetscher (2) of "big renin" in the plasma of a patient with Wilms' tumor. In addition, Mimran et al. (38) have reported that acidification of extracts of a juxtaglomerular cell tumor caused considerable increase in renin activity. While plasma inactive renin levels were not reported, these findings suggest that increased levels may be expected in at least some cases of renal tumors that secrete renin.

The demonstration that extrarenal renin-secreting tumors produce inactive renin may support the view that this substance is a biosynthetic precursor of renin. In addition to the patients described herein, Soubrier and co-workers (39) have recently reported the presence of inactive renin in tumor extracts from the patient with a renin-secreting pulmonary carcinoma described originally by Genest et al. (40). Thus, in each of the three established cases of renin-secreting neoplasms of nonrenal origin, inactive renin was produced together with the active form. Since, after neoplastic transformation, the expression of genes not normally expressed by the parent cells is likely to be a random process, the simultaneous production of active and inactive renin by neoplasms derived from tissues that normally do not produce renin can be taken as evidence of tight genetic linkage. The extreme case of tight linkage, i.e., the possibility that inactive renin is the product of a single gene that also codes for the active form, would be consistent with a precursor-product relationship.

These observations do not, of course, exclude the possibility that inactive renin is, instead, an enzyme/inhibitor complex. The present data would suggest, however, that if this were the case the inhibitor must either be a ubiquitous tissue component or be encoded for by a gene closely associated with the renin gene. At present, there is no evidence to support either of these possibilities. The single piece of evidence that could suggest that inactive renin represents such a complex is the reversible nature of acid-activation. Thus, it has been proposed (17) that inactive renin may be analogous to the large molecular weight complexes of renin and a binding protein isolated from renal cortex of several species. But this analogy is not convincing for at least two reasons. First, the complexes described in most studies are enzymatically active, and dissociation of the binding protein leads at best to only modest increases in enzymatic activity (20).

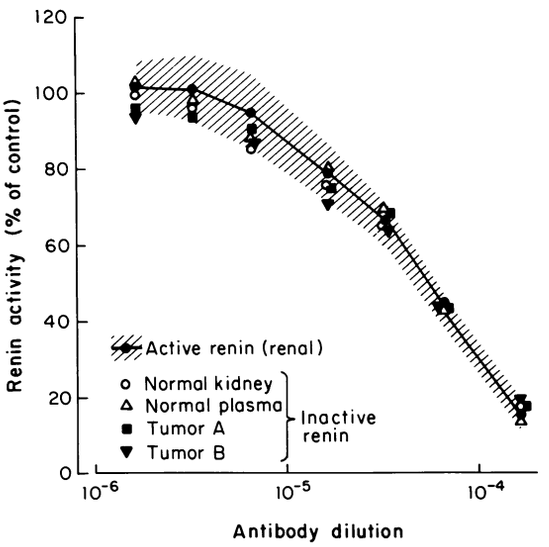


Figure 9. Inhibition of active or trypsin-activated inactive renins by monospecific antirenin antibodies. The symbols are the same as used in Figs. 7 and 8. The line and shaded area indicate the mean±SD of five determinations for active renal renin.

More significantly, the renal cortical binding substance appears to be acid-labile, so that the reduction in molecular size that results from acidification is not reversible (20). The studies described in the present report demonstrate that the process of reversal of acid-activation of inactive renin obeys first-order kinetics, with a reaction half-time that is independent of the initial concentration (Fig. 5 *B*). This speaks strongly against a bimolecular reaction (e.g., the reassociation of renin and an inhibitor). In addition, Hsueh et al. (37) have shown that acidification of crude plasma actually increases the M_r (i.e., Stokes' radius) of inactive renin as determined by gel filtration. These observations are therefore more consistent with the hypothesis that acidification of inactive renin induces a reversible conformational change, thereby exposing the catalytic site. In certain enzyme precursors, such as trypsinogen (41), electrostatic interaction between the cleavage peptide and the remainder of the molecule are important in preventing access of substrate to the active center. Therefore, it is conceivable that pH alteration could reversibly expose the catalytic site of an enzyme precursor. On the other hand, irreversible activation would be expected to involve a proteolytic process, as appears to be the case for inactive renin.

The present results indicate that inactive renin consistently has a greater apparent molecular weight than the active form derived from the same tissue source (see Table III). While proteolytic activation of inactive renin in vitro produces an enzyme that resembles active renin in all other respects, previous studies have shown that this is not accompanied by an appropriate reduction in M_r (8, 12–14). Aside from the likelihood that activation in vitro might not mimic the in vivo processing of a presumed precursor, it is presently uncertain what the expected M_r of the activation product ought to be, since larger forms of circulating active renin have been described (8, 12, 13, 42). In this regard, it is of interest that the active forms of renin isolated from tumor tissue appear to be slightly larger than that of normal kidney (Table III).

Added to the complexity of these molecular weight interrelationships is our previous finding that, like the active form, inactive renin derived from normal plasma has a greater M_r than inactive renin extracted from kidney (13). This finding has been confirmed recently by Chang et al. (43), who have suggested that the lower M_r of kidney inactive renin could reflect partial proteolytic degradation during extraction from protease-rich tissues such as kidney. While this is conceivable, it should be noted that the substance can be isolated in a completely inactive form (13), whereas the small molecular weight reduction produced by trypsin in vitro leads to irreversible activation. In our experience, neither the amount nor the apparent M_r of inactive renin isolated from kidney is significantly altered by additional protease inhibitors such as aprotinin, sodium tetrathionate, *N*-ethylmaleimide, 8-hydroxyquinoline, or pepstatin A (unpublished data). Another possible explanation for this molecular weight discrepancy (13, 24) is that inactive or active renin secreted into the circulation may differ from that found in tissue extracts in terms of molecular configuration or carbohydrate composition, factors that are known to influence M_r estimation by gel filtration. It is noteworthy that, in contrast to normal subjects, plasma inactive renin from patients with extrarenal malignancies was not appreciably larger than that present in their tumor extracts (Fig. 4, Table III). There is evidence that glycosylation of certain polypeptide hormones may be directly linked to the normal secretory process (44). Therefore, it is possible that the slightly lower M_r of plasma inactive renin in patients with tumors reflects an abnormality of the secretory process in neoplastic tissue.

Another finding that is consistent with ineffective precursor processing in extrarenal tumors is the abnormal elevation in the proportion of circulating inactive renin in patients bearing such tumors. The analysis in Fig. 1 *B* suggests that as active renin secretion by the normal or ischemic kidney increases, the proportion of circulating inactive renin falls. Assuming that inactive renin is a precursor, this implies that the conversion (or activation) process may play an important role in determining the active renin secretory rate. As implied above, it is likely that the machinery for intracellular proteolytic conversion, as well as for packaging and secretion, might be aberrant in neoplastic tissues. Therefore, the extraordinary increase in the proportion of circulating inactive renin in patients with ectopic production is consistent with the idea that, in normal subjects, inactive renin is activated intracellularly. This observation does not rule out the possibilitty that activation might also occur to some extent in the extracellular space of tissue of origin (i.e., kidney), but it implies that such a process is in some manner linked to the normal secretory process.

There has been considerable interest in the possible role of kallikreins in regulating the conversion of inactive to active renin, since both glandular (20, 45, 46) and plasma (47, 48) kallikreins are able to activate inactive renin in vitro. Although there is no experimental evidence that kallikrein-mediated activation occurs in vivo, several indirect observations are consistent with this possibility (see reference 8 for review). Most investigators feel, however, that the abundant protease inhibitors in blood would prevent substantial activation of circulating inactive renin by plasma kallikrein under normal circumstances (8). The present results support that view, since circulating plasma kallikrein ought to be able to act in patients with ectopic production of inactive renin as well. On the other hand, activation by tissue (glandular) kallikrein at or near the site of inactive renin secretion is conceivable. Although recent immunohistochemical studies suggest that renal kallikrein is not present within the juxtaglomerular complex itself (49), it is not impossible that kallikrein (or other proteases) could act on this substance in the extracellular space of the kidney.

In summary, these studies have demonstrated that extrarenal renin-secreting malignancies also secrete unusually large amounts of inactive renin. High plasma levels of inactive renin are therefore likely to be a biochemical marker for renin production by neoplastic tissue, providing a useful test in the differential diagnosis of high-renin hypertensive states. These findings also support the contention that inactive renin might be a

biosynthetic precursor. They suggest, in addition, that if conversion of inactive to active renin plays a role in the normal physiological control of circulating active renin levels, this process is more likely to occur predominantly in the kidney. Thus, inactive renin may be, in the main, the secreted form of an intracellular precursor rather than a circulating zymogen in the classic sense. If this is so, it raises the important teleological question of why such a large proportion of a renin precursor appears to be released normally by the kidney. Numerous hypothetical possibilities could be imagined for a role of a circulating "prorenin" that do not depend on its conversion to active renin in the blood, and hopefully additional studies will shed light on this intriguing biological question.

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