Asynchronous Changes in Prorenin and Renin Secretion after Captopril in Patients with Renal Artery Stenosis

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SUMMARY An assay of plasma prorenin was developed in which the conversion to renin occurred under apparently optimal conditions. Some characteristics of the assay were 1) prorenin was activated by Sepharose-bound trypsin at 4°C; 2) the concentration of activator was not critical provided that incubation was prolonged until renin activity had reached a plateau; and 3) this plateau was stable and had the same height as after maximal activation with acid, pepsin, plasmin or urokinase. Maximal activity with Sepharose-bound trypsin at 4°C was higher than with cryoactivation, and optimal conditions were more readily reproduced than with trypsin at 37°C or with acid-activation. The assay was used for measurements in peripheral and renal vein plasma after captopril in hypertensive patients with unilateral renal artery stenosis. Peripheral renin rose within 30 minutes after a first dose of captopril, 50 mg orally, and it remained high with chronic treatment. In contrast, peripheral prorenin fell initially and rose after 4 hours. These changes in peripheral plasma were related to changes in the secretion rates of the two forms of renin from the affected kidney. Thus chronic, but not acute, stimulation of renin release was associated with an increased secretion rate of prorenin. The late rise in prorenin is probably an indication of enhanced synthesis in the kidney, so that more prorenin is available for conversion. The data suggest that prorenin is indeed a biosynthetic precursor of renin and that, at least under certain circumstances, a major proportion of circulating prorenin originates from the kidney. (Hypertension 5: 244–256, 1983)

KEY WORDS • captopril • converting enzyme • prorenin • renal artery stenosis • renin

About 80% of the renin in normal human plasma is thought to circulate in an inactive form.1-3 Inactive renin is often called "prorenin" because it can be converted in vitro to active renin.4 It is not certain that prorenin is a precursor of naturally occurring renin, however.

Activation of the factor XII-kallikrein pathway causes irreversible prorenin-to-renin conversion after dialysis of plasma against acid followed by restoration of pH to neutral (acid-activation)5,6 and possibly also in plasma that is stored at low temperature (cryoactivation).7 Prorenin can also be activated by adding trypsin or pepsin to plasma. These exogenous activators act independently of factor XII and kallikrein.

Prorenin is measured by functional assays in which prorenin is converted to renin. The difference in renin activity before and after activation is taken as a measure. The implicit assumptions are that all prorenin molecules are converted and that one molecule of activated prorenin has the same enzymatic activity as one molecule of naturally occurring renin. Cryoactivation, however, often leads to incomplete prorenin-to-renin conversion.3 Acid activation appears more complete but careful adjustment of pH is of critical importance.11,12 For trypsin activation, high concentrations of trypsin are required to overcome the inhibitors in plasma but such high concentrations may destroy renin.11,12 Trypsin may also attack renin substrate and may interfere with the radioimmunoassay of angiotensin I, the final step in the assay of renin. Soybean trypsin inhibitor (SBTI) has therefore been used to prevent this. However, some commercial SBTI preparations appear to have angiotensinase activity resulting in considerable loss of angiotensin I during the assay.13

The present paper describes an assay of prorenin in which it is activated by trypsin that is bound to Sephar-
molecular weight markers for gel chromatography, ribonuclease A (Mr 13,700), chymotrypsinogen A (Mr 25,000), ovalbumin (Mr 43,000), and human serum albumin (Mr 67,000) were purchased from Pharmacia, Uppsala, Sweden. ^14C-ovalbumin (Mr 46,000) and ^14C-bovine serum albumin (Mr 69,000) were also used as molecular weight markers and were obtained from the Radiochemical Centre, Amersham, England.

**Buffer Solutions**

**Phosphate Buffer, pH 7.5**

This buffer contained 12.2 mM NaH2PO4, 86.7 mM Na2HPO4, 75.9 mM NaCl and 1.0 mM disodium ethylenediamine-tetraacetate (EDTA).

**Glycine/HCl Buffers, pH 3.3 or pH 4.0**

These buffers contained 50 mM glycine, 94.9 mM NaCl, and 5.1 mM EDTA. The pH was adjusted with concentrated HCl.

**Tris/Acetate Buffer, pH 7.5**

This buffer contained 0.1 M Tris, 0.35% bovine serum albumin, 0.1% lysozyme, and 0.2% neomycin sulphate. The pH was adjusted with glacial acetic acid.

**Preparation of Plasma**

Blood was collected in chilled plastic tubes containing EDTA in a final concentration of 5 mM. It was centrifuged at 3000 × g and 4°C immediately after collection. Plasma was kept frozen at −20°C before use.

**Preparation of Immobilized Trypsin and Pepsin**

The enzymes were covalently bound to CNBr-activated Sepharose 4B in a ratio of 30–40 mg of protein per g of dry Sepharose according to the directions of the manufacturer. In this manner more than 97% of protein was bound to Sepharose. Sepharose-bound trypsin was diluted in phosphate buffer pH 7.5. Suspensions of Sepharose-bound pepsin were stored in glycine/HCl buffer pH 3.3.

**Activation of Prorenin**

**Activation by Immobilized Trypsin**

Dilutions of Sepharose-bound trypsin (100 µl, 0.05–4.0 mg trypsin) were added to 1 ml plasma. The mixtures were incubated at 37°C and at 4°C for various time periods as indicated in the results section. Trypsin was removed by centrifugation. The supernatants were checked for amidolytic activity with the chromogenic substrate N-benzoyl-L-isoleucyl-L-glutamyl-L-arginine-p-nitroanilide (S 2222, Kabi, Stockholm, Sweden). For this purpose 0.1 ml of the supernatant of the incubates was mixed with 0.2 mM substrate (about 10 times Km for trypsin) in 0.1 M Tris/HCl buffer pH 8.2 in a total volume of 1.0 ml. The linear release of p-nitroanilide was followed for 1–2 minutes at 405 nm in a 1-cm semi-microcuvette at 37°C. With this method the remaining trypsin activity in the supernatants was found to be less than 0.1% of the original activity in the incubates.
Acid Activation

Plasma samples (2 ml) were dialyzed against glycine/HCl buffer pH 3.3 for 24 h at 4°C, followed by dialysis against phosphate buffer pH 7.5, containing 6% polyvinylpyrrolidone, again for 24 hours at 4°C. Polyvinylpyrrolidone had been added to the buffer to prevent dilution of the plasma due to colloid-osmosis. The dialysis bags were emptied in calibrated plastic tubes and rinsed with phosphate buffer pH 7.5 and the volume was adjusted to 2 ml with the same buffer.

Activation by Immobilized Pepsin

Plasma samples (2 ml) were dialyzed against glycine/HCl buffer pH 3.3 for 24 h at 4°C. To 1 ml of the dialyzed plasma was added Sepharose-bound pepsin (100 μl, 0.3 mg pepsin). The mixture was incubated at 32°C for various time periods, as indicated in the Results. Pepsin was removed by centrifugation and pH was restored to 7.5 with 1 M NaOH.

Activation by Plasmin and Urokinase

Plasmin was dissolved at a concentration of 20 casein units/ml in phosphate buffer pH 7.5. Urokinase was dissolved in this buffer at a concentration of 1000 Ploug units/ml. The plasma samples were dialyzed against glycine/HCl buffer pH 4.0 for 24 hours at 4°C and pH was restored to 7.5 with 1 M NaOH. The activator solutions (100 μl) were added to 1 ml of the pH 4.0-pretreated samples and incubated at 4°C for various time periods as indicated in the Results.

Assay of Naturally Occurring Renin and Prorenin that is Activated In Vitro

For this assay, 0.10–0.25-ml samples were added to 0.5 ml of renin substrate, and the volume was adjusted to 1.0 ml with phosphate buffer pH 7.5. The final concentration of renin substrate in the mixture was 2.5 μM Ile<sup>3</sup>-Al equivalents, which corresponds to about 10 times km (see Results). After addition of protease inhibitors, i.e., 10 μl of 0.34 M 8-hydroxyquinoline, 5 μl of 0.28 M phenylmethylsulphonyl-fluoride in ethanol, and 10 μl aprotinin (10,000 kallikrein-inhibiting units/ml), the mixtures were incubated at 37°C. The incubation time was 3 hours except when stated otherwise. The renin-containing samples had been diluted in such a way that no more than 5% of the substrate was cleaved during incubation. Parallel incubations at 4°C served as blanks. Incubations of dilutions of standard human kidney renin at 37° and 4°C were run in each assay batch. The concentration of homologous substrate in the incubation mixtures was less than 0.2 μM Ile<sup>3</sup>-Al equivalents. Previous studies have shown that this concentration of homologous substrate did not interfere with the reaction of renin and the heterologous substrate. Previous study showed that this concentration of homologous substrate did not interfere with the reaction of renin and the heterologous substrate. The reaction was stopped by adding 1 ml of 0.15 M NaCl followed by heating for 10 minutes in a boiling water bath. The precipitate was removed by centrifugation. The concentration of AI in the supernatant was measured by radioimmunoassay, using <sup>125</sup>I-labeled Ile<sup>3</sup>-Al and rabbit anti-Ile<sup>3</sup>-AI anti-serum. Renin concentration is expressed as micro-units of the renin standard per ml (μU/ml). Prorenin was measured as the difference between the renin concentration after activation of the test sample ("total renin") and the concentration before activation. For routine measurements in plasma, 1.0 ml plasma was incubated with 100 μl Sepharose-bound trypsin in a final concentration of 0.25 mg trypsin/ml for 24 hours at 4°C. The reasons why this procedure was selected are presented and discussed in the results and discussion sections.

Interassay variability was evaluated by weekly measurements of naturally occurring active renin and prorenin in a normal plasma pool (stored at -20°C) during a 9-month period. The mean value of naturally occurring active renin was 27 μU/ml (36 assays) with a standard deviation of 3 μU/ml (coefficient of variation 11%). The mean value of "total renin" was 254 μU/ml, with a standard deviation of 26 μU/ml (coefficient of variation 10%). The mean value of prorenin was 227 μU/ml with a standard deviation of 24 μU/ml (coefficient of variation 11%). In normal plasma the contribution of naturally occurring active renin to total renin is small but it becomes greater after stimulation of renin release. The coefficient of variation of prorenin measurements then also becomes greater since prorenin is taken as the difference between total renin and naturally occurring active renin.

In 17 healthy men (aged 24–45 years) who were recumbent for at least 1 hour before blood sampling, naturally occurring renin had a mean value of 23 μU/ml (antilog of arithmetic mean after logarithmic transformation of data) with a range of 14 to 43 μU/ml. The mean value of prorenin was 196 μU/ml with a range of 138 to 312 μU/ml; the mean value of the proportion of renin that was in the active form was 10.9%, with a range of 4.3% to 17.5%.

Gel Filtration

Untreated plasma (2 ml) or trypsin-activated plasma (2 ml) was applied to 2.6 × 90 cm columns of Sephadex G-100 equilibrated with 0.01 M Tris/HCl buffer pH 7.0 containing 0.15 M NaCl and 1 mM EDTA. The same buffer was used for elution. Flow rate was adjusted to 10 ml/hr and 1.5-ml fractions were collected. The columns were calibrated with ribonuclease A, chymotrypsinogen A, human albumin and ovalbumin. Blue dextran 2000 was used for determining void volume. <sup>14</sup>C-ovalbumin and <sup>14</sup>C-BSA were used as internal standards. Gel filtration was carried out at 4°C.

Affinity Chromatography

Untreated plasma (4 ml) or trypsin-activated plasma (4 ml) was applied to 1.6 × 25 cm columns of Blue Sepharose CL-6B equilibrated with 0.02 M phosphate buffer pH 7.1. Elution was performed with this buffer in 3 steps, i.e., without added NaCl, with 0.2 M NaCl and with 1.4 M NaCl added to the buffer. Flow rate was 50 to 60 ml/hr and 2.5-ml fractions were collected. Affinity chromatography was carried out at 4°C.
Studies in Patients

Fifty-four hypertensive patients were studied after they had given their informed consent. All had unilateral renal artery stenosis as demonstrated by renal arteriography. Treatment was stopped at least 3 weeks before the studies began. The patients were recumbent for at least one hour before blood sampling.

Group 1 (n = 14)
Renal arteriography had already been performed before this study. The patients received a first dose of captopril, 50 mg orally. Peripheral venous blood was sampled from an indwelling needle for renin and prorenin measurements before and at different time intervals after captopril. Renin before captopril was 81 μU/ml (antilog of arithmetic mean after logarithmic transformation of data) with a range of 19 to 250 μU/ml, and prorenin was 190 μU/ml with a range of 77 to 410 μU/ml. The patients were then treated with captopril, 50 mg 3 times a day, and blood was taken after 1, 2, and 4 weeks 1–2 hours after the morning dose.

Group 2 (n = 15)

Blood was sampled from both renal veins and from the abdominal aorta before and 30 minutes after a first dose of captopril, 50 mg, just before renal arteriography. Because some time elapsed between sampling of the renal vein of one side and sampling at the other side, two aortic samples were taken each at exactly the same time that a renal vein sample was collected. Peripheral vein renin before captopril was 51 μU/ml (range 16–190 μU/ml) and prorenin was 170 μU/ml (range 57–320 μU/ml).

Group 3 (n = 15)

Blood was sampled from both renal veins and from the abdominal aorta 16 hours after a first dose of captopril, 50 mg, just before renal arteriography. This time interval was chosen because studies in Group 1 had shown that peripheral prorenin was significantly increased after 16 hours. Peripheral vein renin before captopril was 58 μU/ml (range 15–480 μU/ml) and prorenin was 150 μU/ml (range 20–490 μU/ml).

Group 4 (n = 10)

Blood was sampled from both renal veins and from the abdominal aorta while the patients were taking captopril, 50 mg three times a day, for 2 weeks. Sampling occurred 1–2 hours after the last 50 mg dose and just before renal arteriography. Peripheral vein renin before captopril was 66 μU/ml (range 30–360 μU/ml) and prorenin was 210 μU/ml (range 72–520 μU/ml).

Results

Activation of Plasma Prorenin by Immobilized Trypsin: Selection of Optimal Conditions

Normal plasma pool was incubated at 4°C and 37°C with Sepharose-bound trypsin at concentrations ranging from 0.05 to 0.50 mg per ml of plasma at 4°C and from 0.05 to 4.0 mg/ml at 37°C. The results at 4°C and 37°C were markedly different (fig. 1). First, the reaction velocity was higher at 37°C than at 4°C, but for a given trypsin concentration, the maximum level of renin activity ultimately obtained was higher at 4°C than at 37°C. Second, with trypsin concentrations ranging from 0.12 to 0.50 mg/ml, this maximum of renin activity was independent of trypsin concentration during incubation at 4°C but not at 37°C. Third, very high concentrations of trypsin were required at 37°C for approaching the maximal renin activity obtained at 4°C but these high concentrations also caused inactivation or destruction of renin.

As shown in figure 1, with the use of 0.25 mg trypsin per ml of plasma at 37°C, activation had reached its maximum after 2 hours of incubation, and at that time the renin activity was about half the maximum obtained with the same concentration of trypsin at 4°C. With this concentration of trypsin the generation of active renin at 37°C had stopped because not enough uninhibited trypsin was left. This was demonstrated by the following experiments. Plasma (1 ml) was incubated with Sepharose-bound trypsin (100 μl) in a concentration of 0.25 mg trypsin per ml of plasma for 4 hours at 37°C. After incubation the plasma was separated from the activator by centrifugation. The supernatant was transferred to a new tube and renin was measured; it was 140 μU/ml as compared to 32 μU/ml before incubation with trypsin. The supernatant was mixed with fresh Sepharose-bound trypsin, again in a concentration of 0.25 mg/ml, whereas the trypsin pellet was resuspended in fresh plasma (1 ml). The mixtures were then incubated for 24 hours at 4°C and renin was measured. Newly added trypsin caused further generation of active renin to its maximal value of 250 μU/ml in the supernatant but addition of fresh plasma to the precipitate was without effect. In contrast, at 4°C maximal activation of prorenin was obtained while active trypsin was still present. This was shown as follows: plasma (1 ml) was incubated with Sepharose-bound trypsin (100 μl) in a concentration of 0.25 mg/ml for 24 hours at 4°C. Plasma was then separated from the activator by centrifugation. The supernatant was transferred to a new test tube and renin was measured; it was 240 μU/ml as compared to 34 μU/ml before incubation with trypsin. Fresh plasma (1 ml) was added to the trypsin pellet and mixed. Renin was generated during subsequent incubation at 4°C, whereas no further activation occurred after addition of fresh trypsin to the supernatant. It is therefore very likely that at 4°C all the prorenin that could be converted by trypsin was indeed converted.

Recovery of Prorenin and Renin during Incubation with Immobilized Trypsin

The possibility that some loss of prorenin has occurred during trypsin treatment at 4°C cannot be excluded but the fact that the same maximum of renin activity was reached irrespective of the trypsin concentration in the incubate argues against such a loss of...
prorenin. Renin activity decreased with prolonged incubation with trypsin at 37°C but not at 4°C, thereby indicating that active renin was inactivated or destroyed at 37°C. This was substantiated by the following experiment (fig. 2). Standard human kidney renin (1000 μU/ml) in phosphate buffer pH 7.5 was incubated at 4° and 37°C with Sepharose-bound trypsin in a concentration of 0.25 mg/ml. After various time intervals trypsin was removed by centrifugation, and renin activity in the supernatant was determined. Incubation for 24 hours at 4°C had practically no effect on renin activity but at 37°C renin was rapidly inactivated or destroyed. It should be noted that these incubates are free of trypsin inhibitors, so that the concentration of uninhibited trypsin is higher under these circumstances than when the same quantity of trypsin is added to plasma.

As shown in figure 2, at 37°C some destruction of renin occurred in the absence of trypsin. This may be due to a change in pH; in some examples it rose during 24 hours of incubation at 37°C from 7.4 to maximally 8.1. Renin has been reported to be destroyed at this temperature at alkaline pH.3

Recovery of Angiotensin I in Trypsin-Treated Plasma

Aliquots of normal plasma (1 ml), to which Ile5-Al had been added in a final concentration of 0.04 μM, were incubated for 24 h at 4°C with immobilized trypsin (100 μl) in a concentration of 0.25 mg/ml or with phosphate buffer pH 7.5 (100 μl). After incubation the immobilized trypsin was removed by centrifugation and 0.5 ml of the plasma samples was incubated with 0.5 ml of sheep renin substrate in the presence of protease inhibitors as described in the methods section. The recovery of added Ile5-Al was 98 ± 10.1% in trypsin-treated plasma and 98 ± 9.3% in untreated plasma (mean ± SEM, n = 5).
Figure 3. Comparison between various activation procedures: (a) incubation of a pool of normal plasma with immobilized trypsin (0.25 mg/ml of plasma) at 4°C; (b) dialysis at pH 3.3 followed by dialysis at pH 7.5 both at 4°C; (c) incubation with plasmin (2 casein units/ml) or urokinase (100 Ploug units/ml) at 4°C following dialysis at pH 4.0 and restoration of pH by 1 M NaOH; and (d) incubation with pepsin (0.3 mg/ml) at pH 3.3 and 32°C followed by restoration of pH by 1 M NaOH. Results are the mean of three experiments.

Comparison between Trypsin Activation of Prorenin and other Activation Procedures

The following procedures were compared (fig. 3): 1) incubation of normal plasma pool at 4°C with immobilized trypsin (0.25 mg/ml); 2) dialysis for 24 hours at pH 3.3 and 4°C followed by dialysis at pH 7.5 again at 4°C; 3) dialysis for 24 hours at pH 4.0 and 4°C followed by restoration of pH to 7.5 with 1 M NaOH and incubation at 4°C with plasmin (2 casein units/ml) or with urokinase (100 Ploug units/ml); and 4) dialysis for 24 hours at pH 3.3 and 4°C followed by incubation at 32°C with immobilized pepsin (0.3 mg/ml) and subsequent restoration of pH to 7.5 with 1 M NaOH. The maximum levels of renin activity obtained with each of these procedures appeared not different. This is an indication that all the prorenin that could be converted to active renin by proteolytic attack was indeed converted.

Comparison between Trypsin-Activated Prorenin and Naturally Occurring Renin

Gel Filtration

The eluates were treated with trypsin for measuring prorenin. The conditions were the same as for plasma, i.e., incubation with immobilized trypsin (0.25 mg/ml) for 24 hours at 4°C. We have not rigorously checked whether the conditions chosen for plasma were also appropriate for measuring prorenin in the column eluates. They probably are because, when normal plasma was treated with immobilized trypsin (0.25 mg/ml) for 24 hours at 4°C before it was subjected to chromatography, the quantity of renin in the eluate was the same as when native plasma was applied to the column and the eluate was subsequently treated with trypsin. This quantity was 15 times higher than the quantity of renin that was recovered from the column, when trypsin-treatment had been omitted both before and after chromatography. The factor of 15, as found in the column eluates, was the same as the ratio between total renin and naturally occurring active renin in the plasma itself.

When plasma was activated prior to chromatography, only active renin was recovered from the column, with its peak appearing at the same elution volume as the peak of naturally occurring active renin (fig. 4). Mr of naturally occurring active renin was 49,000 and Mr of prorenin was 56,000 (mean value of three experiments).

Dye-Ligand Affinity Chromatography

The eluates were treated with immobilized trypsin in the same way as after gel filtration. When native plasma was subjected to affinity chromatography, the bulk of active renin passed through the column, while prorenin was eluted with 0.2 M NaCl. The quantity of renin that was recovered after trypsin-treatment of the eluate was about 15 times higher than before trypsin-treatment, which agrees with the ratio between total renin and naturally occurring active renin in the plasma itself. This suggests that the method of trypsin-treat-
Enzyme Kinetics of Naturally Occurring Renin and Prorenin that is Activated by Trypsin

Under the conditions of the renin assay the generation of AI from sheep renin substrate by both forms of renin proceeded linearly with time and was proportional to the concentration of active renin. The activity of human kidney renin (MRC standard) was not influenced by the addition of untreated or trypsin-treated normal plasma, indicating that substances interfering with the reaction between renin and its substrate were absent.

Active renin was isolated from plasma of a patient with hypertension and renal artery stenosis. The concentration of naturally occurring renin in this plasma was very high, 1200 μU/ml. Renin was isolated by Sephadex G-100 gel filtration and further purified by DEAE-Sepharose ion exchange chromatography followed by affinity chromatography on Blue-Sepharose. The preparation was free of renin substrate and prorenin. Prorenin was isolated from normal plasma by Sephadex-G-100 gel filtration followed by chromatography on Blue Sepharose. The preparation was free of renin substrate and renin. Prorenin was then activated with immobilized trypsin as described above. The renin preparations were incubated with sheep renin substrate for 1 hour at 37°C and AI that had been determined was appropriate for measuring prorenin in the column eluates.

When plasma had been activated by trypsin prior to chromatography, all the renin appeared to pass through the column, since no renin was detected both before and after trypsin treatment of the eluates at 0.2 M NaCl and at 1.4 M NaCl (fig. 5).

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When plasma had been activated by trypsin prior to chromatography, all the renin appeared to pass through the column, since no renin was detected both before and after trypsin treatment of the eluates at 0.2 M NaCl and at 1.4 M NaCl (fig. 5).
formed was determined for constructing Lineweaver-Burk plots (fig. 6). Km-values for naturally occurring plasma renin and standard human kidney renin and for plasma prorenin that was activated by trypsin appeared similar; they ranged from 0.21 to 0.28 μM.

**pH-Optimum Curves of Naturally Occurring Renin and Prorenin that is Activated by Trypsin**

The sources of naturally occurring renin and of prorenin that was activated by trypsin were the same as in the previous experiment. The renin preparations and sheep renin substrate were dialyzed for 24 hours at 4°C against phosphate buffers with pH values ranging from 4.5 to 9.0. The renin preparations were then incubated with sheep renin substrate or with human renin substrate at these various pH-values for 1 hour at 37°C. The concentrations of sheep substrate in these incubates was 2.5 μM AL equivalents/ml, and the concentration of human substrate was 0.4 μM AL equivalents/ml. The pH optimum curves of naturally occurring renin and of prorenin that was activated by trypsin appeared not different (fig. 7). The pH-optimum was 7.5 for the reaction with sheep substrate and 5.8 with human substrate.

**Measurements of Plasma Renin and Prorenin in Patients**

**Group 1**

Results are shown in figure 8. Renin in peripheral plasma rose within 30 minutes after the first dose of captopril and reached a peak value after 1–2 hours. It remained high with chronic treatment. Prorenin fell initially (p < 0.05; paired t test) but with long-term treatment it reached a value as high or higher than renin.

**Groups 2, 3, and 4**

Basal values of peripheral vein renin and prorenin were comparable in the three groups (see Methods section). Blood pressure fell from 208 ± 6/112 ± 5 mm Hg to 164 ± 10/94 ± 6 mm Hg 30 minutes after captopril in Group 2. Blood pressure was 211 ± 7/110 ± 6 mm Hg before captopril and 198 ± 8/104 ± 7 mm Hg 16 hours after captopril in Group 3. In Group 4 blood pressure was 202 ± 9/109 ± 4 mm Hg before captopril and 143 ± 6/91 ± 5 mm Hg 1–2 hours after the last dose of captopril. Data collected at the time of renal vein sampling are shown in figure 9 and in table 1. Renin in the renal vein on the affected side, but not contralaterally, was higher than in the aorta before captopril and 30 minutes after a first dose of captopril (p < 0.001, Group 2), and at 16 hours after a first dose of captopril (p < 0.001, Group 3), and also with chronic captopril treatment (p < 0.001, Group 4). Prorenin in the renal vein on the affected side was significantly higher than in the aorta before and 16 hours after a first dose of captopril (p < 0.05 and p < 0.01 in Groups 2 and 3 respectively), and also with chronic captopril treatment (p < 0.01, Group 4). Contralaterally the prorenin levels in the renal vein and aorta were not different neither before captopril nor...
FIGURE 8. Effects of a first dose of captopril, 50 mg orally, and of chronic captopril treatment, 50 mg three times a day, on blood pressure and on renin and prorenin in peripheral plasma. Prorenin at 30 and 60 minutes after the first dose of captopril was significantly below the control value (p < 0.05, paired t test). At 4 and 16 hours it was above control (p < 0.001). During chronic treatment blood was sampled 1 to 2 hours after the morning dose of captopril. Prorenin in these samples was 3 times higher than control (p < 0.001). Values in normal plasma (n = 17) are 23 μU/ml (range 14-43 μU/ml) for renin and 196 μU/ml (range 138-312 μU/ml) for prorenin.

TABLE 1. Renin and Prorenin in Plasma of Renal Vein and Aorta in Patients with Renal Artery Stenosis

<table>
<thead>
<tr>
<th>Patient group*</th>
<th>Study design</th>
<th>Blood sampling time</th>
<th>Aorta</th>
<th>Renal vein</th>
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<th>Renal vein</th>
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<td>Group 2</td>
<td>Captopril 50 mg</td>
<td>Before first dose of captopril</td>
<td>1.73</td>
<td>2.09</td>
<td>1.72</td>
<td>1.79</td>
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<td>Captopril 50 mg</td>
<td>16 hrs after first dose of captopril</td>
<td>2.66</td>
<td>3.12</td>
<td>2.70</td>
<td>2.71</td>
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<tr>
<td>(n = 15)</td>
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<td>30 min after first dose of captopril</td>
<td>0.11</td>
<td>0.13</td>
<td>0.11</td>
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<tr>
<td>Group 4</td>
<td>Captopril 50 mg</td>
<td>1-2 hrs after last dose of captopril</td>
<td>2.02</td>
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*For details see Methods section. †p < 0.05. ‡p < 0.01. §p < 0.001 for difference from 1.00.
**Figure 9.** Effects of a first dose of captopril, 50 mg orally, and of chronic captopril treatment, 50 mg three times a day, on plasma renin and prorenin in the renal vein and the abdominal aorta. Statistics of these data and details on study design are presented in table 1.

**Table 1.** (Continued)

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<th>Aorta</th>
<th>Renal vein</th>
<th>Contralateral</th>
<th>Renal vein-to-aorta ratio of renin</th>
<th>Renal vein-to-aorta ratio of prorenin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>Renal vein</td>
<td>Aorta</td>
<td>Renal vein</td>
<td>Affected side</td>
</tr>
<tr>
<td>2.23</td>
<td>2.26</td>
<td>2.21</td>
<td>2.21</td>
<td>2.38§</td>
</tr>
<tr>
<td>0.04</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.21</td>
</tr>
<tr>
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<td>1.93</td>
<td>2.01</td>
<td>1.89</td>
<td>3.25§</td>
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<td>0.14</td>
<td>0.19</td>
<td>0.50</td>
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<tr>
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<td>2.57</td>
<td>2.41</td>
<td>2.42</td>
<td>2.72§</td>
</tr>
<tr>
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<td>0.13</td>
<td>0.12</td>
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</tr>
<tr>
<td>2.99</td>
<td>3.16</td>
<td>3.01</td>
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<td>3.39§</td>
</tr>
<tr>
<td>0.06</td>
<td>0.06</td>
<td>0.05</td>
<td>0.05</td>
<td>0.48</td>
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</table>
after captopril. High levels of renin were measured in
the aorta 30 min after a single dose of captopril and
with chronic treatment. High levels of prorenin were
measured in the aorta with chronic captopril. This is in
agreement with the measurement of peripheral vein
renin in Group 1.

Discussion

Methodological Aspects

For the results of functional assays of prorenin to be
valid, conversion of the prorenin in the test sample
should be complete and the enzymatic activities of
equimolar quantities of activated prorenin and naturally
occurring renin should not differ. These criteria are
likely to be met when immobilized trypsin is used for
prorenin activation in human plasma under the assay
conditions we have worked out. Several lines of evi-
dence support this conclusion. First, identical results
were obtained with acid-activation, with activation by
pepsin, plasmin, and urokinase and with immobilized
trypsin, provided that optimal conditions were select-
ed. A plateau of maximal renin activity was obtained,
and the level of this plateau had the same height with
each of these procedures.

Second, Km-values and pH-optimum curves for the
reactions of trypsin-activated prorenin and naturally
occurring renin with sheep renin substrate did not ap-
ppear different. Third, the behavior of trypsin-activated
prorenin on Sephadex-G100 and Blue Sepharose chro-
matography columns was similar to that of naturally
occurring renin. The Mr-values of naturally occurring
active renin (49,000) and prorenin (56,000) as estimat-
ed by gel filtration, are in agreement with those report-
ed by others19 M  and confirm that the Mr of these plas-
ma renins is greater than the Mr of human kidney renin
(42,000).

Maximal activation of prorenin was observed, when
plasma was incubated with trypsin at 4°C. Other work-
M ers have incubated at 37°C.14 Our results indicate that it
is difficult, if not impossible, to obtain complete acti-
M ation of prorenin at this temperature. Moreover, the
results at 37°C are strongly dependent on the concen-
tration of trypsin and on the incubation time. At 4°C
these variables are less critical, provided that incuba-
tion is prolonged until renin activity has reached a
plateau. At 37°C a larger proportion of added trypsin is
inhibited by plasma than at 4°C, and at 37°C the re-
M maining uninhibited trypsin causes progressive inacti-
M vation or destruction of renin. Both the inhibition of
trypsin, which leads to incomplete activation of pro-
renin, and the inactivation of renin by trypsin are the
cause of measuring falsely low prorenin values. Low
values have also been obtained after cryoactivation. It
is therefore not surprising that the results of prorenin
measurements reported in the literature are widely
different. A literature search showed that the mea-
sured percentage of renin in normal plasma is highest with cryoactivation (35%–53%),8, 21-23 whereas
as it is intermediate with acid-activation (20%–
50%),1, 2, 10, 11, 13, 22, 24-32 and lowest with trypsin (12%–
34%).3, 12, 14, 33 In our study, 11% of the renin in normal
plasma was in the active form.

The methodological difficulties are amplified when
prorenin is measured after maneuvers that are known
to increase circulating renin. The accuracy of renin
assays expressed as absolute values is inversely corre-
lated to the height of the measured value. Thus, the
higher the renin, the less accurate is its measurement.
This causes problems particularly when the difference
in renin before and after activation of plasma is small.
It explains why there is confusion on whether, after
certain stimuli, increments in circulating renin are
associated with decrements in prorenin.1, 2, 25, 27, 30, 31, 34, 35

Changes in Prorenin after Captopril

As shown by the present study on patients with renal
artery stenosis, stimulation of renin release by angio-
tensin converting enzyme inhibition with captopril
causes a precipitous rise in circulating renin with ini-
tially no change or even a fall in prorenin. Later also
prorenin begins to rise. A transient fall in plasma pro-
renin after captopril has recently been reported36, 37
whereas earlier studies had failed to demonstrate such a
fall.38-40

Before stimulation with captopril we found a renal
vein-to-aorta prorenin ratio of 1.19 ± 0.08 (mean ±
SEM) on the affected side and of 1.04 ± 0.08 contralat-
M erally; the value on the affected side was just signif-
M icantly different from 1.00 (p < 0.05). Also in earlier
studies renal secretion of prorenin was difficult to dem-
M onstrate under basal conditions.2, 11, 25, 27, 41 It has there-
therefore been postulated that prorenin is formed by extrarenal
inactivation of intrarenally produced renin. However,
M  since prorenin appears to have a longer plas-
M ma half-life than renin,11 a relatively low secretion rate
of prorenin may suffice to maintain a relatively high
plasma level. It is possible therefore that the veno-
arterial difference in prorenin across the kidney is often
too small to detect with an assay that has an accuracy
that is not better than 10%.

After acute stimulation with captopril the secretion
of prorenin by the affected kidney was not significantly
increased, despite a tenfold rise in the secretion of
renin. With chronic stimulation however, the veno-
arterial difference in prorenin became large enough to be
easily detectable. Thus it appears that the kidney is
indeed capable of secreting prorenin. This finding is
probably not an artifact since secretion could be dem-
M onstrated on the affected side but not contralaterally.
In fact, our data indicate that the changes in peripheral
prorenin concentration after captopril are a conse-
quence, at least in part, of corresponding changes in
the rate of prorenin secretion from the affected kidney.
Both the concentration of prorenin in peripheral plas-
ma and its secretion are increased with prolonged stimu-
lation of renin release but not with acute stimulation.
An extrarenal source of plasma prorenin, however,
cannot be entirely ignored since low to normal concen-
trations of prorenin are present in the plasma of neph-
rectomized subjects.11, 25, 42
This pattern of changes in renin and prorenin after captopril is similar to that of insulin and proinsulin following an oral glucose load; insulin rises within a few minutes but proinsulin begins to rise not earlier than after 1–2 hours. The late rise in proinsulin is a manifestation of an increased rate of synthesis in the pancreas. More prohormone is then available for conversion to the active hormone before it is released into the circulation. These points of resemblance between the two hormonal systems should not distract from the fact that under normal basal conditions the concentration of insulin in peripheral plasma is 8–9 times higher than that of proinsulin, whereas the reverse is true for renin and prorenin.

Our results do not answer the question whether or not prorenin is a storage form of renin but they do suggest that prorenin enters the circulation either by leakage from the juxtaglomerular cells or by corelease before it is converted to renin. In conclusion, these data indicate that prorenin is indeed a biosynthetic precursor of renin and that under some circumstances, if not mostly, a major proportion of prorenin in the circulation originates from the kidney.

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