Characterization of Inactive Renin from Human Kidney and Plasma

EVIDENCE OF A RENAL SOURCE OF CIRCULATING INACTIVE RENIN

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ABSTRACT An inactive form of renin has been isolated from human plasma. It has been suggested that this may represent renin precursor secreted from the kidney. However, early studies failed to isolate inactive renin from human renal tissue. In this investigation, rapid processing of human kidney cortex at temperatures below 4°C in the presence of protease inhibitors followed by cibacron-blue affinity chromatography allowed us to extract a totally inactive form of renal renin. Furthermore, we found that in kidney inactive renin constituted from 10 to as much as 50% of the total renin concentration. Biochemical characterization of the inactive renin from plasma and from kidney indicates that they are structural homologues and, when activated, have enzymatic properties that resemble active renal renin. Renal and plasma inactive renin were found to have the following properties in common: (*a*) a pH optimum of activation of 3.3; (*b*) reversible activation by acid dialysis on return to pH 7.4, 37°C; (*c*) pH optima of enzyme activity of 7.8 with sheep angiotensinogen and 5.5 and 6.7 (biphasic) with human angiotensinogen; (*d*) Michaelis-Menten constants, K_m, of 0.29–0.34 μM with sheep angiotensinogen, and 0.99–1.25 μM with human angiotensinogen; (*e*) an antibody to human renal renin mean inhibitory titer of 1:30,000 with 1×10^{-4} Goldblatt units of activated renal or plasma inactive renin; (*f*) gel filtration profiles consisting of two peaks with apparent molecular weights of 56,000±1,500 and 49,200±1,000. Activation of plasma and kidney inactive renin by acid plus renal kallikrein was not accompanied by a change in gel filtration elution patterns. To determine whether inactive renin is released by the kidney, we measured inactive renin in samples obtained simultaneously from both the renal veins and inferior vena cava below the origin of the renal veins. In eight consecutive patients, inactive renin concentration was significantly higher in renal venous blood than in inferior vena caval blood. These data indicate that human kidney contains and secretes significant quantities of inactive renin. Thus, the kidney appears to be a major source of inactive renin in human plasma.

INTRODUCTION

Pulse labeling studies in isolated canine glomeruli (1) and studies in cell-free translation systems using mouse submaxillary gland and kidney (2–4) have demonstrated that renin, like most enzymes and polypeptide hormones, is synthesized as a proform, i.e., prorenin. Precursor renin is larger in molecular size than active renin, but little more is known about its biochemistry. In general, proenzymes have no biologic activity and are converted to active enzymes by limited proteolysis (5). Early investigations have been unable to document the presence of a renin zymogen in human kidney tissue (6). However, in man an inactive form of renin constitutes more than half of the total renin concentration in plasma (6). This inactive renin has a larger apparent molecular weight than active renin and can be activated to the same degree by either acid or pro-

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teolytic treatment of plasma (7). It has been postulated that circulating inactive renin may be renin precursor. However, definitive proof is lacking, and the origin of inactive renin is unknown. Furthermore, characterization of inactive renin has been difficult because of the small quantities in plasma.

Successful identification of an inactive form of renin in the kidney and demonstration of a venous-arterial gradient would provide strong support that plasma inactive renin is, at least in part, of renal origin. In addition, the finding of high levels of inactive renin in the kidney would provide a source for its purification, which is necessary for further studies to critically examine the possible precursor role of inactive renin. Recent understanding of the properties and stability of inactive renin has lead to improved conditions of extraction of inactive renin from tissue sources. As a result, Atlas et al. (8), Chang et al. (9), and our laboratory (10) have reported the isolation of a completely inactive form of renin from human renal cortex. The purpose of this study was (*a*) to provide a detailed analysis of the quantitative relationship between active and inactive renin within the kidney and plasma; (*b*) to compare activation characteristics and biochemical properties of renal and plasma inactive renin; and (*c*) to determine whether renal inactive renin is released into the plasma.

Based on six criteria, we found that renal and plasma inactive renin shared remarkable similarities. The use of different activation techniques may have resolved some of the molecular weight discrepancies previously reported between plasma and renal inactive renin (8, 9). Several methods used in this study also emphasize the heterogeneity of human inactive renin. In addition, use of converting enzyme inhibition allowed the first demonstration of a highly significant gradient of inactive renin across the kidney.

METHODS

Extraction of inactive renin

Blood. Plasma was obtained (11) from five volunteers on unrestricted sodium intake. Inactive renin was extracted from plasma by affinity chromatography on cibacron-blue agarose (Bio-Rad Laboratories, Richmond, CA) and eluted with a stepwise increase in NaCl concentration (12, 13).

Kidney tissue. Fresh human kidney tissue was obtained from three patients undergoing radical nephrectomy for a renal tumor. Cadaver kidney was obtained at autopsy performed within 24-h postmortem. The tissue was washed in saline and immediately frozen at −40°C in a nonfrost-free freezer until use. Upon thawing, the tissue was chopped, homogenized, and sonified in 0.025 M phosphate buffer, pH 7.1, containing 4 mM EDTA, 2 mM phenymethylsulfonyl fluoride, 5 mM 8-OH quinolol sulfate, 1.6 mM dimercaprol, and 20 kallikrein inhibitor units of aprotinin/ml. In these concentrations, this combination of inhibitors did not interfere with the renin assay. The homogenate was centrifuged at 3,000 g for 15 min. The supernate was desalted on Sephadex G-15 (Pharmacia Fine Chemicals, Piscataway, NJ) and 6 ml were applied to cibacron-blue agarose affinity column (30-ml bed volume). All tissue processing occurred at 4°C.

Renin measurements and activation

Active renin. The concentration of active renin was measured in untreated samples by radioimmunoassay of angiotensin I (AI)[1] after incubation at 37°C pH 7.5 with nephrectomized sheep plasma (14) containing angiotensinogen (final concentration 1 mM) and angiotensinase inhibitors (15). Mixtures of sample and substrate (20–100 μl) were incubated for four or more time periods between 0 and 2 h (generating 0.1–1.0 ng AI/aliquot) to ensure linearity of AI concentration as a function of time. In this assay, generation of 1.2 × 10^5 ng AI/ml per h was equal to 1 Goldblatt unit (GU) of renin as determined against Medical Research Council (MRC) renal renin (68-356).

Total renin concentration. This is the measurement of renin following 18 h of dialysis of the sample against pH 3.3, 0.05 M glycine buffer at 4°C and titration to pH 7.1 with 1.0 M phosphate buffer, pH 8.0. Following this procedure, plasma renin is totally activated, but activation is reversible (15). Therefore, samples were incubated with sheep angiotensinogen for five time periods between 0 and 0.5 h at pH 7.5, and the slope of AI concentration vs. time was determined by linear regression analysis (an r value ≥ 0.96 was acceptable). In undiluted plasma we have previously shown that measurements of total renin either by (*a*) acid dialysis and neutralization by titration; (*b*) acid dialysis followed by neutral dialysis; (*c*) acid dialysis and pepsin treatment; (*d*) acid dialysis neutralization by titration and renal kallikrein treatment; or (*e*) trypsin treatment yield the same values (7, 15).

The effect of renal kallikrein on human inactive renin was evaluated by incubating samples with partially purified human renal kallikrein (2.3 IU/ml sample) at 25°C for 1 h, pH 7.5 (16).

Preparation of renin-free human angiotensinogen

Human angiotensinogen from plasma of a subject ingesting oral contraceptives was prepared by ammonium sulfate precipitation (17). Plasma containing 5 mM EDTA was adjusted to 1.5 M $(NH_4)_2SO_4$ by addition of solid ammonium sulfate, stirred for 18 h and centrifuged at 5,000 *g* for 30 min. The precipitant was washed twice with 1.5 M $(NH_4)_2SO_4$. The supernates were combined, adjusted to 2.3 M $(NH_4)_2SO_4$ and stirred for 4 h. Following centrifugation, the precipitate was dissolved in distilled water and exhaustively dialyzed against distilled water. All processing occurred at 4°C. The final preparation had a substrate concentration of 5,400 ng AI/ml and did not possess renin activity.

Angiotensinogen concentration was determined by the method of Skinner et al. (18).

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[1] *Abbreviations used in this paper*: AI, angiotensin I; GU, Goldblatt unit; HC, the kidney with higher concentration of inactive renin; IVC, inferior vena cava; K_{av}, partition coefficient; MRC, Medical Research Council.

Characterization of human inactive renin

Cibacron-blue preparations of plasma inactive renin and renal inactive renin were used for characterization studies. The plasma preparation was concentrated by dialysis against dextran (T-70, Pharmacia Fine Chemicals) resulting in a total renin concentration of 40 ng/ml per h and an active renin concentration < 2 ng/ml per h. The kidney preparation had a total renin concentration of 60 ng/ml per h and an active renin concentration < 2 ng/ml per h.

Effect of acid. To test the optimum pH of activation, samples containing renal or plasma inactive renin were dialyzed for 24 h at 4°C against 0.05 M glycine that had been adjusted to pH values ranging from 2.8 to 7.5. To test the reversibility of acid activation of renal active renin, samples that had been dialyzed to pH 3.3 were neutralized by titration with 1.0 M phosphate buffer, pH 8.0 and incubated for 2 h at 37°C, the optimum conditions for reversal of acid activation of plasma inactive renin (15).

Enzyme activity. To obtain a stable activated form of renin, cibacron-blue preparations of renal and plasma inactive renin were dialyzed to pH 3.3 for 18 h at 4°C, titrated to pH 7.1 with 1.0 M phosphate buffer pH 8.0, and incubated for 2 h at 25°C with 2.3 IU/ml renal kallikrein.

The optimum pH of enzyme activity with both human and sheep angiotensinogen were compared for renal and plasma activated renin and active renal renin (MRC). The angiotensinogen preparation was dialyzed against 0.2 M citrate, from pH 4.4 to 5.5, 0.2 M phosphate from pH 5.5 to 7.5, and 0.2 M Tris from pH 7.5 to 9.0. The pH of the activated renin preparations was adjusted by addition of buffer. In the incubation mixture of AI generation, the final concentration of sheep angiotensinogen was 1.4 μM and of human angiotensinogen, 10.0 μM. Samples were incubated for at least four time periods at each pH to ensure linearity of AI generation with time.

The Michaelis-Menten constant, K_m, was determined by incubating MRC renin, activated inactive plasma renin (2.2×10^{-4} GU) or activated inactive renal renin (3.7×10^{-4} GU) with increasing concentrations of sheep angiotensinogen (0.06–0.63 μM) at pH 7.5, or human angiotensinogen (0.19–2.00 μM) at pH 7.0. The protocol was designed to yield <3% of substrate hydrolysis at all substrate concentrations. In addition, three time periods of AI generation were used to ensure constant velocity during the reaction. Lineweaver-Burke analysis was applied to the data, and each K_m was determined in triplicate using the statistical method of Wilkinson (19).

Gel filtration. Renal or plasma inactive renin isolated by cibacron-blue affinity chromatography was placed on a 2.5 × 85-cm column of Sephadex G-100 (Pharmacia Fine Chemicals) at pH 7.5 with a flow rate of 12 ml/h at 4°C. Elution was expressed as the partition coefficient, K_{av} (20). Standards used for column calibration included bovine serum albumin, ovalbumin, α-chymotrypsinogen (Sigma Chemical Co., St. Louis, MO) with molecular weights of 67,000, 44,000, and 23,200, respectively. Apparent molecular weight was estimated from a semilogarithmic plot of K_{av} vs. molecular weight.

Inhibition by anti-human renal renin antibody. Renin specific antibody (R1723) was obtained in a rabbit immunized with human renal renin, purified to homogeneity by a modification of our previously described method (21). The modifications included the addition of an α-casein-Sepharose column at step 5 and DEAE cellulose chromatography to the final step. The final product demonstrated a single band on polyacrylamide gel electrophoresis and sodium dodecyl sulfate gel electrophoresis. Antiserum R1723 has a 50% inhibitory titer of 1:30,000 against 1×10^{-4} GU of MRC human renal renin. The antiserum is specific for renin as demonstrated by immunodiffusion and immunoelectrophoresis analysis. Furthermore, it had no effect on the enzymatic activities of a series of nonrenin proteases such as renal cathepsins D and B, trypsin, renal kallikrein, or pepsin.

Inhibitory activity of this antibody against active renin or activated (acid plus renal kallikrein) inactive renin was determined by the addition of 100 μl of antiserum or preimmune serum (control) to the above standardized activity of active renin or activated inactive renin. After incubation for 1 h at 37°C, pH 7.4, sheep angiotensinogen was added, and the residual renin activity of the mixture was assayed as described above.

Inactive renin in renal vein and inferior vena caval blood

Inactive renin levels were determined in inferior vena cava (IVC) samples obtained simultaneously from both above and below the origin of the renal veins and from the renal veins in eight patients undergoing venous catheterization. To enhance inactive renin secretion, four of the patients were given Captopril, 50 mg per os t.i.d., for at least 3 d before the study. Inactive renin was measured in duplicate following dialysis of the sample against pH 3.3 and the pH 7.5 buffer (15). Intraassay coefficient of variation for the inactive renin measurement was 15%. Statistics were calculated using a paired t analysis.

RESULTS

Inactive renin in human renal cortex

In homogenates from three surgical specimens of renal cortex, inactive renin as demonstrated by acid activation comprised from 10 to 46% of the total renin concentration. In three cadaver kidneys as much as 53% of the total renin was inactive (Table I).

A representative cibacron-blue elution pattern (specimen 1B in Table 1) of active and inactive renin in the homogenates of fresh kidney is shown in Fig. 1A. Most of the active renin did not bind to cibacron-blue, and negligible amounts of inactive renin were detected in the unbound fractions. In all specimens, the bound inactive renin eluted at 0.5 M NaCl. In specimen 1 from fresh tissue and specimen 1 of cadaver tissue, which contained the highest percentages of inactive renin, little or no active renin was detected in the bound fractions. In specimens 2 and 3 from fresh tissue, approximately half of the bound renin was active, but rechromatography of the bound fractions on cibacron-blue decreased the percentage of active renin that bound to the column. Recovery of active and inactive renin from the cibacron-blue column ranged from 47 to 100%. Chromatography on cibacron-blue enhanced the specific activity of activated inactive renal renin 7- to 40-fold. Trypsin treatment of the bound fractions, using the procedure of Atlas et al.

TABLE I
Active and Inactive Renin in Human Renal Cortex and Plasma

	Homogenate			Cibacron-blue eluates*					
				Bound		Unbound		Recovery	
	Active renin	Inactive renin	Percent inactive	Active	Inactive‡	Active	Inactive	Active	Total
								%	
Fresh kidney§									
1‖ (A)	64	55	46	101	2,648	1,055	83	67	96
(B)	87	64	42	0	2,520	1,280	0	46	49
2‖ (A)	1,435	150	10	2,420 (705)	1,850 (2,727)	1,434	0	95	85
(B)	1,193	200	14						
3 (A)	570	255	31	2,500 (620)	1,750 (2,580)	1,535	0	100	100
(B)	423	252	37						
Cadaver kidney§									
1	1,306	1,449	53	0	6,000	4,679	0		
2	16	25	37						
3	268	131	33						
Plasma¶									
Normal	0.1	0.4	73	0	52.0	1.3	0	54	76

* Values given for inactive and active renin represent the sum of the inactive and active renin in the bound and unbound fractions, respectively.

‡ Numbers in parentheses represent rechromatography of the bound pooled eluates on cibacron-blue.

§ Units of renin measurements are nanograms AI per milligram protein per hour.

‖ Fresh kidney tissues were homogenized and processed two separate times, A and B.

¶ Units of renin in plasma are nanograms AI per milliliter per hour.

(8), demonstrated a peak of inactive renin similar to the peak demonstrated by acid treatment. Subsequent characterization of renal inactive renin was performed on renin obtained from specimen 1.

We also measured inactive renin in a 1-kg preparation (~20 kidneys) of human renal cortex (21). Either acid or trypsin treatment after homogenization and DEAE batch chromatography yielded significant increases in renin activity. Inactive renin comprised 40–80% of the total renin concentration.

Inactive renin in human plasma

When plasma from a normal subject, containing an active renin concentration of 14 ng/ml per h, was chromatographed on cibacron-blue, active renin in plasma did not bind, but inactive renin bound and eluted at 0.5 M NaCl (Fig. 1B). Inactive renin was separated from human serum albumin, which eluted at 1.5 M NaCl. The specific activity of activated plasma renin increased 130-fold by cibacron-blue affinity chromatography. Further characterization of plasma inactive renin was based on a pool of the bound fractions from this plasma.

Inactive renin concentration in plasma of the three patients with renal tumor before nephrectomy ranged from 40 to 60 ng/ml per h (Table II), consistent with normal values (20). Therefore, the renal tumors in these patients did not appear to secrete large amounts of inactive renin into the circulation.

Effect of acid and renal kallikrein on renal and plasma inactive renin

Both renal and plasma inactive renin were maximally activated by dialysis against pH 3.3 buffer at 4°C for 24 h. A graph of pH of dialysis vs. percent total renin resembled a titration curve with an approximate pK_a of 3.5 (Fig. 2). The curves for renal and plasma inactive renin were superimposable.

When renal inactive renin is dialyzed to pH 3.3 buffer for 24 h at 4°C, neutralized by titration with 1 M phosphate buffer, pH 8.1, and incubated at 37°C for 2 h (conditions for reversal of acid-activation of renin in plasma), renin activity decreased to levels approaching that of renin in nonacid-treated control samples. Repeat dialysis to pH 3.3 fully activated the renin. Addition of renal kallikrein did not alter renin

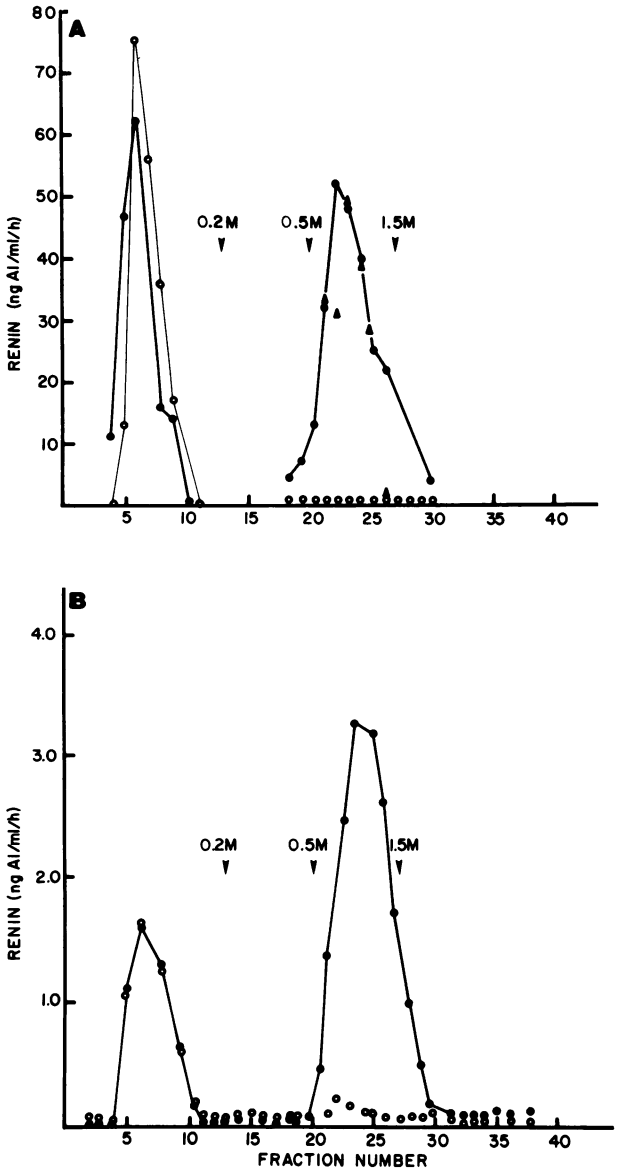

FIGURE 1 Cibacron-blue affinity chromatography of human kidney homogenate, A, and human plasma, B. Eluates were assayed for active renin concentrations (O), dialyzed to pH 3.3, neutralized by titration, and then assayed for renin concentration (total), (●), or trypsin treated and then assayed (▲).

activity, unless the renal inactive renin had been dialyzed to pH 3.3 and subsequently neutralized by titration before renal kallikrein treatment. Activation resulting from acid dialysis and renal kallikrein treatment was equal to that achieved with acid dialysis alone; however, renal kallikrein treatment prevented reversal of acid activation. These data are summarized in Fig. 3 and are identical to the behavior of plasma inactive renin (15).

TABLE II

Renin Levels in Plasma of Patients with Renal Cell Tumors

Patient*	Active renin	Inactive renin
	ng/ml/h	
1	4	41
2	3	42
3	3	65

* Patient number corresponds to the fresh kidney tissue number in Table I.

Characterization of inactive renin

pH optimum of enzyme activity. With sheep angiotensinogen the pH optimum of activity of both renal plasma inactive renin activated by acid plus renal kallikrein treatment was 7.8 (Fig. 4). Activated renins from both sources demonstrated two peaks of activity with human angiotensinogen, a broad peak around pH 5.5 and a sharper peak around pH 6.7. MRC renal renin had a pH optima with sheep and human angiotensinogen that were the same as the pH optima demonstrated in the activated plasma and renal renin preparations.

K_m. Kinetic properties of activated renin from kidney and plasma were identical. The K_m of kallikrein-treated renal and plasma inactive renin with human angiotensinogen was 0.99±0.26 and 1.25±0.16 μM

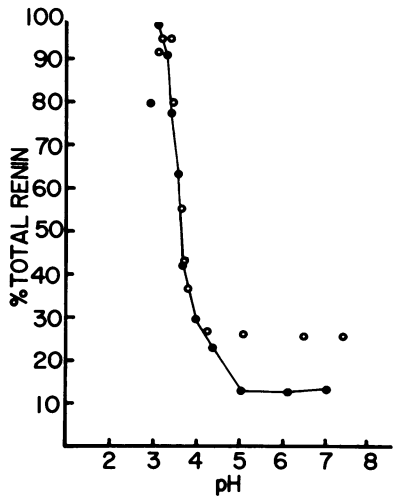

FIGURE 2 Effect of pH on renin activation. Preparations of renal (O), or plasma (●), inactive renin were dialyzed against the indicated pH, neutralized by titration, and immediately assayed for renin.

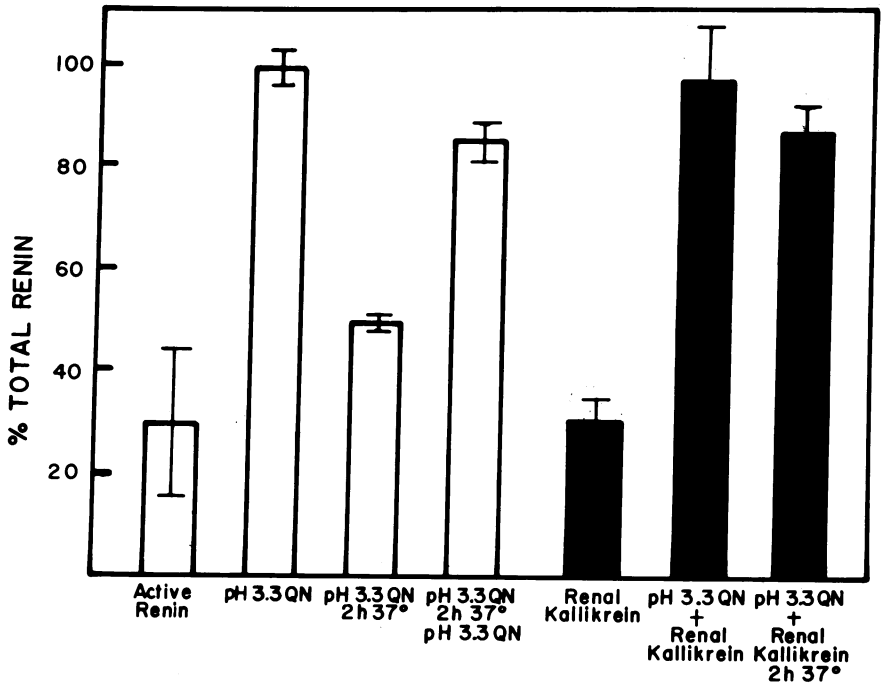

FIGURE 3 Effect of acid and renal kallikrein on renal inactive renin. The renal inactive renin preparation from cibacron-blue was (*a*) assayed for active renin; (*b*) dialyzed to pH 3.3 and quickly neutralized (QN) by titration; (*c*) dialyzed to pH 3.3, quickly neutralized, incubated for 2 h at 37°C, (*d*) dialyzed to pH 3.3, and neutralized; (*e*) incubated with renal kallikrein and then assayed; (*f*) dialyzed to pH 3.3, quickly neutralized and treated with renal kallikrein; (*g*) dialyzed to pH 3.3, quickly neutralized and treated with renal kallikrein and incubated for 2 h at 37°C.

(mean±SEM), respectively, determined at pH 7.5. The K_m with sheep angiotensinogen was 0.34±0.05 μM and 0.29±0.05 μM, respectively, determined at pH 7.0. The K_m of MRC renin was 1.39±0.20 μM with human angiotensinogen and 0.30±0.05 μM with sheep angiotensinogen.

Antibody inhibition studies. Inhibitory concentration curves of anti-human renin antisera against the various renins are depicted in Fig. 5. Each point represents the mean of four determinations measured in duplicate. The curves for activated plasma and renal inactive renin and MRC renal renin were virtually superimposable. The mean dilution of antisera, which results in 50% inhibition of renin activity (MIC_{50}), was 1:30,000.

Gel filtration. Elution patterns of cibacron-blue preparations of renal and plasma inactive renin on Sephadex G-100 are illustrated in Fig. 6. When fractions were dialyzed to pH 3.3 and subsequently neutralized by titration, two definite peaks of inactive renin were seen in both kidney and plasma preparations. In three elutions of renal inactive renin from specimen 1 and one elution of specimen 2 on Sephadex G-100, the first peak had an apparent mol wt of 56,300±1,500 (mean±SEM) and the second peak had an apparent mol wt of 49,200±1,000. Recovery of total renal renin following gel filtration was 90±12%. Activation of renal inactive renin by acid plus renal kallikrein treatment resulted in no change in the elution pattern from Sephadex G-100. In addition, a small shoulder of activity eluted immediately ahead of the first peak in both chromatograms. The significance of this is unclear at present. In plasma, the peaks of inactive renin had an apparent mol wt of 58,000 and 49,000, respectively. Recovery of total renin from plasma was 90%. The presence of inactive renin under these peaks was confirmed by activation of renin in the eluates with acid plus pepsin treatment (7). When pools of each of the two peaks of renal inactive renin and plasma inactive renin were stably activated by acid plus renal kallikrein, the renin activity of all peaks were completely inhibited by anti-human renal renin antibody in a 1:20,000 dilution.

Tryspin activation of the Sephadex G-100 eluates (8) demonstrated a single peak of inactive renin in both plasma and kidney preparations. The apparent

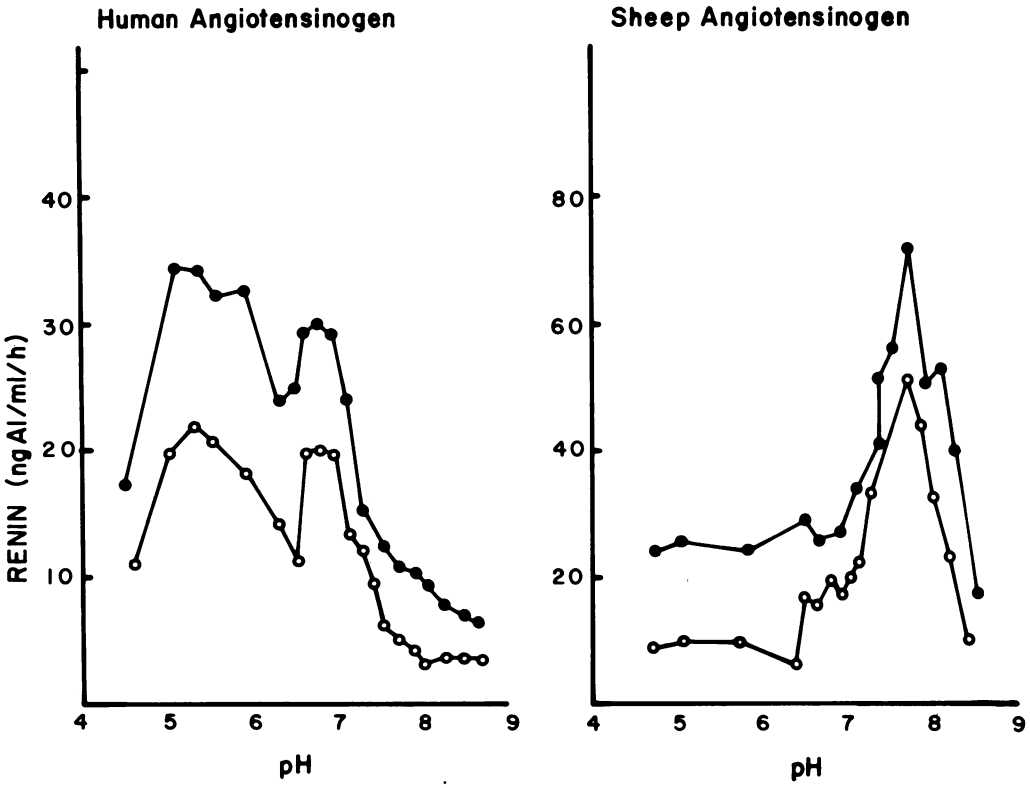

FIGURE 4 pH optimum of enzyme activity was determined for acid plus renal kallikrein-treated renal inactive renin (●), and plasma inactive renin (○), with both human and sheep angiotensinogen. Each point represents the slope of AI generated at the indicated pH for four time periods in duplicate.

mol wt of the peak was 55,000 for plasma (recovery 58%) and 50,000 for kidney (recovery 100%).

Determination of inactive renin concentration in IVC and renal veins

In eight patients undergoing venous catheterization inactive renin concentration was invariably higher in venous blood from one or both kidneys than blood obtained from the IVC below the renal veins. Venous level of inactive renin from the kidney with the higher concentration (HC) was 69.9±11.5 (mean±SE), from the contralateral side was 31.6±10.6, from the IVC above the origin of the renal veins: 50.9±6.7, and IVC below the origin of the renal veins: 31.5±6.2 ng/ml per h. Thus, a gradient of inactive renin exists between the IVC above and below the kidneys ($P < 0.025$) as well as between the renal vein of the HC kidney and the IVC below the kidney ($P < 0.005$). Individual differences between the IVC below and the HC renal vein are shown in Fig. 7. As shown in this figure, chronic inhibition of converting enzyme markedly enhanced the differences in inactive renin concentration. A 2.5-fold increase in inactive renin concentration was observed in the HC renal venous samples as compared with the IVC sample.

DISCUSSION

Until recently, the source of circulating inactive renin has been obscure, and attempts to isolate inactive renin from the kidney have failed. The reasons for this failure could include (*a*) low pH used during tissue extraction (22); (*b*) absence of protease inhibitors (23); (*c*) prolonged exposure to temperatures just above freezing, which is known to activate renin in plasma (24); and (*d*) problems in techniques of activation (6, 15). In this study, we found that inactive renin comprised from 10 to 50% of the total renin concentration in human renal cortex, and even greater percentages were found in large scale processing of human kidneys. These values may underestimate the actual quantities of renin in the kidney, since during isolation, some

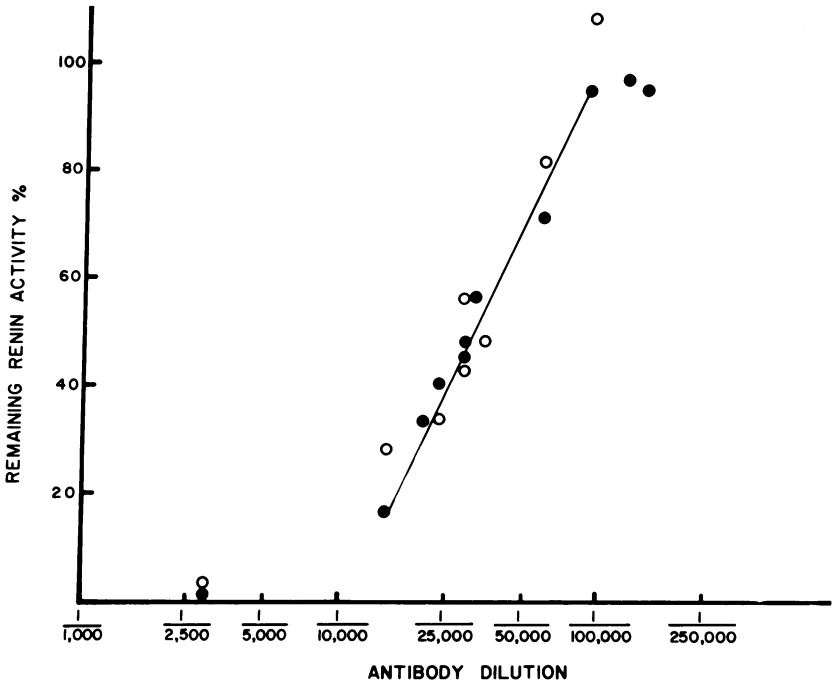

FIGURE 5 Inhibitory dilution of rabbit antisera containing anti-human renal renin antibody with acid plus kallikrein-treated plasma inactive renin (O), and renal inactive renin (●). The mean inhibitory concentration is 1:30,000. This was also the mean inhibitory concentration for active renal renin.

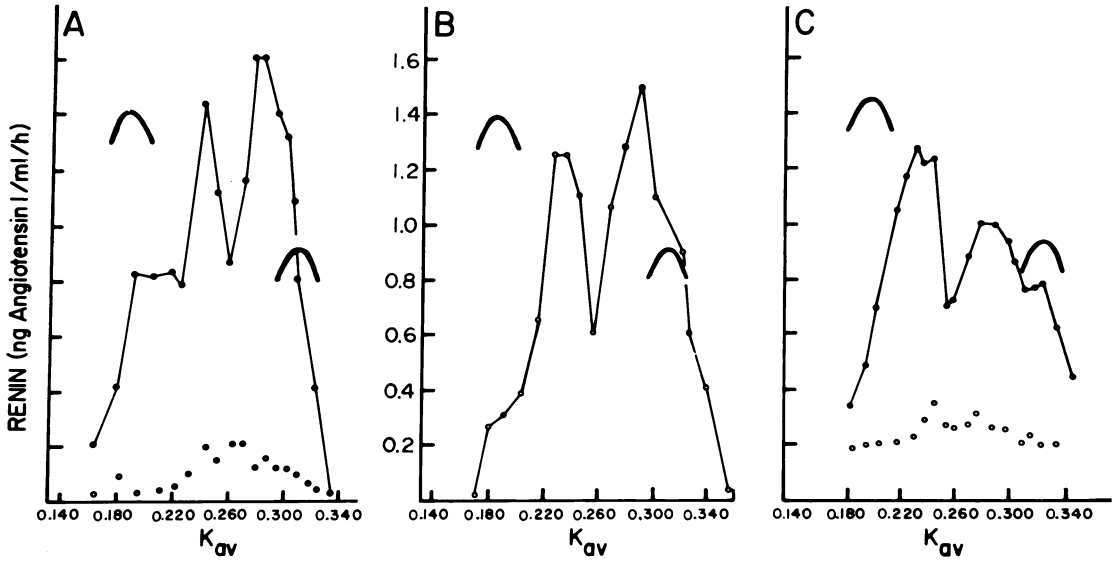

FIGURE 6 Gel filtration of inactive renin extracted from human kidney and plasma: untreated eluate (O), acid-treated eluate (●). Background peaks represent human serum albumin, K_{av} = 0.191, and ovalbumin, K_{av} = 0.309. (A) Renal inactive renin, apparent molecular weight of peaks 56,300±1,500 (mean±SEM, n = 3) and 49,100±1,000. (B) Acid and renal kallikrein treatment of renal inactive renin before chromatography, apparent molecular weight of peaks, 58,000 and 47,500. (C) Plasma inactive renin, apparent molecular weight of peaks 58,000 and 49,000.

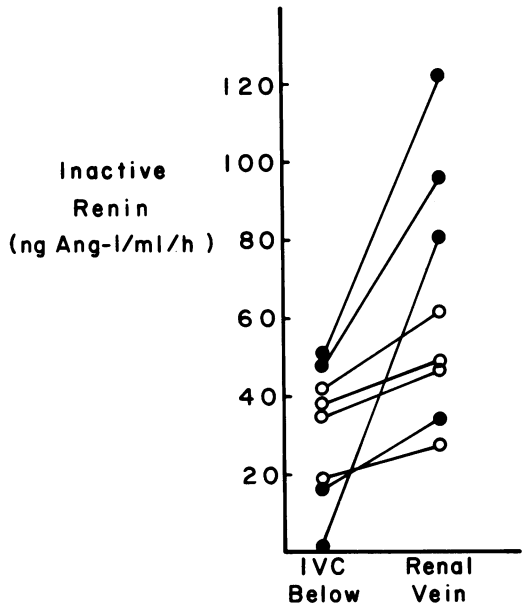

FIGURE 7 Inactive renin concentration in IVC blood below the origin of the renal veins vs. renal vein blood ($P < 0.005$ for the group): patients in the basal state (○); patients on chronic converting enzyme inhibition (●).

TABLE III
Summary of Identical Physico-Chemical Properties between Renal and Plasma Inactive Renin

	Renal	Plasma
Effect of acid		
pH of maximal activation	3.3	3.3
Reversible acid activation	+	+
Kinetic activity		
pH optimum		
Sheep angiotensinogen	7.8	7.8
Human angiotensinogen	5.5, 6.7	5.5, 6.7
K_m (mean±SEM)		
Sheep angiotensinogen	0.34±0.05 μM	0.29±0.05 μM
Human angiotensinogen	0.99±0.25 μM	1.25±0.16 μM
Inhibition by anti-human renal renin antibody	1:30,000 1×10^{-4} GU	1:30,000 1×10^{-4} GU
Gel filtration		
Apparent molecular weight	56,300±1,500	58,000
	49,200±1,000	49,000

activation of renin may have occurred by several endogenous renal enzymes (25, 26).

We have also demonstrated that renal and plasma inactive renin are virtually identical in physiochemical, kinetic, and immunogenic properties (Table III). These similarities have emerged despite the use of only partially purified preparations of plasma and renal inactive renin, which could contain variable quantities of enzymes and other proteins that might affect activation and characterization. These findings are consistent with the postulate that plasma inactive renin arises from the kidney and are supported (*a*) by the present observations demonstrating a gradient of inactive renin in IVC and renal vein blood and (*b*) by the report of Atlas et al. (8) who found inactive renin in the perfusate of an isolated human kidney.

Recent investigations have shown that reversible acid activation is a unique property of plasma inactive renin (15, 27), which is probably due to a conformational change in human renin (15). Renal kallikrein, implicated as a potential in vivo activator of renin (28), can prevent reversal of acid activation at neutral pH by maintaining acid-exposed renin in an active conformation. Since our results demonstrate that the effects of low pH and renal kallikrein on renal inactive renin are the same as that on plasma inactive renin, it would appear that the tertiary structure of both inactive renins is similar.

Antibody to pure human active renal renin completely neutralized the activity of activated plasma and renal inactive renin also suggesting a high degree of structural homology between these molecules. The demonstration that the inhibitory titer of the antibody for both active MRC renin and activated renal and plasma inactive renin were indistinguishable, strongly suggests that the active sites of the enzymes are essentially identical.

Kinetic and antibody inhibition studies also suggest that an enzyme homologous to endogenous active renal renin may be derived from in vitro activations of inactive renin. Activation of both renal and plasma inactive renin yielded an active enzyme with similar kinetic behavior to a standard preparation of active renal renin (MRC). All three renins displayed the same patterns of enzyme activity with two different substrates prepared from sheep and human plasma. Two substrates were used in order to eliminate the possibility that results were biased because of peculiarities of the substrate preparation and to evaluate the renin reaction with two commonly used substrates in which renin hydrolyzed different peptide bonds. Renin splits a valine-leucine bond in human angiotensinogen and a leucine-leucine bond in other mammalian substrates to liberate the same decapeptide (29). The K_m of all three renins with each of the substrates was the same, again suggesting that the active site of the renins originating from different sources is not significantly different. The K_m with sheep substrate was 0.29–0.34 μM and with human substrate was 0.99–1.39 μM; these agree with previously reported values for active forms of plasma and renal renin (14, 30–32).

In addition to enzyme activity, investigation of pH optima and gel filtration lend strong support to the identity of plasma and renal inactive renin. Another important observation stemming from these studies is the demonstration that human plasma and renal inactive renin exist in multiple forms. Activated renal and plasma inactive renin had a pH optimum of 7.8 with sheep angiotensinogen, and pH optima of 5.5 and 6.7 with human angiotensinogen. This biphasic pattern with human substrate could result from the presence of several forms of inactive renin or from heterogeneity of the renal substrate in plasma of women ingesting oral contraceptives (33).

Gel filtration displayed two distinct peaks, and possibly a third peak, of inactive renin in both plasma and kidney. In previous studies, in which we applied normal plasma to a Sephadex G-100 column, only a single peak of inactive renin of 55,000-mol wt was described (7). In this study, improved resolution of potential renin peaks was possible since partially purified inactive renin was placed on the G-100 column. The peaks in plasma correlated to those in kidney, 56,000±1,500 and 49,200±1,000. In contrast, Atlas et al. (8) and Chang et al. (9) described a single peak of plasma and renal inactive renin of 56,000- and 50,000-mol wt, respectively. Both groups used trypsin treatment to define inactive renin, and we have confirmed these estimates using trypsin. Because excess trypsin destroys renin (6), its optimum concentration for renin activation varies from eluate to eluate due to variable elution of trypsin inhibitors and other proteins. Using trypsin, multiple peaks of inactive renin may be difficult to detect. We have defined inactive renin in column eluates by a modified acid-activation technique, which does not appear to be mediated by proteases (15). This approach yields good recoveries of inactive renin. Therefore, it may have advantage over other methods accompanied by proteolytic destruction of renin.

Both peaks of inactive renin have immunoidentity to active renal renin. These peaks could represent two distinct forms of inactive renin that differ in amino acid composition. Alternatively, differences in glycosylation or conformation could contribute to variations in Stokes' radius to account for differences in apparent molecular weight. A less likely possibility is that the peaks represent partially degraded inactive renins following extraction and chromatography. Renal and plasma inactive renin have previously been shown to have similar sets of isoelectric points (9), and active renin has similarly been demonstrated to be heterogenous (31, 34). More recent studies from our laboratory using pulse-labeling techniques show that multiple forms of renin are synthesized and released (35, 36). The current findings help to explain the discrepancy generated by observations reporting significant differences in apparent molecular weight between plasma and renal inactive renin and may represent biochemical counterparts to the pulse-labeling experiments. However, data derived from the present studies cannot distinguish whether inactive renin is a precursor of active renin or an active renin-inhibitor complex.

To strengthen the hypothesis that the kidney is a source of plasma inactive renin, we looked for differences in inactive renin concentration in IVC and renal vein blood. Since there is no evidence that there are differences in renin concentration in arterial blood and IVC blood below the renal veins, the renin concentration in the IVC below the renal veins has been generally accepted to reflect the renal arterial concentration (37). We found a small but significant increase in inactive renin in renal venous blood compared with IVC below the kidney, similar to some previous reports (38, 39). Other studies, however, have been less consistent in demonstrating such a renal gradient (40). Since chronic inhibitor of converting enzyme induces a fourfold increase in circulating inactive renin (41), we also measured renal vein inactive renin in four patients during chronic administration of captopril. The exact mechanism by which captopril stimulates inactive renin production is unclear. Nevertheless, inactive renin concentration across the kidney increased by ~250% as compared with 38% in the unstimulated group. In view of the 15% intraassay coefficient of variation, the increase in inactive renin during stimulation is highly significant. Therefore, we have demonstrated a consistent gradient of inactive renin across the kidney.

In summary, extensive characterization of plasma and renal inactive renin and measurement of a renal gradient of inactive renin indicate that the kidney is a likely source of circulating inactive renin. Therefore, plasma levels of active and inactive renin may reflect renal secretion of the two forms of renin. Certain physiologic and pathologic conditions are accompanied by alterations of the inactive/active renin ratio in plasma (42–44). Thus, further investigation of the relationships between plasma and tissue active and inactive renins promises to be important in defining the pathophysiology of renin production in man.

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