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Improved Immunoradiometric Assay for Plasma Renin

Jaap Deinum,* Frans H.M. Derkx, and Maarten A.D.H. Schalekamp

Background: **Our renin IRMA overestimated renin in plasmas with high prorenin-to-renin ratios. We suspected that the overestimation of renin was caused less by cross-reactivity of the renin-specific antibody with prorenin than by a conformational change of prorenin into an enzymatically active form during the assay.**
Methods: **Because the inactive form of prorenin converts slowly into an active form at low temperature, we raised the assay temperature from 22 °C to 37 °C, simultaneously shortening the incubation time from 24 to 6 h. The former IRMA was performed in <1 working day with these modifications.**
Results: **The comeasurement of prorenin as renin was eliminated. Reagents were stable at 37 °C, and the new and old IRMAs were comparable in terms of precision and accuracy. The functional lower limit of the assay (4 mU/L) was below the lower reference limit (9 mU/L). The modified IRMA agreed closely with the activities measured with an enzyme-kinetic assay. Results were not influenced by the plasma concentration of angiotensinogen. At normal angiotensinogen concentrations, the IRMA closely correlated with the classical enzyme-kinetic assay of plasma renin activity.**
Conclusion: **The modified IRMA, performed at 37 °C, avoids interference by prorenin while retaining the desirable analytical characteristics of the older IRMA and requiring less time.**

The aspartic protease renin is rate-determining in the proteolytic cascade that leads to generation of angiotensin (Ang) II,[1] a key factor in the regulation of blood pressure and the maintenance of water and salt homeostasis *(1)*. Renin is produced exclusively in the kidney from its enzymatically inactive precursor, prorenin. Prorenin is produced not only in the kidney, but also in other organs, such as the adrenal gland *(2)*, the ovary *(3)*, the testis *(4)*, and the uteroplacental unit *(5)*. Its secretion into the blood is continuous, in contrast to the tightly controlled release of renin. The concentration of prorenin in nondiseased plasma is approximately ninefold higher than that of renin and is much less subject to short-term fluctuations than renin *(6)*.

Enzyme-kinetic measurements of the so-called plasma renin activity (PRA) are often used to assess the in vivo activity of the renin-angiotensin system, rather than the difficult and labor-intensive measurement of Ang II. In the PRA assay, plasma is incubated at 37 °C. During this incubation, renin acts on an endogenous renin substrate, angiotensinogen, to generate Ang I. The degradation of Ang I and its conversion to Ang II are blocked by protease inhibitors that have been added to the plasma. The Ang I generated in vitro is quantified by radioimmunoassay. Because angiotensinogen circulates at a concentration roughly equal to the K_m, the rate of Ang I generation in the PRA assay depends as much on the concentration of renin as on that of angiotensinogen. Plasma angiotensinogen concentrations may vary; therefore, PRA is not always a good measure of renin release by the kidney. To overcome this problem, exogenous angiotensinogen can be added to the incubation mixture at a saturating concentration. This is the principle of the enzyme-kinetic plasma renin concentration (PRC) assay.

Recently, our group described the clinical validation of an IRMA for plasma renin *(7)*. This assay measures renin directly, is better to standardize, and is less labor-intensive than the classical enzyme-kinetic PRA and PRC assays. Our report, however, sparked a polemic on the specificity of the new assay *(8–10)* because in the IRMA some prorenin is measured as renin, and therefore, the reliability of the assay at low renin concentrations might be insufficient. We therefore set out to improve our method to minimize the comeasurement of prorenin.

We hypothesized that the problem was caused less by cross-reactivity of the renin-specific antibody with native, enzymatically inactive prorenin than by inadvertent activation of prorenin during the assay procedure. It is

Department of Internal Medicine I, University Hospital Dijkzigt, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands.
*Author for correspondence. Fax 31 10 4634531; e-mail deinum@inw1.azr.nl.
[1] Nonstandard abbreviations: Ang, angiotensin; PRA, plasma renin activity; PRC, plasma renin concentration; mAb, monoclonal antibody; IRP, International Reference Preparation; and cpm, counts per minute.
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known that prorenin spontaneously undergoes a conformational change by which it becomes enzymatically active (active prorenin). This change does not require the proteolytic cleavage of the prosegment and does not necessarily lead to generation of renin. The nonproteolytic activation of prorenin is time- and temperature-dependent. Activation occurs mainly at low temperature (4 °C) *(11)*. Our original IRMA was performed at room temperature. Spontaneous activation of prorenin over time has been detected at room temperature, but is virtually absent at 37 °C *(12)*. We therefore attempted to prevent the inadvertent activation of prorenin by shortening the incubation time of the assay and raising the incubation temperature to 37 °C.

Materials and Methods

ANTIBODIES

The monoclonal antibodies (mAbs) R3-36-16 and R1-20-5 against renin and prorenin were purchased in a kit from Nichols Diagnostics. Briefly, the primary mAb, R3-36-16, is a biotinylated monoclonal mouse anti-human antibody that binds renin as well as active and inactive prorenin. The secondary mAb, R1-20-5, is radio-iodinated (^{125}I) and binds only renin and active prorenin. Detailed information on the antibodies is given in Ref. *(13)*. mAb F258-37-B1 was provided by Dr. S. Mathews (Hoffmann-La Roche, Basle, Switzerland) and was elicited against the C-terminal portion of the propeptide of prorenin. The epitope of this region of the propeptide is hidden in native, enzymatically inactive prorenin, but is expressed on the active form of prorenin *(14)*. Like mAb R3-36-16, mAb F258-37-B1 was biotinylated *(15)*.

AVIDIN-COATED PLASTIC BEADS, WASHING BUFFER, AND SHEEP SERUM

Biotinylated bovine serum albumin was coupled to 8-mm diameter polystyrene beads (Precision Plastic Balls), after which the beads were coated with avidin *(15)* and stored at 4 °C. The washing buffer, phosphate-buffered saline containing 0.1 mL/L Triton X-100, was provided by Nichols Diagnostics. Serum from healthy sheep was heat inactivated for 1 h at 56 °C.

RENIN CALIBRATORS

Recombinant human renin was provided by Nichols Diagnostics. We calibrated this renin preparation against the WHO human kidney renin standard, International Reference Preparation (IRP), lot no. 68/356 (National Institute for Biologic Standards and Control, Potters Bar, Hertfordshire, UK) *(16)* in the enzyme-kinetic PRC assay (see below). Serial dilutions of human recombinant renin to be used for constructing the calibration curve were made up in undiluted sheep serum. The sheep serum content of the renin calibrators was, therefore, near 100%, contrary to what was stated in our previous report *(7)* on a renin IRMA. The dilutions of the renin calibrators were then lyophilized and stored at 4 °C. Before assay, the lyophilisates were reconstituted in distilled water.

RENIN INHIBITOR

Remikiren, an active site-directed renin inhibitor, was obtained from Hoffmann-La Roche *(17)*. Remikiren is a nonpeptide transition-state analog with a M_r of 726. The IC_{50} for purified human renin is 0.7×10^{-9} mol/L, and the K_i for the reaction with human renin is 3×10^{-10} mol/L *(17)*. Only freshly prepared solutions of remikiren were used.

ANGIOTENSINOGEN

Angiotensinogen was prepared from the plasma of nephrectomized sheep *(18)*. The partially purified preparation was dialyzed against 0.15 mol/L phosphate-buffered saline, pH 7.4, and neomycin sulfate was added (final concentration, 2 g/L). The final preparation had an angiotensinogen concentration of 1.2×10^{-6} mol/L, determined as described below.

NONPROTEOLYTIC ACTIVATION OF PRORENIN

Prorenin was treated with the renin inhibitor remikiren to change the molecular conformation from an inactive form into a form that is recognized by the mAb specific for renin *(19)*. We refer to this remikiren-induced conformational change of prorenin as "activation", despite the fact that the prorenin-remikiren complex lacks enzymatic activity. To activate all the prorenin in plasma, we added 1 volume of remikiren (10^{-3} mol/L) to 10 volumes of plasma and incubated the mixture for 24 h at 4 °C *(19)*.

ASSAYS

Enzyme-kinetic assays of renin. To measure PRC, we used our in-house assay, as described previously *(20, 21)*. In short, we added 250-μL aliquots of sheep angiotensinogen solution to 25- to 250-μL aliquots of plasma and adjusted the volume to 500 μL with 0.15 mol/L phosphate-buffered saline, pH 7.4, and added 17.5 μL of a protease inhibitor solution consisting of two volumes of 0.34 mol/L 8-hydroxyquinoline sulfate, one volume of 0.287 mol/L phenylmethylsulfonyl fluoride in ethanol, two volumes of 0.5 mol/L disodium EDTA, and two volumes of aprotinin (10 000 kallikrein-inhibiting units/mL). We incubated the mixture at 37 °C for at least two different time periods between 0.25 and 3 h to verify linear Ang I generation. The final concentration of sheep angiotensinogen in the incubation mixture was 6×10^{-7} mol/L (K_m, 2×10^{-7} mol/L). Only incubations in which <5% of the angiotensinogen was hydrolyzed were accepted for calculating the concentration of naturally occurring renin. Parallel incubations at 0 °C served as blanks. The generated Ang I was measured by RIA *(22)*. Results of the PRC measurements were expressed as mU/L, as calibrated with the international renin standard. Under the conditions of the assay, 1 mU of renin

generated 163 pmol (212 ng) of Ang I per hour. The lower limit of detection was 1 mU/L.

For the PRA assay, we used a modification of the method proposed by Sealey *(23)*. The method was modified to include blank subtraction, and the 18-h incubation for low renin samples was eliminated. In this assay, 50 μL of maleic acid, pH 5.7, and 12.5 μL of a protease inhibitor solution consisting of one volume of 0.287 mol/L phenylmethylsulfonyl fluoride in ethanol, two volumes of 0.5 mol/L disodium EDTA, and two volumes of 100 g/L neomycin sulfate were added to 0.5 mL of plasma. The mixture was then incubated at 37 °C for at least two time periods between 0.5 and 3 h to check for linear generation of Ang I. The generated Ang I was quantified by RIA *(22)*. Results were expressed as nmol Ang I/L per hour. The lower limit of detection was 0.08 nmol Ang I/L per hour.

Assay of angiotensinogen. The concentration of angiotensinogen in plasma was determined as the maximal quantity of Ang I that was generated during incubation at pH 7.4 and 37 °C of plasma to which recombinant human renin had been added at a final activity of 20 000 mU/L. The results were expressed as nmol/L *(21)*.

IRMAs. To 200-μL aliquots of untreated plasma, remikiren-treated plasma, or renin calibrators, we added 100 μL of a 1:1 mixture, by volume, of biotinylated mAb R3-36-16 (0.5 mg/L) and radiolabeled [~250 000 counts per minute (cpm)] mAb R1-20-5. We incubated this mixture with a polystyrene, avidin-coated bead in duplicate for either 24 h at room temperature (IRMA 22 °C, 24 h) or for 6 h at 37 °C (IRMA 37 °C, 6 h). After incubation, we washed the beads three times and then transferred the washed beads to clean tubes. The radioactivity of the bound antibody was counted for 5 min in a gamma counter. The assay measures renin in the untreated plasmas and renin plus prorenin in the remikiren-treated plasmas. The difference between the results of the two measurements was the prorenin.

To test our hypothesis that comeasurement of prorenin as renin was caused by a conformational change in the prorenin, we also performed another, two-step sandwich assay. This assay was a modification of an assay we described earlier *(14)* and measured prorenin directly after remikiren activation. In this assay, an avidin-coated bead carrying 1 μg of biotinylated F258-37-B1 was incubated for 6 h at 37 °C or for 24 h at 22 °C with plasma from a patient with high plasma prorenin (prorenin, 2757 mU/L; renin, 53.1 mU/L). The F258-37-B1-coated bead bound the exposed propeptide of the active form of prorenin *(14)*, but did not bind renin or native, inactive prorenin. The half-time of binding of active prorenin to the bead at 37 °C was ~12 min. After incubation, we rinsed the bead three times with 2 mL of phosphate-buffered saline containing 1 g/L bovine serum albumin to remove all renin and inactive prorenin. We then incubated the bead with sheep serum containing remikiren (10^{-4} mol/L) and radiolabeled R1-20-5 for 24 h at 22 °C. The tracer antibody bound the active prorenin trapped on the bead. We added remikiren in this second step to ensure that any active prorenin captured on the bead in the first step was kept in its active conformation. After incubation, we washed the beads with 3 × 2 mL of washing buffer and counted bound radioactivity after the bead was transferred to a clean tube. A standard line of remikiren-treated recombinant human prorenin in sheep serum was used *(14)*. The lower limit of detection of this assay was ~10 mU/L active prorenin. The interassay CV at <100 mU/L active prorenin was ~10%.

COLLECTION OF PLASMA SAMPLES

Plasma samples were from healthy subjects, patients with essential hypertension either not receiving therapy or receiving enalapril, patients with renovascular hypertension either not receiving therapy or receiving enalapril, patients with hepatic cirrhosis, and patients with primary aldosteronism (Conn syndrome). Individuals with conditions where a high prorenin-to-renin ratio is not unusual, i.e., pregnant women, women with ovarian hyperstimulation, women with preeclampsia, women taking oral contraceptives, and patients with diabetes mellitus, were also studied. Blood sampling was performed according to our in-house protocol. Blood samples were collected from subjects after cannulation of an antecubital vein and 30–45 min of supine rest. The samples were collected into tubes containing 0.2 mL of 0.646 mol/L citrate per 10 mL of blood and centrifuged immediately at room temperature. The plasma was stored at −20 °C. Shortly before each assay, plasma samples were thawed rapidly and kept at room temperature.

Results

PERFORMANCE CHARACTERISTICS OF THE RENIN IRMA AT 37 °C

Calibration curve. Fig. 1 depicts a comparison of the calibration curve of IRMA 37 °C, 6 h with the calibration curves of IRMA 37 °C, 24 h and IRMA 22 °C, 24 h. The results, expressed as cpm, were linear over a wide range of renin concentrations. IRMA 37 °C, 24 h and IRMA 22 °C, 24 h produced identical results. It appears, therefore, that the reagents were stable at 37 °C. The calibration curve of IRMA 37 °C, 6 h was parallel to those of the 24-h IRMAs, but the values were 25% lower. This difference was fully explained by the first-order rate constant for the binding of the radiolabeled mAb to renin at 37 °C (0.23 h^{-1}; data not shown). For practical purposes, we chose IRMA 37 °C, 6 h instead of IRMA 37 °C, 24 h as our routine assay, although the quantity of radiolabeled mAb trapped by renin was somewhat lower after 6 h than after 24 h.

We tested the linearity of the assay by preparing dilutions of plasma samples with 5.7–2106 mU/L renin. Dilutions were made up in the same matrix, i.e., sheep serum, that was used for the calibration curve. Plots of the results, expressed as mU/L, against the dilution factor gave straight lines with the correct slope (Fig. 2).

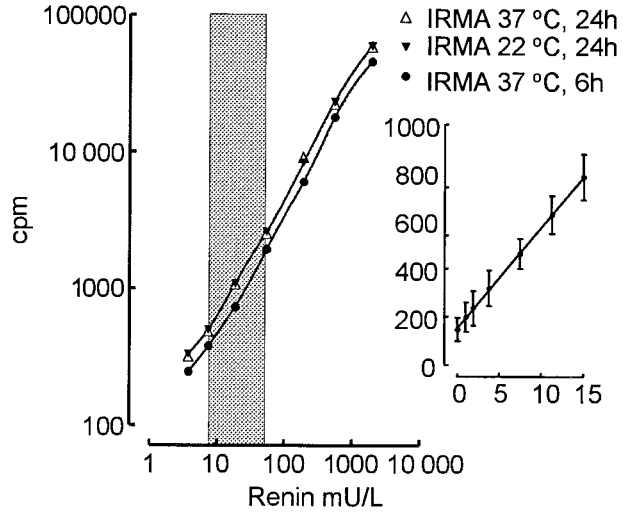

Fig. 1. Calibration curves for the IRMAs.

Ordinate, total binding of labeled antibody. *Abscissa*, renin activity. The *shaded area* represents the reference interval. *Inset*, low range of the calibration curve for IRMA 37 °C, 6 h (mean ± 3 SD).

Analytical recovery. The human kidney renin IRP was added to plasma samples containing 7.4–155 mU/L renin. As shown in Table 1, IRMA 37 °C, 6 h demonstrates excellent analytical recovery of both low and high quantities of added renin. The mAbs we used are capable of inhibiting the Ang I-generating activity of renin *(13)*. To investigate whether angiotensinogen might interfere with the binding of renin to these antibodies, we added the human kidney renin IRP to a plasma obtained from a pregnant woman. This plasma (plasma D in Table 1) had an angiotensinogen concentration of 3208 nmol/L, which is approximately threefold higher than the reference value. Angiotensinogen at this high concentration did not influence the results of IRMA 37 °C, 6 h (see Table 1).

Table 1. Recovery of human kidney renin added to plasma.

Plasma	Renin added, mU/L	Renin concentration before addition of renin, mU/L	Renin concentration after addition of renin, mU/L	Recovery of added renin, %
A	10	7.4	17.1	97
B	20	10.1	30.2	102
C	50	24.5	75.6	102
D	50	25.1	75.6	101
E	50	28.1	80.1	107
F	10	37.0	46.5	95
G	20	42.3	61.9	98
H	70	53.1	115.4	89
H	130	53.1	203.9	116
I	130	155	265.5	85
I	260	155	446	110

Assay imprecision and detection limits. As shown in Fig. 1, the detection limit of IRMA 37 °C, 6 h, defined as 3 SD above the zero calibrator, was 1.3 mU/L. The radioactivity of the zero calibrator of this calibration curve was ~150 cpm, and that of the 4 mU/L calibrator was ~300 cpm. Thus, the result for the calibrator was close to the blank. This raised concern about the reliability of the IRMA renin measurements at the lower end of the concentration range. This issue is addressed in Fig. 3, which shows the results, expressed as cpm, for 4 mU/L human recombinant renin and for nonspecific binding (blank) in 32 individual assays. Fig. 3 shows adequate separation between the 4 mU/L calibrator and the blank in each of

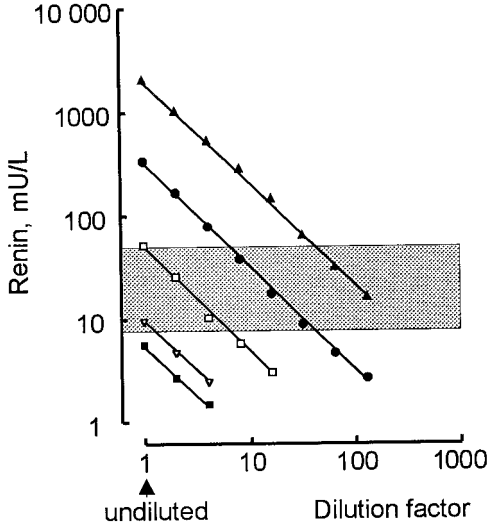

Fig. 2. Plasma dilution tests for plasmas from a patient with renal artery stenosis treated with enalapril (▲), a patient with untreated renal artery stenosis (●), a pregnant woman (□), a patient with low-renin hypertension (▽), and a patient with Conn syndrome (■).

Ordinate, results obtained with IRMA 37 °C, 6 h. The *shaded area* represents the reference interval.

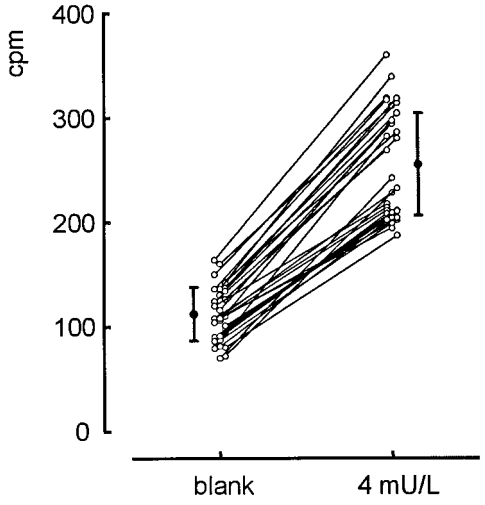

Fig. 3. Difference between the blank (nonspecific binding) and 4 mU/L renin calibrator in all assays (IRMA 37 °C, 6 h) performed from January 1, 1998, to September 1, 1998.

Ordinate, radioactivity bound to the bead. The mean (± SD) nonspecific binding is shown on the *left*; the mean (± SD) binding of the 4 mU/L calibrator is shown on the *right*.

Table 2. Intra- and interassay CVs determined in plasma samples.

	Intrassay CV, % n = 6			Interassay CV, % n = 10		
Renin,[a] mU/L	IRMA 22 °C, 24 h	IRMA 37 °C, 6 h	Enzyme-kinetic PRC assay	IRMA 22 °C, 24 h	IRMA 37 °C, 6 h	Enzyme-kinetic PRC assay
3.6	11	9.5	8.2	25	21	14
7.3	5.9	7.6	6.2	13	16	9.6
34.3	4.9	3.9	3.6	6.6	5.2	5.1
154	3.9	3.8	3.7	5.7	5.3	4.6

[a] Measured with enzyme-kinetic PRC assay.

the 32 assays. The variability of the duplicate blanks or the duplicate 4 mU/L calibrators was low. The difference between the duplicate blanks, given as median (range), was 13.5 cpm (1–38 cpm), or 11% (1–35%) of the mean blank (n = 32). The difference between the duplicate 4 mU/L calibrators was 14 cpm (1–32 cpm), or 6% (0–15%) of specific binding.

The intra- and interassay CVs for IRMA 37 °C, 6 h; IRMA 22 °C, 24 h; and the enzyme-kinetic PRC assay at low, medium, and high renin activities in plasma are compared in Table 2. The intraassay CVs were similar for the three assays. The interassay CVs for the plasmas with low renin activity were, however, higher for the IRMAs than for the enzyme-kinetic PRC assay. The "functional sensitivity", i.e., the minimum renin activity that could be measured from assay to assay with <20% CV, was ~4 mU/L (see Table 2).

COMEASUREMENT OF PRORENIN

The results for a plasma pool from healthy subjects measured with IRMA 37 °C, 6 h; IRMA 22 °C, 24 h; and the enzyme-kinetic PRC assay are compared in Table 3. The renin activity in the plasma pool measured with IRMA 37 °C, 6 h was equal to that measured with the enzyme-kinetic PRC assay and was ~25% lower than the activity measured with IRMA 22 °C, 24 h. In contrast, the results obtained with IRMA 37 °C, 6 h and with the enzyme-kinetic PRC assay in a dilution of the human kidney renin IRP in sheep serum were not different from the results obtained with IRMA 22 °C, 24 h. This is an indication that prorenin interferes with the measurement of renin in IRMA 22 °C, 24 h. To further demonstrate that a conformational change in prorenin at 22 °C is the source of renin overestimation, we also used a two-step IRMA (at 22 and 37 °C) that measures the active conformation of prorenin. When the first step of the assay incubation was performed at 37 °C for 6 h, very little active prorenin was measured in the second step (18 mU/L, <1% of total prorenin and close to the detection limit of the assay). In contrast, when the same plasma was incubated with the same beads at 22 °C for 24 h, the active prorenin measured in the second step was 149 mU/L, i.e., 5.4% of total prorenin. This indicates that in the course of incubation, prorenin is activated and then captured by the F258-37-B1-coated bead at 22 °C, but not at 37 °C. The results were the same when the assay was repeated with the same plasma, and similar results were observed for three other plasmas with high prorenin. Activation of prorenin at 22 °C, but not at 37 °C, also explains the results presented in Fig. 4, in which we compare the results obtained with IRMA 22 °C, 24 h and IRMA 37 °C, 6 h with the results obtained with the enzyme-kinetic PRC assay for plasmas having different prorenin-to-renin ratios. Overestimation of renin was frequent in IRMA 22 °C, 24 h, and was most pronounced at high prorenin-to-renin ratios (Fig. 4, left-hand plot). Overestimation of renin was not seen in IRMA 37 °C, 6 h. Fig. 5 shows a Bland-Altman plot comparing IRMA 37 °C, 6 h with the enzyme-kinetic PRC assay. Because the renin activity ranged over three orders of magnitude and the differences were proportional to the activity, the mean of the two assays was log-transformed and the difference was corrected for the renin activity. The plot demonstrates the good agreement between IRMA 37 °C, 6 h and the enzyme-kinetic PRC assay for the entire range of renin activity in plasma.

In Table 4, the results presented in Figs. 4 and 5 are grouped according to diagnostic categories. Table 4 also compares the three types of renin measurement (IRMA 22 °C, 24 h; IRMA 37 °C, 6 h; and enzyme-kinetic PRC assay) with the measurement of PRA in different patient groups. The percentage of overestimation that was seen in IRMA 22 °C, 24 h was most pronounced in patients with diabetes mellitus, in pregnant women with or without preeclampsia, and in gonadotropin-treated women. These were the patient groups with the highest prorenin-to-renin ratio. IRMA 22 °C, 24 h did not overestimate renin in patients with renovascular hypertension treated with enalapril and patients with liver cirrhosis. In these patient groups, the prorenin-to-renin ratio was abnormally low.

Table 3. Renin concentration[a] determined in a pool of human plasma and in a dilution of human kidney renin in sheep serum.

	IRMA 22 °C, 24 h, mU/L	IRMA 37 °C, 6 h, mU/L	Enzyme-kinetic PRC assay, mU/L
Plasma pool	30.6 ± 3.6	24.5 ± 1.5	25.1 ± 1.6
Kidney renin	50.2 ± 2.9	51.1 ± 2.8	50.0 ± 1.5

[a] Values are means ± SD, n = 3. Plasma samples from eight healthy subjects were pooled; the prorenin concentration of this pool was 238 ± 13 mU/L (IRMA 37 °C, 6 h).

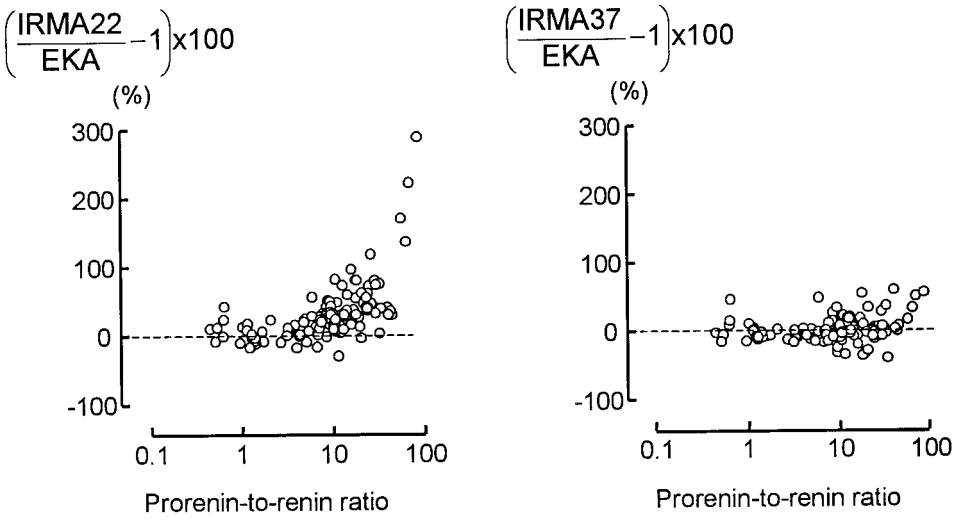

Fig. 4. Comparison of IRMA 22 °C, 24 h (*IRMA 22; left*) and IRMA 37 °C, 6 h (*IRMA 37; right*) with the enzyme-kinetic PRC assay (*EKA*).

Ordinate, difference between each IRMA and the enzyme-kinetic PRC assay, expressed as a percentage of the enzyme-kinetic PRC assay. *Abscissa*, prorenin-to-renin ratio as measured with IRMA 37 °C, 6 h. The slopes of the regression lines for IRMA 22 and IRMA 37 (not depicted) are 41 and 5.9 ($P < 0.001$ and not significant, respectively, for comparison with slope = 0). The *y*-intercept is −7.2 for IRMA 22 and −3.7 for IRMA 37. The *x*-variable is log[prorenin-to-renin ratio]. The rightmost outlier was omitted from analysis.

Many laboratories measure PRA instead of renin concentration. The results of the PRA assay are determined not only by the concentration of renin, but also by the concentration of angiotensinogen in plasma. We compared the results obtained with IRMA 37 °C, 6 h with those obtained with the PRA assay. Fig. 6 shows the results for healthy subjects, for patients with untreated essential hypertension, and for patients with Conn syndrome. The angiotensinogen concentration in these plasma was normal, and the renin was normal or low. The results of the PRA assay (with nmol Ang I · L^{-1} · h^{-1} as the units) were 0.07 times the values found with the IRMA 37 °C, 6 h (with mU/L as the units). A similar correlation was seen in other patient groups having normal renin and angiotensinogen concentrations in their plasma (see Table 4). As expected, the PRA-to-IRMA ratio was higher in patients with abnormally high angiotensinogen, and the ratio was lower in patients with abnormally low angiotensinogen.

Discussion

The original IRMA for renin that we described previously was not 100% specific for renin *(7)*. It also measured a small fraction (0.5–2%) of prorenin. Measurements in plasma therefore led to an overestimation of renin. In healthy subjects and in patients with essential or renovascular hypertension, renin was overestimated by 20% or less, but the deviation can be larger in situations in which plasma prorenin comprises >90% of total renin, such as in low-renin essential hypertension, pregnant women, in patients with Conn syndrome, and in patients with diabetes mellitus.

The present study demonstrates that this problem is solved in the improved assay. The tertiary structure of renin and prorenin has the form of two lobes separated by a cleft that contains the enzyme's active site *(24, 25)*. In enzymatically inactive intact prorenin, the propeptide is folded in this cleft, and angiotensinogen cannot reach the active site. This so-called closed form of prorenin is in dynamic equilibrium with an open form in which the propeptide, although still covalently linked with the renin portion of the molecule, has been displaced from the cleft, and angiotensinogen can now reach the active site *(14)*. The transition of the closed form into the open, active form takes hours, and the equilibrium is shifted toward

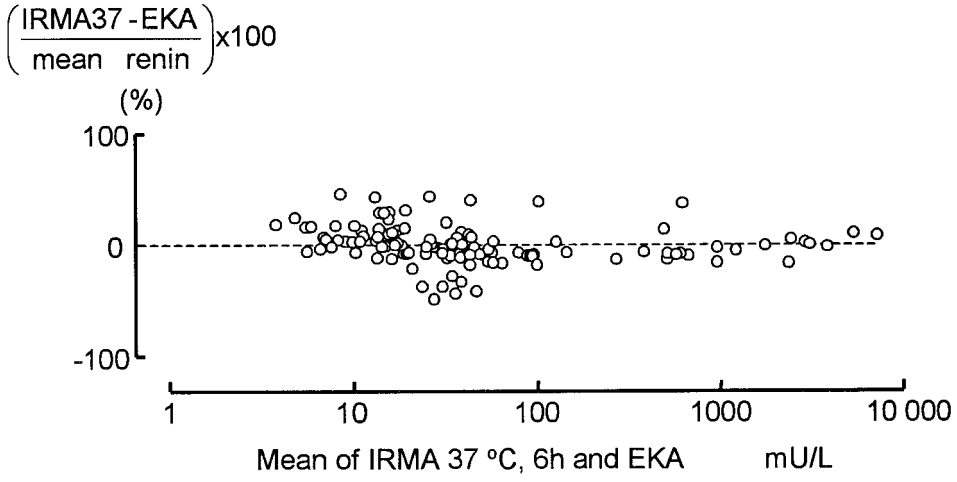

Fig. 5. Bland-Altman plot comparing IRMA 37 °C, 6 h (*IRMA 37*) with the enzyme-kinetic PRC assay (*EKA*).

Ordinate, difference between IRMA and the enzyme-kinetic PRC assay, expressed as a percentage of the renin activity (mean renin = mean of IRMA and EKA). *Abscissa*, mean of IRMA and enzyme-kinetic PRC assay. The mean of the difference between IRMA 37 °C, 6 h and the enzyme-kinetic PRC assay is 0.4%; SD = 17%; n = 121.

Table 4. Plasma renin, renin plus prorenin, angiotensinogen, and PRA in healthy subjects and in various groups of patients.

		Renin[a]			Renin plus prorenin[a]				
Subject group	n	IRMA 22 °C, 24 h, mU/L	IRMA 37 °C, 6 h, mU/L	Enzyme-kinetic PRC assay, mU/L	IRMA 22 °C, 24 h, mU/L	IRMA 37 °C, 6 h, mU/L	PRA, nmol Ang I · L^{-1} · h^{-1}	Angiotensinogen, nmol/L	PRA-to-renin ratio
Controls	10	29.7[b,c]	24.9	23.3	198	202	2.01	918	0.079
		15.9–62.2	14.6–38.9	10.2–36.2	131–347	123–344	1.15–6.09	823–1003	0.064–0.118
Essential hypertensives									
No treatment	20	11.0[b,c]	9.1	8.5	139	138	0.58	1159	0.073
		6.7–21.9	5.4–16.1	5.0–16.9	49–527	48–486	0.19–1.58	729–1628	0.029–0.114
Enalapril	9	38[b,c]	30.9	32.7	346	330	2.42	876	0.077
		20.7–73.3	17.8–58.0	13.2–68.2	213–559	219–470	1.06–5.97	627–1189	0.029–0.114
Renovascular hypertension									
No treatment	8	100.2[b,c]	86.3	94.7	426	411	7.43	1334	0.079
		73.3–158	58–140	68.1–149	346–475	385–445	5.97–9.00	1189–1628	0.061–0.093
Enalapril	9	527	496	533	1233	1171	31.9	804	0.060[d]
		153–1392	128–1188	124–1238	405–2034	383–1923	11.8–78.1	526–1139	0.038–0.095
Hyperaldosteronism (Conn syndrome)	2	4.4, 9.4	4.1, 6.4	3.4, 5.4	44.0, 64.1	43.7, 68.2	0.33, 0.57	1105, 1244	0.081, 0.080
Liver cirrhosis	12	1892	1847	1777	ND[f]	3569	27.73	252[d]	0.015[e]
		525–6821	528–7436	456–6830		807–4605	13.53–70.61	115–626	0.007–0.040
Diabetes mellitus (insulin-dependent)	8	61.6[b,c]	36.8	38.3	691	678	3.28	903	0.076
		45.4–82.4	24.4–54.4	25.0–57.5	381–904	381–859	1.79–7.08	784–1025	0.061–0.117
Pregnancy	10	67.7[b,c]	52.5	56.2	648	658	9.62	3535[d]	0.157[e]
		33.7–127	29.6–121	30.7–147	367–1609	426–1348	5.35–20.8	2631–4675	0.065–0.210
Preeclampsia	9	27.3[b,c]	18.5	19.8	484	485	2.83	2501[d]	0.113[e]
		7.6–56.3	5.4–40.4	4.2–44.0	139–1137	129–1137	1.09–4.93	1723–3711	0.064–0.189
Gonadotropin-treated women	12	47.2[b,c]	27.9	27.2	ND	914	3.31	2707[d]	0.122[e]
		19.9–110	14.0–57.8	12.4–56.7		355–2345	1.54–7.64	1563–3981	0.076–0.161
Women on oral contraceptives	10	18.7[b,c]	16.9	15.9	122	130	2.01	2884[d]	0.133[e]
		12.1–36.8	11.0–34	9.2–33.0	95–152	101–135	0.85–4.68	1390–6776	0.092–0.187

[a] Values are means and 95% confidence intervals.
[b] Significantly different from IRMA, 37 °C, 6 h: $P < 0.01$.
[c] Significantly different from enzyme-kinetic PRC assay: $P < 0.01$.
[d,e] Significantly different from control subjects: [d]$P < 0.05$; [e]$P < 0.01$.
[f] ND, not determined.

the open form by lower temperatures (cryoactivation) *(12)* or by the addition of active site-directed renin inhibitors *(19)*. Our results, especially those of the two-step IRMA that captures active prorenin, demonstrate that the comeasurement of prorenin we observed in the original IRMA 22 °C, 24 h, is caused by the formation of open prorenin. This problem is almost completely overcome in IRMA 37 °C, 6 h.

IRMAs usually are performed at room temperature. One may therefore wonder whether the reagents we used are stable at 37 °C. Our observation that the calibration curves for IRMA 37 °C, 24 h and IRMA 22 °C, 24 h were identical strongly suggests that the reagents are stable at 37 °C.

The renin calibrator was diluted in sheep serum to minimize nonspecific binding of the mAbs. Theoretically, the binding characteristics in the sheep serum matrix might be different from those in human plasma. The recovery of renin calibrator added to human plasma was close to 100%, and serial dilutions of human plasma produced decrements of binding that were linearly correlated with the dilution factor. The close agreement between the results of IRMA 37 °C, 6 h with those of the enzyme-kinetic PRC assay also indicates that the difference in the reaction milieu between plasma samples and calibrators did not affect the results.

For plasma samples with normal or increased renin, IRMA 37 °C, 6 h was as precise as the enzyme-kinetic PRC assay (interassay CV <10%). For plasma samples with low renin activity (<10 mU/L), the precision of IRMA 37 °C, 6 h was less than that of the enzyme-kinetic PRC assay. The functional sensitivity, defined as the minimum activity that could be measured from assay to assay with <20% CV, was ~4.0 mU/L. Our results show this to be sufficient for measuring renin in plasmas from patients with suppressed renin, such as patients with Conn syndrome or low-renin essential hypertension.

Many laboratories still use the enzyme-kinetic PRA assay. From our results, it appears that the patients who were diagnosed as having a low plasma renin activity on the basis of results obtained with IRMA 37 °C, 6 h also had abnormally low PRA.

The improved IRMA is specific for renin and appears

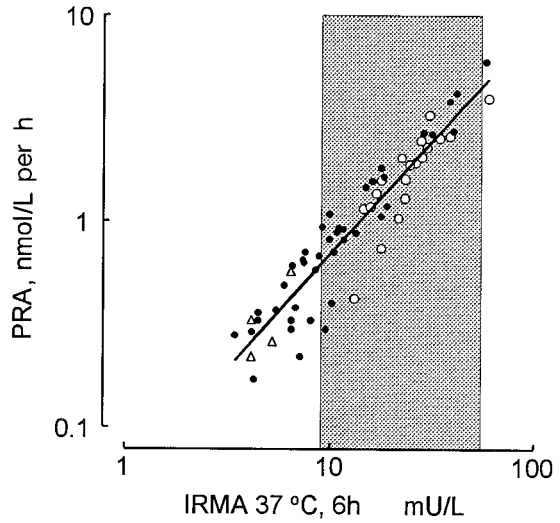

Fig. 6. Comparison of results obtained with IRMA 37 °C, 6 h with results obtained with the enzyme-kinetic PRA assay for patients with Conn syndrome (△), patients with essential hypertension (●), and control subjects (○).

The regression line was obtained by least-squares regression analysis after logarithmic transformation of the results. The *y*-intercept (given as the antilog) is 0.056; the slope is 1.097; n = 65. The *shaded area* represents the reference interval.

to be a simple, precise, and accurate method for measurements in clinical plasma samples. This assay is easier to standardize than the enzyme-kinetic assays that rely on endogenous angiotensinogen, such as the PRA assay. The results obtained with the improved IRMA are expressed in terms of the internationally recognized human renin standard and, therefore, permit ready comparison between different laboratories.

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