Control of enzymatically inactive renin in man under various pathological conditions: implications for the interpretation of renin measurements in peripheral and renal venous plasma

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Summary

1. Human plasma contains two types of renin: one is active in its native form (active renin), the other has renin-like activity after exposure to low pH (inactive renin). Reactions of acid-activated plasma renin and kidney renin with either homologous or heterologous substrate showed identical $K_m$ values.

2. Peripheral venous values for active and inactive renin in essential hypertension ($n = 22$), renovascular hypertension ($n = 14$), primary aldosteronism ($n = 12$), adrenal insufficiency ($n = 6$) and control subjects ($n = 13$) were directly correlated. But the percentage of renin that was active varied widely.

3. After bilateral nephrectomy in 12 patients both active and inactive plasma renin fell, but did not completely disappear. Estimates of half-life in two patients were 30–80 min for active renin and 150–165 min for inactive renin.

4. Renal vein to peripheral vein ratios of active and inactive renin in ten patients with essential hypertension (19 determinations) ranged from 0·96 to 1·60 and from 0·68 to 1·44 respectively with mean values (±SEM) of 1·21 ± 0·04 and 1·06 ± 0·05.

5. The renal vein to peripheral vein ratio of active renin on the affected side in 13 out of 17 patients with renovascular hypertension was above the range found in essential hypertension. Six of them also had an elevated ratio of inactive renin on that side, which indicated renal release of this form of renin into the circulation. But, in contrast to the renal vein to peripheral vein ratio of active renin, the mean value of the ratio of inactive renin on the affected side was not significantly higher than on the contralateral side. The results suggest a renal mechanism not only for controlling the total quantity of circulating renin but also for modulating its degree of activation.

Key words: adrenal insufficiency, anephric subjects, essential hypertension, primary aldosteronism, renin, renovascular hypertension.

Abbreviations: AI, angiotensin I; All, angiotensin II.

Introduction

Techniques for measuring plasma renin are currently a subject of vigorous debate. All methods are based on the generation in vitro of angiotensin I. An essential step, therefore, is the inactivation of plasma angiotensinases. It is further recognized that various factors besides renin (EC 3.4.99.19), such as renin substrate and some inhibitors, may influence the generation of angiotensin (Poulsen, 1973). In principle renin assays can be divided into two categories. In so-called plasma renin activity
methods, angiotensin is generated in the presence of an unknown quantity of endogenous substrate. In plasma renin concentration methods a large excess of exogenous substrate is added to the plasma, in order to attain zero-order kinetics with respect to the substrate concentration. A low pH step is frequently used both for inactivating angiotensinases and for destroying endogenous renin substrate (Brown, Davies, Lever, Robertson & Tree, 1964; Skinner, 1967). However, this procedure may artificially increase the enzymatic activity of plasma renin. From recent studies, there is some evidence that at least two types of renin are present in human plasma (Skinner, Cran, Gibson, Taylor, Walters & Catt, 1975; Derkx, Wenting, Man in 't Veld, Van Gool, Verhoeven & Schalekamp, 1976; Leckie, McConnell, Grant, Morton, Tree & Brown, 1977). One type is enzymatically active at neutral pH (active renin). Another type is not active in its native form, but can be activated by treatment at low pH. There has so far been no proof that this so-called inactive renin is indeed structurally related to active renin, and its pathophysiological significance is also unknown. But obviously its presence in plasma could have important implications for the interpretation of plasma renin measurements. In an attempt to clarify these points, we have studied (1) the activation in vitro of renin at different pH values, (2) the reactions of active renin and acid-treated inactive renin with homologous and heterologous substrates, (3) the enzyme kinetic properties of acid-activated plasma renin and purified renal renin, (4) the occurrence of active and inactive renin in anephric subjects, and (5) the concentrations of active and inactive renin in peripheral and renal venous plasma of normal subjects and patients with various pathological conditions.

Material and methods

Reagents

Glycine/HCl buffers ranging from pH 2.0 to pH 3.5, and citric acid/phosphate buffers ranging from pH 4.0 to pH 8.0 were used for treating plasma in studies on the activation in vitro of renin (Skinner, 1967). For the incubation of renin with substrate a phosphate buffer, pH 7.4, containing disodium EDTA (1 mmol/l) was used. For the radio-immunossay of angiotensin I a Tris/acetate buffer, pH 7.4, was used. It contained 0.1% lysozyme, 0.2% neomycin sulphate and 0.35% bovine serum albumin. For the separation of antibody-bound radioactivity from free radioactivity dextran-coated charcoal was suspended in barbital buffer, pH 7.4.

Asp1-Ile2-angiotensin I (Schwarz–Mann, Orangeburg, N.Y. 10962, U.S.A.) was dissolved in Tris/acetate buffer, pH 7.4, containing 0.35% bovine serum albumin. It was stored at a concentration of 40 nmol/ml at -20°C. The preparation was not pure. Its strength was therefore tested by bioassay in the rat against Asp1-Val3-angiotensin II (Hypertensin, Ciba–Geigy Ltd, Basle, Switzerland). When allowance was made for the impurities present in the angiotensin I standard, as stated by the manufacturer, the pressor activity of both standards on a molar base was found to be equal. This agrees with the results of Tree (1973). Partly purified human kidney renin (MRC standard 68/356) was kindly supplied by the WHO International Laboratory for Biological Standards, National Institute for Biological Standards and Control, Holly Hill, Hampstead, London (Bangham, Robertson, Robertson, Robinson & Tree, 1975). The renin was dissolved in Tris/acetate buffer, pH 7.4, containing 0.35% bovine serum albumin. It could be stored at a concentration of 104 μunits/ml at -20°C for up to 3 months without loss of activity.

Renin substrate was prepared from human, pig, rat or sheep plasma, according to Skinner (1967). Sheep substrate was purified as described by Skinner, Dunn, Mazzetti, Campbell & Fidge (1975). Plasma from anephric subjects who were on chronic intermittent haemodialysis was used for the preparation of human substrate. Pig and sheep substrates were prepared from plasma which was taken 4 days after bilateral nephrectomy. Rat substrate was prepared from plasma which was taken 24 h after bilateral nephrectomy. Angiotensinase activity of the substrates was very low; recovery of added Asp1-Ile2-angiotensin I ranged from 86 to 102%. The substrates showed, however, some endogenous renin activity, as will be described in the Results section.

Radio-iodination of Asp1-Ile2-angiotensin I was performed according to the procedure of Greenwood, Hunter & Glover (1963), with chloramine-T. Free radioactivity was removed by chromatography over a 7 cm x 0.6 cm column of Dowex AG1-X8 ion-exchange resin, 50–100 mesh, acetate form (Bio-Rad Laboratories). The column was pre-washed with acetic acid (0.1 mol/l) containing 0.1% bovine serum albumin. The labelled angiotensin was eluted with the same solution and
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collected in 0.5 ml fractions. Two or three fractions with the highest radioactivity (40–60% of initial radioactivity) were pooled and fractionated over a 90 cm x 1.5 cm column of Bio-Gel P-4 (Bio-Rad Laboratories) with acetic acid (0.05 mol/l) containing 0.1% bovine serum albumin, 0.01% thiomersalate, 0.001% neomycin sulphate and NaCl (0.1 mol/l). The highest fractions of the first peak of radioactivity (23–46% of initial activity) were stored in 0.2 ml portions at -20°C. Specific radioactivity was 330-600 μCi/μg of angiotensin I.

Anti-(angiotensin I) antibodies were raised in rabbits by injections of Asp1-Ile5-angiotensin I-bovine serum albumin complex (Goodfriend, Levine & Fasman, 1964). Antiserum was diluted 1:100 with Tris/acetate buffer, pH 7.4, containing 0.35% bovine serum albumin, and stored at -20°C.

Procedures

Blood was collected in chilled tubes containing disodium EDTA (5 mmol/l) as an anticoagulant. After centrifugation at 4°C, the plasma was stored at -20°C. After thawing, 2 ml of plasma was dialysed for 24 h at 4°C in Visking sacs against glycine/HCl buffers ranging from pH 2.0 to pH 3.5, or in citric acid/phosphate buffers ranging from pH 4.0 to pH 8.0. pH was then restored by dialysis for 24 h at 4°C against phosphate buffer, pH 7.4, containing 6% polyvinylpyrrolidone. The kallikrein inhibitor Trasylol (Bayer), 200 units, and neomycin sulphate, 4 mg, were added to the dialysed plasma, and the volume was restored to 2 ml with phosphate buffer, pH 7.4.

Standard renin or pretreated plasma (0.1–0.5 ml) was adjusted with phosphate buffer, pH 7.4, to a total volume of 0.5 ml, and 0.5 ml of substrate was added. Incubation was performed for 3–24 h in a shaking water bath at 37°C. The incubate was then diluted with 1 ml of NaCl solution (0.15 mol/l), heated for 10 min in a boiling-water bath, and centrifuged at 8000 g for 15 min. The angiotensin I concentration of the supernatant was determined by radioimmunoassay. Parallel incubations of the same quantities of renin and substrate at 4°C served as blanks.

Angiotensin I was measured as described by Stockigt, Collins & Biglieri (1971).

Plasma renin concentration is expressed as μunits of standard human kidney renin (MRC standard 68/356)/ml of plasma (μunits/ml). Plasma renin activity is expressed as nmol of Asp1-Ile5-angiotensin I (AI) generated by 1 ml of plasma during 1 h (nmol of AI h−1 ml−1). Plasma renin substrate concentration is expressed as the maximal quantity of Asp1-Ile5-angiotensin I (nmol) which can be generated by 1 ml of plasma in the presence of a large excess of renin (nmol of AI/ml). Statistical significance was assessed by unpaired t-test.

Patients and control subjects

Patients with the following conditions were studied: essential hypertension (n = 22), renovascular hypertension (n = 17), primary aldosteronism (n = 12), adrenal insufficiency (n = 6) and the anephric state (n = 12, seven males). Seven normal subjects and six normotensive patients with a healing uncomplicated peptic ulcer served as control subjects. Both the patients with essential and renovascular hypertension and the control subjects were hospitalized and had a fixed sodium intake of 50 mmol/day. Renovascular hypertension was diagnosed on the basis of radioisotope renography and arteriography in each case. A diagnosis of primary aldosteronism was made on the basis of hypertension associated with consistent hypokalaemia, high plasma aldosterone (>0.70 nmol/l) and low plasma renin concentration (<40 μunits/ml). The patients with adrenal insufficiency had morning plasma cortisol concentrations below 220 nmol/l, which were unresponsive to synthetic β24-corticotrophin (Synacthen, Ciba, 250 μg intramuscularly). The patients gave their informed consent.

Results

Acid activation of plasma renin

Pooled plasma from normal subjects was treated at different pH values by dialysis against the appropriate buffers for 24 h at 4°C. pH was then restored by dialysis against phosphate buffer (pH 7.4) for 24 h. Portions of pretreated plasma (0.25 ml) were incubated at pH 7.4 with sheep renin substrate (0.548 nmol of AI/ml of incubate) for 3 h at 37°C. Under these circumstances zero-order kinetic conditions with respect to the concentration of renin substrate were approached; substrate concentration exceeded five times the Michaelis–Menten constant for the reaction of renin with sheep substrate, and the percentage of the converted substrate was very low (less than 4%). As judged from the quantity of angiotensin I generated during incubation, lowering the pH from 8.0 to 4.0 during pretreatment of the plasma had
no significant effect on renin activity (Fig. 1). From pH 4·0 to pH 2·5, however, a threefold increase of renin activity was observed. In the pH 2·5–8·0 range, the recovery of purified human kidney renin (MRC standard 68/356), which was added to the plasma before dialysis (50 μunits of standard renin/ml of plasma), was not significantly different from 100%. Below pH 2·5 the apparent renin activity of the plasma declined. This might be caused, at least partly, by destruction of active renin, as was indicated by the reduced recovery of added kidney renin at this low pH. Generation of angiotensin I was maximal after treatment of the plasma at pH 3·3. Renin activation had reached its maximum after 12–16 h of dialysis against a buffer of this pH. This was not only true for normal plasma, but also for plasma with a high renin content from a patient with adrenal insufficiency, as well as for plasma with a low renin content from a patient with an aldosterone-producing adrenal adenoma. Increasing the temperature during pH 3·3 dialysis from 4°C to 37°C did not alter the maximal degree of renin activation. Recovery of standard angiotensin I (0·028 nmol/ml), which was added to the plasma pool after the dialysis steps had been completed, was 99% in plasma treated at pH 3·3, 94% at pH 4·5 and 82% at pH 8·0. Endogenous renin substrate in the pretreated plasma samples was determined by adding 1000 μunits of human kidney renin (MRC standard 68/356) to 0·01 and 0·02 ml portions of the samples after the dialysis steps had been completed. The mixtures, which had a pH of 7·4, were incubated for 3, 6, 12 and 24 h at 37°C. Under these conditions angiotensin I concentration had reached its plateau after 6–12 h of incubation. Acid treatment was not found to have a significant influence on the concentration of endogenous renin substrate as long as pH was above 4·0. Further decrease of pH to 3·5, however, caused a complete destruction of the substrate, which agrees with the results of Skinner (1967).

There is some evidence that an enzyme might be involved in the acid-activation process (Morris & Lumbers, 1972). Thus the degree of activation attained by low pH might not only depend on the quantity of inactive renin available for activation, but also on the concentration of this hypothetical activator. We therefore prepared serial dilutions (v/v) of the plasma pool: plasma/NaCl (0·16 mol/l), 20/0, 18/2, 16/4 etc. to 2/18. The diluted plasma was dialysed against pH 3·3 buffer, and 0·5 ml portions were incubated at pH 7·4 with sheep substrate (0·548 nmol of Al/ml of incubate) for 3 h at 37°C. The dilution of plasma would reduce the concentration of both inactive renin and the activator. Measurements of angiotensin I in these incubates showed that the degree of activation was not altered by diluting the plasma. Thus, under the conditions of this experiment, the activating enzyme, if it exists, did not appear to be a rate-limiting factor.

Reactions of native active plasma renin and acid-activated plasma renin with homologous and heterologous substrates

Pooled plasma from normal subjects was treated at pH 4·5 or at pH 3·3, as described above. After pH had been restored to 7·4, portions of pretreated plasma (0·25 ml) were incubated with sheep renin substrate (0·548 nmol of Al/ml of incubate), pig substrate (0·381 nmol of Al/ml of incubate), rat substrate (0·314 nmol of Al/ml of incubate) or human substrate (0·325 nmol of Al/ml of incubate) for 3 h at 37°C. Similar incubates, in which the pretreated plasma was replaced by phosphate buffer, pH 7·4, were prepared for determining the endogenous renin activity of the substrates. It was found to be 0·21 × 10⁻³ nmol of...
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AI h⁻¹ ml⁻¹ in rat and pig substrates 0·11 × 10⁻³ nmol of AI h⁻¹ ml⁻¹ in human substrate, and 0·07 × 10⁻³ nmol of AI h⁻¹ ml⁻¹ in sheep substrate. With these substrates the quantities of angiotensin I generated by the plasma pool were respectively 3·1, 3·3, 2·3 and 4·1 times larger after pH 3·3 treatment than after pH 4·5 treatment (Fig. 2).

As demonstrated by the acid-activation studies, endogenous substrate is not affected at pH 4·5, whereas it is completely destroyed at pH 3·3. Thus, for pH 4·5-treated plasma, the incubate contained endogenous and exogenous substrate. The presence of homologous substrate might interfere with the reaction between renin and heterologous substrate. We therefore added increasing quantities of pH 4·5-treated plasma from normal subjects or from anephric patients to a fixed quantity of pH 3·3-treated plasma (0·2 ml of plasma pool from normal subjects) or to 10 or 100 μunits of human kidney renin (MRC standard 68/356). The mixtures were then incubated at pH 7·4 with sheep renin substrate (0·548 nmol of Al/ml of incubate) for 3 h at 37°C. Concentrations of human substrate ranged from 0·060 to 0·480 nmol of Al/ml of incubate. The results showed that the presence of endogenous or exogenous human substrate did not influence the generation of angiotensin I from sheep substrate.

Reaction kinetics of acid-activated plasma renin and purified human kidney renin

Fixed quantities of pH 3·3-treated pooled plasma from normal subjects or purified human kidney renin (MRC standard 68/356) were mixed with various quantities of either sheep substrate or human substrate, and incubated at pH 7·4 for 1 h at 37°C. During this period of incubation angiotensin I generation was approximately linear. Results were plotted according to Lineweaver–Burk for calculating the Michaelis–Menten constant (Km). Acid-activated plasma renin and purified human renal renin showed identical Km values, both when reacting with sheep substrate and with human substrate (Fig. 3). The relatively low Km value for the reaction of human renin with sheep substrate will favour zero-order conditions with respect to the substrate concentration. Such conditions are probably not met in the reaction with human substrate.
Concentrations of active and inactive renin in peripheral venous plasma of normal subjects and patients

Plasma samples were treated at pH 4.5 or pH 3.3. Portions of these samples were then incubated at pH 7.4 with sheep substrate (0.548 nmol of AI/ml of incubate) for 3 h at 37°C. The quantity of angiotensin I generated was compared with the production of angiotensin I by human kidney renin (MRC standard 68/356), which was incubated with sheep substrate under the same experimental conditions as the plasma samples. The quantity of angiotensin I produced by pH 4.5 pretreated plasma was used as an index of the concentration of active plasma renin, the quantity of angiotensin I produced by pH 3.3 pretreated plasma as an index of the total concentration of plasma renin. Thus the concentration of inactive renin was calculated by subtracting the value obtained after pH 4.5 treatment from the pH 3.3-treatment value. The inter-assay reproducibility was 8.0% for active renin and 10.6% for inactive renin.

Plasma concentration of active renin in the control subjects averaged 19 μunits/ml; plasma concentration of inactive renin averaged 91 μunits/ml (Fig. 4). Both active and inactive renin were significantly lower in anephric patients ($P < 0.001$). Bilateral nephrectomy in two patients with terminal renal failure caused a rapid fall of both active and inactive renin to very low values, which remained nearly constant after 48 h. In Fig. 5 these have been subtracted from the actual values measured during the first 12 h, in order to correct for renin of extrarenal origin. In this way the approximate half-life values for active and inactive plasma renin were calculated to be 50–80 and 150–165 min respectively. The plasma values of active and inactive renin in untreated patients with primary aldosteronism were as low as in the anephric patients. The ranges of both forms of renin in untreated essential and renovascular hypertension were wider than in the control subjects. The mean active renin value in the present series of patients with essential hypertension was not significantly different from normal, but the mean inactive renin value was lower ($P < 0.005$). Patients with renovascular hypertension had significantly elevated active renin ($P < 0.05$), whereas inactive renin did not significantly differ from normal. In this heterogeneous series of patients inactive renin increased with increasing active renin (Fig. 6a). However, the percentage of inactive renin tended to decrease with increasing active renin. This was also observed in individual patients (Fig. 6b). It should be noted that for a given active renin
value the concentration of inactive renin showed a wide range of values.

Concentrations of active and inactive renin in renal venous plasma

Renal vein catheterization was performed in 24 hypertensive patients who were off treatment for at least 2 weeks. The studies were carried out as part of the diagnostic investigations for renal hypertension in patients who had uncontrollable hypertension or showed a unilateral renal abnormality on intravenous urography. Renal vein sampling was always combined with simultaneous sampling of peripheral venous blood. Arteriography was performed after this procedure. Renovascular hypertension was diagnosed in 14 patients. A diagnosis of essential hypertension was made in the remainder. Thirteen patients had unilateral renal artery stenosis with either a relatively normal kidney (nine patients) or a small contracted kidney on the affected side. One patient had bilateral stenosis. Data obtained during surgery in three additional patients with unilateral renal artery stenosis are also included.

The renal vein to peripheral vein ratios (Fig. 7) of active and inactive renin in essential hypertension ranged from 0.96 to 1.60 and from 0.68 to 1.44 respectively. Mean values in these patients were 1.21 ± SEM 0.04 and 1.06 ± 0.05. In 13 patients with renovascular hypertension the renal vein to peripheral vein ratio of active renin on the
affected side was above the range found in essential hypertension. Six of them also had an elevated ratio of inactive renin on that side, indicating renal release of this form of renin into the circulation. The mean value of the active renin ratio in renovascular hypertension was found to be elevated on the affected side (3.71 ± SEM 0.76) and suppressed on the contralateral side (1.04 ± 0.04), as compared with essential hypertension (P < 0.001 and P < 0.005 respectively; data obtained during surgery not included). The mean values of the inactive renin ratio both on the affected and contralateral side (1.26 ± 0.21 and 1.06 ± 0.05 respectively) were not significantly different from the value in essential hypertension.

Discussion

Assays of inactive and active renin

Acid pretreatment of plasma samples (pH 3.3) from normal subjects and from patients with various disorders resulted in a marked increase of the velocity of angiotensin I formation, when these samples were allowed to react at neutral pH with homologous or sheep, pig and rat renin substrates. For routine purposes sheep substrate was chosen because of the low \(K_m\) value. The apparent increase of renin-like activity cannot be explained by angiotensinase inhibition, since inhibition with EDTA was almost complete when plasma was not treated at this low pH. It is also not due to destruction of a factor which inhibits the reaction of active renin with its substrate, since the recovery of added renin from human kidneys (MRC standard 68/356) was not significantly different from 100%, whether or not plasma was treated at low pH. Exposure of plasma to pH < 4.0 caused a destruction of endogenous renin substrate. The presence of endogenous substrate in plasma treated at higher pH might therefore interfere with the reaction between renin and sheep substrate, which is known to have a high affinity for renin. Such interference, however, could not be demonstrated. Thus the increased renin activity after acid treatment of plasma is not caused by the absence of substrate competition. The effect of dialysis at pH < 4.0 can best be explained by the activation of a non-dialysable plasma factor which is enzymatically not active in its native form.

Our calculation of the plasma concentration of this so-called inactive renin is based on the assumption that renin activity after pH 3.3 treatment of the samples reflects the total quantity of renin present in these samples. Acid activation of inactive renin was found to be maximal at this pH. The activation, however, might not have been complete. Part of the inactive renin could have been destroyed during the activation procedure. These possibilities cannot be excluded as long as inactive renin can only be detected by virtue of its potential enzymatic activity. As compared with other procedures for activating renin, which involve exposure to proteolytic enzymes such as pepsin and trypsin, acidification seems to give identical or even higher results (Morris & Lumbers, 1972; Day & Luetscher, 1975; Cooper, Murray & Osmond, 1977).

Chemical and physiological relationships between inactive and active renin

There is little information on the physico-chemical differences between the two forms of renin. Molecular weight of enzymatically active renal and plasma renin from different species, including man, is known to be about 40 000 daltons (Skeggs, Lentz, Kahn, Levine & Dorer, 1972; Leckie, 1973; Boyd, 1974; Day & Luetscher, 1975; Boyd, 1977). Higher-molecular-weight renin-like factors have been identified in kidney extracts and in plasma both in animals and in man (Leckie, 1973; Boyd, 1974; Day & Luetscher, 1975; Boyd, 1977; Mailing & Poulsen, 1977). Some of these factors can be activated at low pH (Day et al., 1975). Using gel filtration Day & Luetscher (1975) separated a plasma fraction which had a molecular weight of 69 000 and had renin-like activity after exposure to low pH. This fraction was found in patients with renal neoplasia and other types of nephropathy, but not in normal subjects. It differs, therefore, from the inactive form of renin we have studied. Our inactive renin probably corresponds to a plasma fraction recently described by Boyd (1977). Acid activation of this fraction was associated with a reduction in molecular weight from 43 000 to 41 000. The higher-molecular-weight renin-like factors have been described as prorenins, analogous to progastrin and pro-insulin (Day & Luetscher, 1975; Day et al., 1975; Sealey, Moon, Laragh & Alderman, 1976; Malling & Poulsen, 1977; Sealey, Moon, Laragh & Atlas, 1977). But it is also possible that inactive renin is formed by binding of active renin to an inhibitor (Leckie & McConnell, 1975). Whatever the precise chemical difference between plasma renin which is active in its native
form, and renin which can be activated by exposure of plasma to low pH, the results of the present study indeed support the hypothesis that these forms of renin are chemically and physiologically closely related. Acid-treated inactive plasma renin showed similar reaction kinetics as purified renin from human kidneys (MRC standard 68/356); $K_m$ values of both forms of renin for the reaction with either human substrate or sheep substrate were identical. The plasma concentration of inactive renin appears to be regulated by factors which are also known to be involved in the control of active renin, for instance sodium balance, posture, $\beta$-adrenergic activity and renovascular abnormalities. Under steady-state conditions the plasma values for the two forms of renin were found to be significantly correlated (Derlx et al., 1976). Our results further show that the amount of plasma inactive renin largely depends on the presence of the kidney. Nephrectomy was found to result in a fall of inactive and active plasma renin to very low values. The renal vein renin determinations indeed indicate that the kidney can release inactive renin into the circulation.

Determinations of the rate of renin decrease after bilateral nephrectomy gave a higher estimate of half-life for inactive renin than for active renin (150–165 and 50–80 min respectively). The renin half-life values reported in the literature range from about 10 to 165 min (Brown, Curtis, Lever, Robertson, De Wardener & Wing, 1969; Devaux, Meyer, Idatte & Milliez, 1969; Hannon, Deruyck, Joossens & Amery, 1969). This wide range can be explained, at least partly, by differences in the methods used for measuring renin. Relatively low values have been obtained by those authors who measured plasma renin without prior acidification (Devaux et al., 1969; Hannon et al., 1969). These methods probably give an index of active rather than inactive renin. The highest half-life values have been obtained by Brown et al. (1969), who included a low-pH step in their method, thereby measuring inactive as well as active renin. Although generally under steady-state conditions a low plasma concentration of inactive renin is associated with low active renin, there are some exceptions. For instance, in a previous study we found active but not inactive renin to be suppressed during chronic $\beta$-adrenergoreceptor blockade in most patients receiving propranolol (Derlx et al., 1976). This agrees with observations made by Amery, Lijnen, Fagard & Reybrouck (1976) on the effect of another $\beta$-adrenergoreceptor-blocking drug, metoprolol, on renin. This drug caused a decrease of renin activity (measured after pH 4.5 treatment of plasma) but not of renin concentration (measured after pH 3.3 treatment). As described in a previous report the reverse had been observed during acute stimulation of renin release; $\beta$-adrenergic stimulation by isoprenaline, the upright posture, or vasodilatation by diazoxide caused a rise of active renin, whilst inactive renin fell or did not change (Derlx et al., 1976). The renal vein renin measurements also indicated that a dissociation of the renal release of active renin and the release of inactive renin can occur. In patients with renovascular hypertension the renal vein to peripheral vein ratio for active renin on the affected but not on the unaffected side was higher than for inactive renin. This may be caused by selectively increased release of active renin on that side.

**Renin activity versus renin concentration**

It is still common practice to distinguish plasma renin activity from renin concentration, depending on whether endogenous or exogenous substrate is used for measuring renin. But now this terminology does not seem to be appropriate. Some plasma renin concentration methods differ from activity assays not only because exogenous substrate has been used but also because inactive renin has been artificially activated. Plasma renin concentration is then a measure of the sum of active and inactive renin, and may be called total plasma renin concentration (TPRC). Under various circumstances TPRC is not a good index for the concentration of circulating active renin. The present study clearly shows that the percentage of total renin which is active differs in different clinical conditions and, as discussed before, this percentage changes during suppression or stimulation of renin release. It is also clear that criteria for renal vein renin ratios, which are frequently used in the diagnostic evaluation of patients with renal artery stenosis, must depend on whether total renin or active renin is measured. Plasma renin activity might be a better guide for diagnostic purposes than TPRC, but we have preferred to measure the plasma concentration of active renin, since the result does not depend on endogenous renin substrate concentration.

Thus in summary the discovery of an inactive form of renin in human plasma has altered current concepts on the methodological aspects of plasma renin assays, which are used in clinical laboratories. This has some implications for the interpretation of their results. Perhaps more intrigu-
ing is the evidence provided by this study that the control of inactive renin might be linked to active renin in a homeostatic physiological mechanism.

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References


