Role of Renin Isoelectric Heterogeneity in Renal Storage and Secretion of Renin

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ABSTRACT
Renin is a glycoprotein that is heterogeneous with respect to carbohydrate content and net charge. In an attempt to clarify the role of renin isoelectric heterogeneity in renal renin storage and secretion, the isoelectric profile of renal renin, secreted renin, and circulating renin were directly assessed and compared under basal and stimulated conditions by the use of an in vivo blood perfused rabbit kidney preparation. Under basal conditions, the kidney preferentially stored and secreted the relatively basic isoelectric forms of renin. Acute stimulation of renin secretion (reduced renal perfusion pressure and angiotensin-converting enzyme inhibition) significantly increased the secretion of the relatively basic isoelectric forms but had very little effect on the secretion of the relatively acidic renin forms. Circulating renin was composed primarily of relatively basic isoelectric forms of renin. Acute stimulation of renin secretion (reduced renal perfusion pressure and angiotensin-converting enzyme inhibition) significantly increased the secretion of the relatively basic isoelectric forms but had very little effect on the secretion of the relatively acidic renin forms. These findings suggest that the isoelectric heterogeneity of renin is important in the cellular processing of renin and can be explained by a two-pool model in which the relatively acidic isoelectric forms represent a regulated pathway in which they are stored and rapidly released in response to acute secretory stimuli. Preferential hepatic extraction of the more basic isoelectric forms has previously been described. Data from this study suggest that the disproportionate increase in circulating basic forms of renin observed after acute stimulation reflects the net effect of preferential renal secretion and preferential hepatic degradation of the more basic renin isoelectric forms. The disproportionate increase in relatively basic circulating renin forms after acute secretory stimulation results in an overall circulating renin activity with a shorter half-life.

Key Words: PRA, renin, isoelectric forms, glycoforms, renal renin, constitutive secretion

Circulating renin is 7% carbohydrate by molecular weight. After synthesis on the ribosome, the polypeptide backbone of renin is glycosylated by the enzymatic attachment of oligosaccharides to specific asparagine residues (N-linked glycosylation). The attached oligosaccharides are subsequently modified in a consistent process (1) such that a reproducible profile of renin glycoforms can be demonstrated. Because carbohydrate attachment to proteins results in the addition of negatively charged carbohydrate moieties, glycoproteins like renin are also heterogeneous with respect to net charge and can also be separated into multiple isoelectric forms on the basis of isoelectric point heterogeneity. Recently, it has been demonstrated that the isoelectric heterogeneity of renin is due, at least in part, to variable glycosylation (2,3) and that the hepatic degradation of renin is a direct function of both isoelectric point (4,5) and glycosylation (6). Thus, the isoelectric heterogeneity and glycosylation of renin appear to be closely related.

The presence of multiple forms of active renin within the kidney and in circulating plasma appears to have many important physiologic consequences. Glycosylation is important for the intracellular sorting and secretion of proteins (1) and could be a determinant of the fate of newly synthesized renin: immediate secretion (constitutive release) or routing to a storage pool (regulated release) (7,8). In support of this hypothesis, it appears that the kidney contains different proportions of renin isoelectric forms and glycoforms than plasma (9,10). Additionally, the kidney preferentially secretes some isoelectric forms and glycoforms depending on the secretory stimulus and its duration, indicating the involvement of mul-
multiple renin forms in stimulus-secreting coupling (4,9,11–15). Although some workers have not found evidence for disproportionate storage or secretion of renin isoelectric forms (5,16), these discrepancies may be the result of artifacts from in vitro preparations or incorrect inferences. As previously mentioned, the hepatic degradation of renin is a direct function of isoelectric point and glycosylation so that each renin isoelectric form has a different plasma half-life (4–6, 17). Thus, differential hepatic extraction of multiple renin forms helps to set circulating PRA. Finally, it appears that renin isoelectric forms may differ with respect to their target organ effects (18–20).

In summary, the presence of multiple forms of active renin appears to affect the intracellular trafficking, stimulus-secretion coupling, hepatic extraction, and target organ effects of renin. However, the bulk of the above data regarding the physiologic significance of renin isoelectric forms and glycoforms is derived from in vitro experimental preparations, in which basal renin secretion is severalfold greater than in vivo, or from indirect in vivo measurements in experimental models and humans. In an attempt to clarify the role of renin isoelectric heterogeneity in renal intracellular trafficking and secretion of renin, the isoelectric profile of renal renin (storage pool), secreted renin, and circulating renin were directly assessed and compared under both basal and stimulated conditions in vivo.

METHODS—EXPERIMENTAL PREPARATION

Nine New Zealand White rabbits of either sex, weighing 3 to 4 kg, fed standard Purina rabbit chow (Purina Mills Inc., St. Louis, MO), and given free access to water, were used in this study, which was conducted in accord with the NIH Guide for the Care and Use of Laboratory Animals. The experimental preparation was nearly identical to that previously reported (21). Briefly, rabbits were sedated with morphine sulfate (2 mg/kg sc). One hour later, a loading dose of pentobarbital sodium was given (25 mg/kg); this was followed by a constant maintenance infusion of 7 mg/kg h. Both were administered via the marginal ear vein.

A tracheostomy was performed, and the trachea was cannulated and connected to a Harvard rodent ventilator (Harvard Apparatus, South Natwick, MA) with a positive end-expiratory pressure of 2 cm H2O. Blood gases were maintained at approximate values of pH, 7.44; PaO2, 110 mm Hg; and PaCO2, 38 mm Hg. Rectal temperature was monitored, and body temperature was maintained near 39°C with the aid of a heating pad. The right femoral vein was cannulated for iv injections and extracorporeal exchange, and the right femoral artery was cannulated for measurement of blood gases (IL-1302 blood gas machine), arterial blood pressure (Statham P 23xL pressure transducer; Guild Inc., Oxnard, California), and arterial renin. The left common carotid artery was also cannulated and used as the arterial blood source for renal perfusion.

A right nephrectomy was performed. A flank incision was then made over the left kidney, and the renal artery and vein were cleared for subsequent cannulation. The left renal vein was catheterized with a 24-gauge teflon catheter for sampling of renal venous blood (21).

An extracorporeal circuit (25 mL fluid volume) was then established in which blood from the left common carotid artery was pumped by a constant-pressure servo-controlled Mohrman pump (22) through a windkessel (bubble trap/pulse pressure damper), an electromagnetic flow probe (Zepeda Instruments, Seattle, WA), and finally, a double-lumen stainless steel cannula tied into the left renal artery. The inner lumen measured renal perfusion pressure at the tip of the cannula via a second pressure transducer. The blood pump was servo-controlled from the renal perfusion pressure signal and was set for either 90 mm Hg (basal period) or 60 mm Hg (stimulation period). The outer cannula lumen conducted blood into the renal artery. Continuous recording of total RBF (electromagnetic flow signal), renal perfusion pressure, and arterial pressure was made by a Gould 2400S chart recorder (Gould Inc., Cleveland, OH).

Before in vivo blood perfusion of the kidney, the rabbit was systematically anticoagulated with sodium heparin, 625 U/kg. This was followed by a continuous infusion of 200 U/kg h, and supplemental morphine sulfate was administered (1 mg/kg). The extracorporeal circuit was filled with 25 mL of 6% hetastarch in 0.9% sodium chloride solution (Hespan; DuPont Pharmaceuticals, Wilmington, DE) and was initially circulated between the left common carotid artery and femoral vein to achieve good mixing with blood.

The renal artery was then cannulated after 30 to 90 s of occlusion, and perfusion pressure was brought up to 90 mm Hg. Ibuprofen (70 mg/kg) was given slowly iv after the initiation of renal perfusion to prevent platelet adherence to the perfusion cannula.

STUDY DESIGN

The isoelectric heterogeneity of multiple renin forms (renin form profile) of the homogenized right kidney (removed before left kidney perfusion) was assumed to reflect the renin form profile of stored renin in the basal period. In this preparation, the renin content of both kidneys has been shown to be equal in the basal period (21).

Renin secretory rate (RSR) determinations were initiated after 20 min of renal artery perfusion at 90
mm Hg. At time 0, the first baseline RSR determination was made, and RSR was then determined every 10 min for 40 min. Immediately after the fifth baseline RSR determination (time = 40 min), renal perfusion pressure was lowered to 60 mm Hg and enalapril maleate (1 mg/kg) was given iv to stimulate renin secretion. RSR was then determined at 5, 10, and every 15 min thereafter for a total of between 55 and 85 min of stimulated renin secretion.

Visual inspection of the RSR versus time curve for each experimental animal was used to select a single basal and single stimulated time at which the RSR appeared to be at a steady state (21). This typically occurred 30 min after the initiation of renal perfusion (basal) and 45 min after the stimulation of renin secretion (stimulated). The isoelectric form profile of active renin in both the arterial and renal venous samples from these selected steady-state basal and stimulated times was then measured and used to determine the separate isoelectric form RSR and plasma isoelectric renin form profiles.

LABORATORY METHODS

Plasma Renin Concentration

Four hundred microliters of blood from an arterial or renal venous source was collected over 1 min, mixed with 20 μL of 5% EDTA, immediately chilled on ice, and centrifuged to separate the plasma component, which was frozen at −25°C until assayed. On the day of the plasma renin concentration (PRC) assay, the plasma was quickly thawed and centrifuged at 3000×g for 30 min at 4°C.

A rabbit angiotensinogen source (concentrations of active renin near zero) was prepared from 24-h bilaterally nephrectomized rabbits. Sixty-five microliters of the angiotensinogen plasma source was combined with the following angiotensinase inhibitors: 5 μL of 1.7% dimercaprol in benzyl benzoate/peanut oil, 5 μL of 6.6% 8-hydroxyquinoline sulfate, and 12 μL of 5% EDTA. The mixture was then incubated at 37°C for 0 and 45 min. After incubation, plasma renin activity was stable in the homogenate, and the mixture was diluted with 165 μL of 0.1 M maleate buffer with 5% BSA (pH = 6). Ten microliters of plasma for PRC determination was added to the resulting diluted angiotensinogen mixture (250 μL), and the mixture was incubated at 37°C for 0 and 60 min. During incubation, angiotensin I was generated in direct proportion to the renin concentration (zero order kinetics) of the 10-μL plasma sample. Angiotensin I concentrations at 60 min were corrected for zero time angiotensin I concentration and for angiotensin I generated in the diluted angiotensinogen solution as the result of residual renin activity in the 24-h bilaterally nephrectomized rabbit plasma. Only 4% or less of the total available angiotensinogen was converted to angiotensin I, and linear generation of angiotensin I over time was always verified (10, 21).

Angiotensin I was assayed by RIA by the use of a modified Dupont angiotensin I RIA kit (Dupont, Wilmington, DE). Major modifications included preparation of angiotensin I standards with the same solution as that described for the PRC samples. Also, activated charcoal was substituted for the second antibody. RIA assay data were linearized with a log-logit transformation, resulting in a correlation coefficient of −0.99 or better. The average labeled angiotensin I bound 40% to the first antibody without competition. The average percentage of error for predicting the standard concentration from the transformed curve was less than 7%. Angiotensin I recovery from spiked samples was a function of the absolute angiotensin I concentration. In general, recoveries were between 94 and 106% at angiotensin I concentrations between 0.1 and 5 ng/mL, but above 5 ng/mL, recovery steadily decreased and equaled 89% at 10 ng/mL. As a result, all assays were kept between 0.1 and 5.0 ng of angiotensin I/mL.

Renin Homogenate

Immediately after right nephrectomy, the kidney was flushed with ice-cold saline, decapsulated, and then snap-frozen in a dry ice/methanol mixture. After thawing partially, the medulla was carefully dissected away and the entire cortex was cut into cubes of approximately 0.7-cm³. Four cortical cubes were randomly chosen, cut into thin slices with the aid of a handheld microtome, and homogenized in 50 vol of 0°C distilled water with a Virtishear (The Virtis Company, Gardiner, NY) electric homogenizer at 70% peak power for 35 s per sample. The homogenates were frozen, thawed twice, and then centrifuged at 3000×g for 20 min at 4°C. The resulting supernatant was then prepared for isoelectric focusing gels (see below). Renin activity was stable in the homogenate, as evidenced by a constant renin activity in the final supernatant during repeated measurements and homogenate focusing gel yields of 90 to 105%.

Isoelectric Focusing

We prepared plasma samples for focusing by mixing them with 3 mg of fluted silicon dioxide (SiO₂) per 100 μL of plasma, incubating them with frequent vortexing for 30 min at 20°C, and then centrifuging them at 10,000×g for 5 min at 20°C. The SiO₂ incu-
bation was used to remove lipoproteins and fibronogen, which would otherwise block renin from completely entering the focusing gels. Renin yields averaged 90% (10).

Renal cortical homogenate supernatants were diluted approximately 100-fold in rabbit plasma derived from 24-h nephrectomized rabbits and containing near-zero renin activity. The homogenate-plasma sample was then prepared for isoelectric focusing exactly as a plasma sample. In this manner, the isoelectric profile of renal cortical renin could be compared with the plasma renin profile under identical focusing conditions (10).

Isoelectric focusing was performed in a manner similar to that initially described by Nguyen and Chrambach (23) and modified by Katz et al. (10). A stable pH gradient was formed in the isoelectric focusing gels by the equalization of the anolyte pH with the pH of the most acidic ampholyte present in the gel and by the use of the isoelectric amino acid solutions as the anolyte and catholyte. The polyacrylamide isoelectric focusing gels contained 0.60% ampholine, pH 5 to 7, and 1.4% ampholine, pH 4 to 6 (Pharmacia LKB, Piscataway, NJ). Each gel contained a volume of 2.6 mL of polyacrylamide solution and was poured to a length of 13 cm. The upper (catholyte) and lower (anolyte) electrode solutions were 0.02 M L-histidine (free base form) and 0.02 M L-asparagin, respectively.

All electrophoresis was carried out at 20°C. The top of the gels facing the catholyte received 50 to 200 μL of SiO2-treated plasma or 125 μL of homogenate-plasma samples. The electrophoresis run time was 23 h. During the first hour, the voltage across the gels was increased gradually to 500 V and remained at 500 V for the duration of the run. Peak wattage per gel was not more than 1 W. After the run, each gel was removed from its tube and immediately frozen in a dry ice-methanol mixture. The frozen gel was then serially sliced into approximately 60 gel segments.

Each gel segment was eluted in an individual polystyrene tube with 125 μL of 0.1 M maleate buffer (pH 6) with 1% BSA and 0.25% EDTA at 4°C for approximately 24 h. The gel segments were then carefully removed, leaving behind approximately 90 μL of eluant (with renin present, if the enzyme had focused in the corresponding gel segment). Each 90 μL of eluant was then combined with 22.6 μL of 24-h bilaterally nephrectomized rabbit plasma as an angiotensinogen source, 1.2 μL of 6.6% 8-hydroxyquinoline, and 1.2 μL of 1.7% dimercaprol with 3.3% benzyl benzoate in peanut oil as angiotensinase inhibitors. The total 115 μL was then incubated at 37°C for 1 to 3 h in order to center the renin activity peaks within the angiotensin I standard curve (range of standard curve, 0.1 to 10 ng of angiotensin I/ml).

During the incubation, rabbit renin forms, if present, hydrolyzed rabbit angiotensinogen to form angiotensin I. The angiotensin I generated in the entire 115-μL incubation mixture was then measured by standard RIA as described for the PRC assay. Zero time angiotensin I and residual renin activity in the substrate were subtracted from all gel segments containing renin activity. This was done by subtracting the baseline renin activity found at the end of each gel where renin activity peaks were absent. Between 90 and 105% of the applied renin activity could be accounted for in either the eluant or removed gel slices.

Plasma focusing samples contain both angiotensinogen and angiotensin I, which could cause renin activity to be overstated within corresponding gel segments. However, neither molecule focuses within the relevant pH range and neither is present in sufficient quantity in the sample to influence the renin measurement (10).

**RENIN FORM QUANTITATION**

Quantitation of the five major renin forms that were resolved in the gel was performed as previously reported (10). The amount of net renin activity in each of the five renin activity peaks (renin forms I to V) was measured by summing the renin activity in each gel segment corresponding to an individual renin activity peak. Identification of each gel peak as a specific renin form was accomplished by identification of the corresponding isoelectric point of the gel segment from identical gels run in parallel and was verified by observation of the relative position of a peak in relation to other peaks in the gel.

The amount of net renin activity in each of the five peaks from a plasma or renal cortical homogenate sample (renin form profile) was initially expressed as nanograms of angiotensin I per milliliter of gel slice eluant per hour of assay incubation. The activity of each of the five renin forms was then expressed as a proportion (percentage) of the total renin activity recovered from the gel. In addition, after correction for any differences in applied sample volume to the focusing gels, the renin form profile of renin secreted from the kidney was calculated by subtracting the corrected net renin activity of each arterial renin form from the corresponding renal vein renin form in matched arterial and renal vein plasma samples. The resulting difference for each renin form (absolute amount) was also expressed as a proportion (percentage) of the total active secreted renin.

**STATISTICAL ANALYSIS**

Comparison of the proportions of the five major renin forms (renin forms I to V) was made between arterial, renal venous, and homogenate samples. Sta-
statistical comparisons were made between the proportions of the two most relatively basic renin forms combined (forms I and II) compared with the two most acidic renin forms combined (forms IV and V). The intermediate renin form was excluded from statistical analyses to eliminate an obligate interrelation between the percentages of the renin forms analyzed. Combining renin forms allowed assessment of statistical differences without reference to specific renin forms, which are a function of the isoelectric pH gradient. Differences in proportions of renin forms were assessed by both parametric and nonparametric tests because the renin form proportions, although appearing normally distributed, did not meet strict criteria for normal distribution because their range was limited to within 0 to 100%. Thus, the data were assessed by use of both a paired t test and Wilcoxon signed ranks test. Changes in arterial PRC and RSR after stimulation and changes in the absolute amount of renin forms were assessed by paired t tests. Results are reported as mean ± 1 SE. Statistical significance was assumed at P ≤ 0.05 (two tailed).

RESULTS

RSR data and corresponding multiple renin form analysis from nine rabbits are presented. Homogenization of the right kidney for determination of the basal renin form profile of storage pool renin was performed in seven animals.

Isoelectric focusing with a pH gradient of 0.12 pH units/cm of gel consistently resolved rabbit active renin into five multiple forms with isoelectric points of 5.20, 5.00, 4.82, 4.70, and 4.55 (renin forms I to V, respectively). A characteristic renin form profile of renal venous blood showing five renin isoelectric forms focused in a 0.12 pH unit/cm of gel is presented (Figure 1A). When the pH gradient of the isoelectric focusing gel was made more shallow with a different ampholine mixture (0.09 pH units/cm of gel) and when the same sample was rerun, as many as 10 renin forms could be identified (Figure 1B).

Basal RSR averaged 3,260 ± 670 ng of angiotensin I/g of renal cortex·h of assay incubation·h of renal perfusion and increased in all animals after reduction in renal perfusion pressure and increased in all animals after reduction in renal perfusion pressure and treatment with enalapril to 23,640 ± 4,880 ng of angiotensin I/g of renal cortex·h of assay incubation·h of renal perfusion, a 7.3-fold increase (Table 1). Under near steady-state basal and stimulated conditions, the concentration of all isoelectric forms of renin increased across the kidney as evidenced by their positive secretory rates (Table 1). Figure 2 presents the proportions of each of the five isoelectric forms of renin secreted from nine perfused rabbit kidneys during both basal and stimulated steady state conditions. During the basal renin secretion period, the relatively basic renin forms (I and II) comprised 51% of the active renin secreted (Table 2). After the stimulation of renin secretion (reduced perfusion pressure and enalapril treatment), the relatively basic renin forms comprised 79% of the active renin secreted. The shift in the profile of secreted renin toward the more basic renin isoelectric forms under stimulated compared with basal conditions was statistically significant (Table 2). The shift in the secreted renin isoelectric form profile after acute stimulation resulted from an increase in the absolute amount of the more basic forms secreted (Table 1; RSR of forms I and II combined increased 10-fold). The absolute amount of the more acidic forms secreted after acute stimulation did not change significantly (RSR of forms IV and V combined increased two-fold). During RSR stimula-
TABLE 1. RSR, PRC, and renal renin content of each of the isoelectric forms of renin under basal and stimulated (RSR and PRC only) conditions

<table>
<thead>
<tr>
<th>Renin Form</th>
<th>RSR Basal</th>
<th>RSR Stimulated</th>
<th>PRC Basal</th>
<th>PRC Stimulated</th>
<th>Renin Content Basal</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1,370 ± 660</td>
<td>12,500 ± 2,800*</td>
<td>4.6 ± 1.4</td>
<td>10.3 ± 2.3*</td>
<td>360,000 ± 125,000</td>
</tr>
<tr>
<td>II</td>
<td>470 ± 190</td>
<td>6,250 ± 1,460*</td>
<td>1.9 ± 0.3</td>
<td>5.2 ± 0.8*</td>
<td>230,000 ± 67,000</td>
</tr>
<tr>
<td>III</td>
<td>300 ± 120</td>
<td>2,560 ± 520</td>
<td>2.0 ± 0.5</td>
<td>3.1 ± 0.6</td>
<td>63,000 ± 22,000</td>
</tr>
<tr>
<td>IV</td>
<td>720 ± 290</td>
<td>1,470 ± 400</td>
<td>3.0 ± 1.1</td>
<td>2.8 ± 0.8</td>
<td>16,000 ± 6,700</td>
</tr>
<tr>
<td>V</td>
<td>400 ± 120</td>
<td>860 ± 390</td>
<td>1.2 ± 0.4</td>
<td>1.7 ± 0.5</td>
<td>3,600 ± 2,700</td>
</tr>
<tr>
<td>Total</td>
<td>3,260 ± 670</td>
<td>23,640 ± 4,800c</td>
<td>12.7 ± 3.5</td>
<td>23.2 ± 4.5c</td>
<td>667,000 ± 192,000</td>
</tr>
</tbody>
</table>

*Values are mean ± SE. RSR = nanograms of angiotensin I per gram of cortex per hour of perfusion per hour of assay incubation; N = 9. PRC = nanograms of angiotensin I per milliliter of plasma per hour of assay incubation; N = 9. Renin content = nanograms of angiotensin I per gram of cortex per hour of assay incubation; N = 7.

bP < 0.004 (combined forms I and II) compared with basal by paired t test.

cP < 0.015 compared with basal by paired t test.

Figure 2. Proportions of the five renin isoelectric forms secreted by the kidney under basal and stimulated conditions. Secreted renin form proportions are derived from the differences of steady-state matched renal vein and arterial renin form profiles. During acute stimulation of renin secretion (filled bars), the proportions of combined renin forms I and II significantly increased and the proportions of combined renin forms IV and V significantly decreased, compared with basal secretion (open bars). See Table 1 for statistical comparisons.

Renal cortical renin content averaged 667,000 ± 192,000 ng of angiotensin I/g of renal cortex-h of assay incubation under near study-state basal conditions (Table 1). Thus, the RSR under basal conditions represented approximately 0.5% of renin content.

The isoelectric form profile of renin from renal cortical homogenate revealed an overwhelming predominance of the more basic renin isoelectric forms, as shown for a representative homogenate in Figure 3. The renin profile of renal cortical homogenates was assumed to represent that of stored renin under basal conditions. Figure 4 compares the basal secretory renin form profile (as also shown in Figure 2) with the corresponding homogenate renin profile. When compared with the profile of secreted renin under either basal or stimulated conditions, the homogenate contained significantly smaller proportions of the more acidic isoelectric forms of renin and significantly greater proportions of the more basic forms (Figure 4; Table 2).

The renin form profile of renal arterial plasma was assessed as a reflection of the renin form profile of circulating blood under basal and stimulated conditions (Figure 5). Under steady-state basal conditions, forms I and II comprised 53.7% and forms IV and V comprised 29.2% of the active renin present. After secretion stimulation, the near steady-state PRC in
Renin Isoelectric Forms: Storage and Secretion

Figure 3. An example of the isoelectric focusing profile of active renin from a renal homogenate. Renin activity (left axis) is expressed as nanograms of angiotensin I per milliliter of gel elution per hour. The corresponding pH profile of the gel (right axis) is also shown.

Figure 4. Proportions of the five isoelectric renin forms secreted by the kidney under basal conditions (open bars) compared with the same renin forms found in corresponding renal cortical homogenates that were assumed to represent basal state stored renin (filled bars). The proportions of renal cortical homogenate renin forms I and II were significantly increased and the proportions of renal cortical homogenate renin forms IV and V were significantly reduced compared with basal secretion of active renin forms. See Table 2 for statistical comparisons.

Table 3. Proportion of active renin represented by relatively basic renin forms (I and II) and acidic forms (IV and V) for circulating arterial renin under basal and stimulated conditions.

<table>
<thead>
<tr>
<th>Renin Isoelectric Forms</th>
<th>I and II (%)</th>
<th>IV and V (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal (N=9)</td>
<td>53.7 ± 4.0</td>
<td>29.2 ± 3.1</td>
</tr>
<tr>
<td>Stimulated (N=9)</td>
<td>67.7 ± 3.6b</td>
<td>18.5 ± 2.7b</td>
</tr>
</tbody>
</table>

a Values are mean ± SE.
b P < 0.01 by paired t test and Wilcoxon signed ranks test compared with basal conditions.

forms are immediately secreted (not stored) and are relatively unresponsive to secretory stimuli. Circulating PRA is composed primarily of relatively basic renin forms, which increase disproportionately after stimulation of renin secretion.

In this study, active renin in plasma and renal homogenate of rabbits was found to be heterogeneous with regard to net charge. Multiple isoelectric forms of active renin have previously been demonstrated in fish, amphibians, reptiles, birds, and mammals, including humans (4,12,14,17,24–26). As demonstrated in this study (Figure 1), the number of isoelectric forms identifiable in plasma (and presumably renal tissue as well) is a function of the pH gradient of the isoelectric focusing gel.

Previous studies (in rat plasma and kidney slices and the ex vivo human kidney) suggest that the kidney preferentially secretes the more basic isoelectric forms of active renin under basal conditions.

arterial plasma increased twofold (P < 0.015) and the proportion of active renin composed of forms I and II rose to 67.7% (P < 0.01) and that composed of forms IV and V decreased to 18.5% (P < 0.01), as shown in Figure 5 and Tables 1 and 3.

DISCUSSION

This study assessed the participation of active renin isoelectric heterogeneity in renal renin storage, in basal and stimulated renin secretion, and in circulating plasma renin levels. The results indicate that the relatively basic renin forms are preferentially stored and secreted under basal and stimulated conditions. The relatively acidic renin isoelectric
(4,5,7,10-12,15). However, the basal renin release rate of kidney slices is severalfold that of in vivo kidneys, and renin secretion from the ex vivo human kidney may have been altered by the perfusion conditions. Thus, no direct data exist regarding the isoelectric form profile of active renin secreted by the kidney under basal conditions in vivo. In the experimental model used in this study (21), the basal renin secretory rate was approximately 0.5% of renal renin content per hour (Table 1), which is comparable to that observed in vivo in dogs (27). In addition, the preparation used here features intact renin secretory mechanisms (renal baroreceptor, macula densa, sympathetic nerves, angiotensin II feedback). By the use of direct arterial and renal venous sampling in this model, the active renin secreted by the blood-perfused in vivo kidney under basal conditions was disproportionately represented by the relatively basic isoelectric forms, as shown in Tables 1 and 2 and Figure 2.

Acute stimulation of renin secretion in this preparation resulted in a 7.3-fold increase in RSR and a shift in the isoelectric form profile of the active renin secreted by the kidney. The increased secretion of renin was due almost solely to the relatively basic renin forms (I and II) because their combined stimulated secretory rate significantly increased 10-fold over the basal secretory rate, whereas the secretory rates of the more acidic renin forms (IV and V) did not change significantly. Similarly, in renal cortical slices, acute stimulation of renin secretion has also resulted in a shift toward secretion of the more basic isoelectric forms of active renin (7,12). However, this study provides the first direct in vivo evidence that the kidney preferentially secretes the more basic isoelectric forms of active renin in response to acute stimulation of renin secretion.

Previous observations in the dog (17), rat (5), and human (4) have shown that the more basic isoelectric forms of active renin are preferentially cleared by the liver and have the shortest plasma half-lives. Thus, the isoelectric form profile of circulating renin (PRA) represents the net effect of renal secretion and hepatic extraction of each isoelectric form. As in a previous human study (14), the determination of the isoelectric form profile of circulating active renin in this study revealed a predominance of basic isoelectric forms under basal conditions and a disproportionate increase in the more basic isoelectric forms after acute stimulation of renin secretion. Because the liver preferentially removes the more basic forms, however, preferential renal secretion of any isoelectric form would not be necessary to explain the renin profile change in circulating plasma after acute stimulation (5). Direct measurement across the kidney in this experimental model revealed that the kidney did indeed secrete a predominance of the basic isoelectric forms under basal conditions and that stimulated renin secretion was due primarily to a further release of the more basic renin forms. Thus, it is clear that preferential renal secretion of the more basic isoelectric forms contributes to the circulating renin isoelectric form profile and that the circulating profile represents a composite of preferential renal secretion and preferential hepatic degradation of the more basic isoelectric forms. This observation has important physiologic implications because the disproportionate increase in relatively basic circulating renin forms after acute secretory stimulation results in an overall PRA with a shorter half-life and allows fine control of PRA in the acutely stimulated state.

Increasing evidence suggests that at least two pools of active renin are available for release from the kidney (7,8,12,14,27,28). One of these, a storage pool, is released in response to acute stimulation (28). Renin released from the storage pool perhaps participates in a regulated secretory pathway and is composed of the relatively basic renin forms (7,12,14). The second smaller, newly synthesized pool is immediately released, and its secretion does not increase in response to acute stimulation. Renin released from the newly synthesized pool is thought to participate in a nonregulated (constitutive) pathway and is composed of relatively acidic renin forms (7,8,11,28). Under basal conditions in this study, the more acidic isoelectric forms represented 3% of the active renin in renal homogenate (storage pool) and 39% of the active renin secreted by the kidney (Figure 4; Tables 1 and 2). Additionally, we observed a large increase in renal secretion of the more basic isoelectric forms and no significant change in renal secretion of the more acidic forms after acute stimulation of renin secretion. These in vivo data are best explained by the hypothesis that the acidic isoelectric forms of renin represent constitutively secreted renin and the more basic forms represent storage pool renin, which is available for release in response to acute stimulation.

In this study, the renin storage and secretory pools could be distinguished on the basis of differences in isoelectric form profile, although there was considerable overlap. Under basal conditions, the kidney secreted both relatively basic and acidic isoelectric forms of renin. Because renin secretion was not suppressed (by design), the basic isoelectric forms could have originated from the regulated pathway. Alternatively, some of the more basic forms may also participate in a constitutive pathway. However, the precise differences among the forms that affect intracellular sorting of renin remain unknown. The forms differ with respect to charge, but charge may not affect cellular sorting directly. More likely, the sorting signal is sugar residues (glycosylation) because glycosylation is known to affect cellular sorting
of secretory glycoproteins (1). Additionally, at least some of the isoelectric heterogeneity of renin has been previously shown to be due to variable glycosylation (2,3), and hepatic degradation of renin is a function of both glycosylation (9) and isoelectric point (4,5,17). Finally, site-directed mutation of the two amino acids involved in renin N-linked glycosylation (which prevented glycosylation) altered renin sorting into storage and secretory pools (29). Thus, although storage and secretory pools of renin can be distinguished on the basis of isoelectric profile, there is some overlap in profiles probably because isoelectric point is not the exact sorting signal of the juxtaglomerular cell.

In summary, data from this study suggest that the intracellular processing of renin depends, in part, on its heterogeneity of charge because the more acidic isoelectric forms appear to be constitutively secreted and the more basic isoelectric forms are preferentially routed to a regulated storage pool. Although the basal secretion of renin is composed of predominately more basic isoelectric forms with a small constitutive renin form component, acute stimulation of renin secretion results in the preferential release of previously stored relatively basic renin. This contributes to the increased circulating plasma levels of these renin isoelectric forms after acute stimulation and, combined with preferential hepatic extraction of the same forms, controls the duration of the physiologic response to acute renin secretion.

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REFERENCES


The importance and extensive prevalence of that form of disease, which, after it has continued for some time, is attended by the peculiar changes in the structure of the kidney, now pretty generally known by the names of 'mottling,' 'white degeneration,' 'contraction,' or 'granulation,' impresses itself every year more and more deeply on my mind; and whether I turn to the wards of the hospital, or reflect on the experience of private practice, I find, on every side, such examples of its fatal progress and unrelenting ravages, as induce me to consider it amongst the most frequent, as well as the most certain causes of death in some classes of the community, while it is of common occurrence in all; and I believe I speak within bounds, when I state, that not less than five hundred die of it annually in London alone. It is, indeed, an humiliating confession, that, although much attention has been directed to this disease for nearly ten years, . . . . . . . . . . . . . . . . . yet little or nothing has been done towards devising a method of permanent relief . . . . .