

Activity Assays and Immunoassays for Plasma Renin and Prorenin: Information Provided and Precautions Necessary for Accurate Measurement

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BACKGROUND: Measurement of plasma renin is important for the clinical assessment of hypertensive patients. The most common methods for measuring plasma renin are the plasma renin activity (PRA) assay and the renin immunoassay. The clinical application of renin inhibitor therapy has thrown into focus the differences in information provided by activity assays and immunoassays for renin and prorenin measurement and has drawn attention to the need for precautions to ensure their accurate measurement.

CONTENT: Renin activity assays and immunoassays provide related but different information. Whereas activity assays measure only active renin, immunoassays measure both active and inhibited renin. Particular care must be taken in the collection and processing of blood samples and in the performance of these assays to avoid errors in renin measurement. Both activity assays and immunoassays are susceptible to renin overestimation due to prorenin activation. In addition, activity assays performed with peptidase inhibitors may overestimate the degree of inhibition of PRA by renin inhibitor therapy. Moreover, immunoassays may overestimate the reactive increase in plasma renin concentration in response to renin inhibitor therapy, owing to the inhibitor promoting conversion of prorenin to

an open conformation that is recognized by renin immunoassays.

CONCLUSIONS: The successful application of renin assays to patient care requires that the clinician and the clinical chemist understand the information provided by these assays and of the precautions necessary to ensure their accuracy.

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Measurement of plasma renin is important for the clinical assessment of hypertensive patients. The most common methods for measuring plasma renin are the plasma renin activity (PRA)⁸ assay and the renin immunoassay. The clinical application of renin inhibitor therapy has stimulated new interest in the measurement of renin and its precursor, prorenin, and in how renin activity and concentration relate to the clinical effectiveness of renin inhibitor therapy (1–3). The nature of renin inhibitor therapy highlights the differences in information provided by activity assays and immunoassays of renin and prorenin. In the absence of renin inhibition, activity assays and immunoassays usually provide equivalent information about the amount of enzymatically active renin in plasma. When an inhibitor is bound to the renin active site, however, renin activity is inhibited in the activity assay, whereas the presence of the inhibitor does not affect the recognition of renin by currently available immunoassays. This review proposes a nomenclature for the different forms of renin and prorenin measured in activity assays and immunoassays (Table 1) and summarizes the

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⁸ Nonstandard abbreviations: PRA, plasma renin activity; Ang I, angiotensin I; Ang II, angiotensin II; ac-PRC, plasma renin concentration measured by activity assay; ac-TRC, total renin concentration measured by activity assay; ac-prorenin, prorenin concentration measured by activity assay; ir-PRC, plasma renin concentration measured by immunoassay; ir-TRC, total renin concentration measured by immunoassay; ir-prorenin, prorenin concentration measured by immunoassay.

Table 1. Nomenclature for different forms of renin and prorenin measured by activity assays and immunoassays.

| Renin form measured | Activity assays | Immunoassays | Units |
|----------------------------------|--|--|-------------------------------------|
| Plasma renin activity (PRA) | PRA: Ang I production by plasma renin enzymatic activity (both renin and prorenin with open conformation) acting on endogenous plasma angiotensinogen. | | μg or nmol Ang I/L per h |
| Plasma renin concentration (PRC) | ac-PRC: Ang I production by plasma renin enzymatic activity (both renin and prorenin with open conformation) acting on exogenous angiotensinogen. ^a | ir-PRC: Renin immunoassay of untreated plasma (measures both renin and prorenin with open conformation). | IU/L, ng/L, or pmol/L |
| Total renin concentration (TRC) | ac-TRC: Ang I production by total plasma renin enzymatic activity (after trypsin activation of prorenin) acting on exogenous angiotensinogen. ^a | ir-TRC: Total renin immunoassay of untreated plasma (measures renin and all prorenin with closed and open conformations). ^b | IU/L, ng/L, or pmol/L |
| Prorenin concentration | ac-prorenin: Calculated from the difference between ac-TRC and ac-PRC. ^a | ir-prorenin: Calculated from the difference between ir-TRC and ir-PRC | IU/L, ng/L, or pmol/L |

^a Activity assays (ac-PRC, ac-TRC, ac-prorenin) can be calibrated against the International Reference Preparation of human renin, and results are expressed in international units per liter [Bangham et al. (45)].

^b ir-TRC can be measured by 2 methods: by immunoassay with a pair of antibodies that recognize both renin and prorenin, or by renin immunoassay after trypsin activation of prorenin or after conversion of prorenin to an open conformation by incubation with a renin inhibitor.

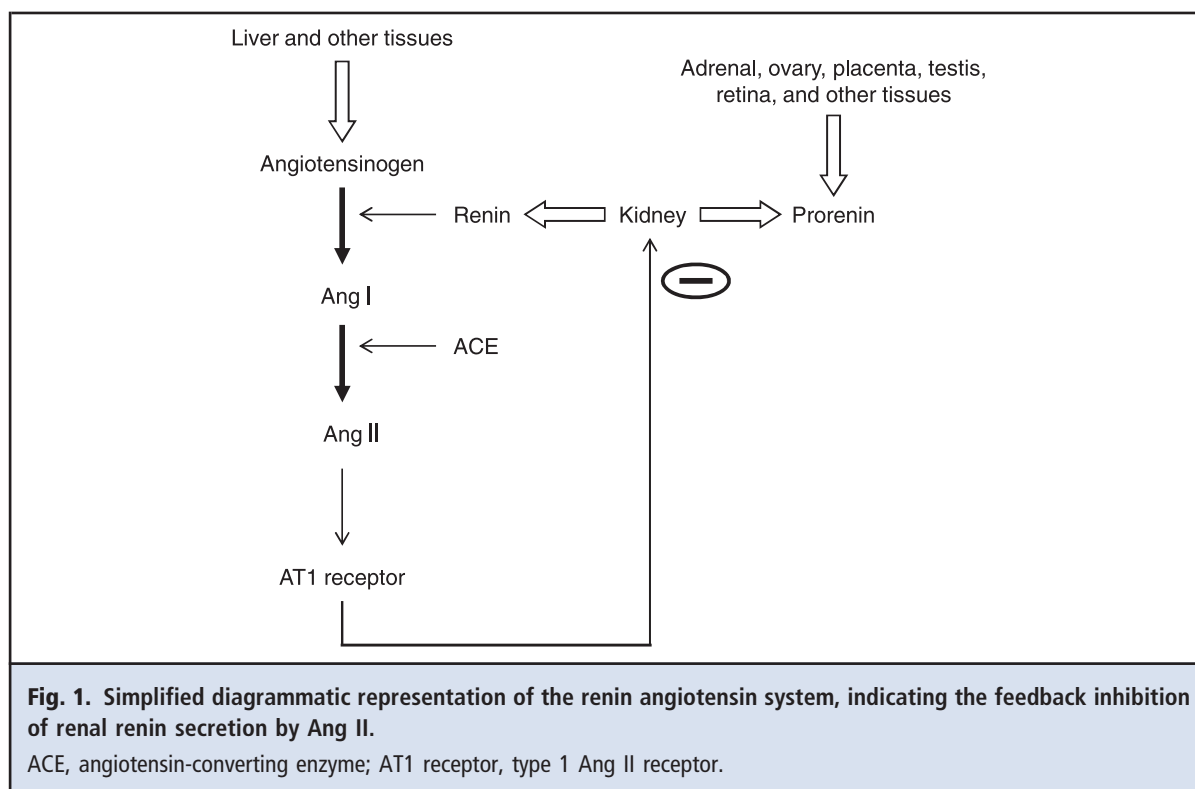
information these assays provide and the precautions necessary to ensure their accuracy.

Renin and Prorenin

Fig. 1 briefly summarizes the renin angiotensin system. Renin is synthesized as the inactive zymogen, prorenin, which contains a prosegment that masks the active site, thereby preventing access by the renin substrate, angiotensinogen (Fig. 2) (4). Whereas renal juxtaglomerular cells are the only known site of production for renin and the kidney produces both renin and prorenin, a number of extrarenal tissues, including the adrenal glands, ovary, testis, placenta, and retina, produce prorenin (5). The importance of these extrarenal sites to prorenin production is highlighted by the plasma prorenin concentrations in anephric individuals, which are approximately half the values in healthy individuals (Table 2). Plasma concentrations of prorenin are usually approximately 10-fold higher than for renin (6, 7) but can be as much as 100-fold higher when renin concentrations are suppressed, as in primary aldosteronism (8). Prorenin exists in 2 different conformations (Fig. 2) (1). More than 98% of plasma prorenin is in a closed conformation in which the prosegment masks the active site; the protein in this conformation is inactive. Less than 2% of plasma prorenin has an open con-

formation. In this conformation, the prosegment no longer masks the active site, which is now accessible to angiotensinogen; this open form of prorenin is enzymatically active (9). Cleavage of the prosegment converts prorenin to renin, and renal juxtaglomerular cells are the only known sites where prorenin is converted to renin in vivo. Schalekamp et al. described 2 forms of open prorenin—an inactive intermediary open conformation and an active open conformation (3). The inactive intermediary open conformation is a transition state of prorenin and is not considered further.

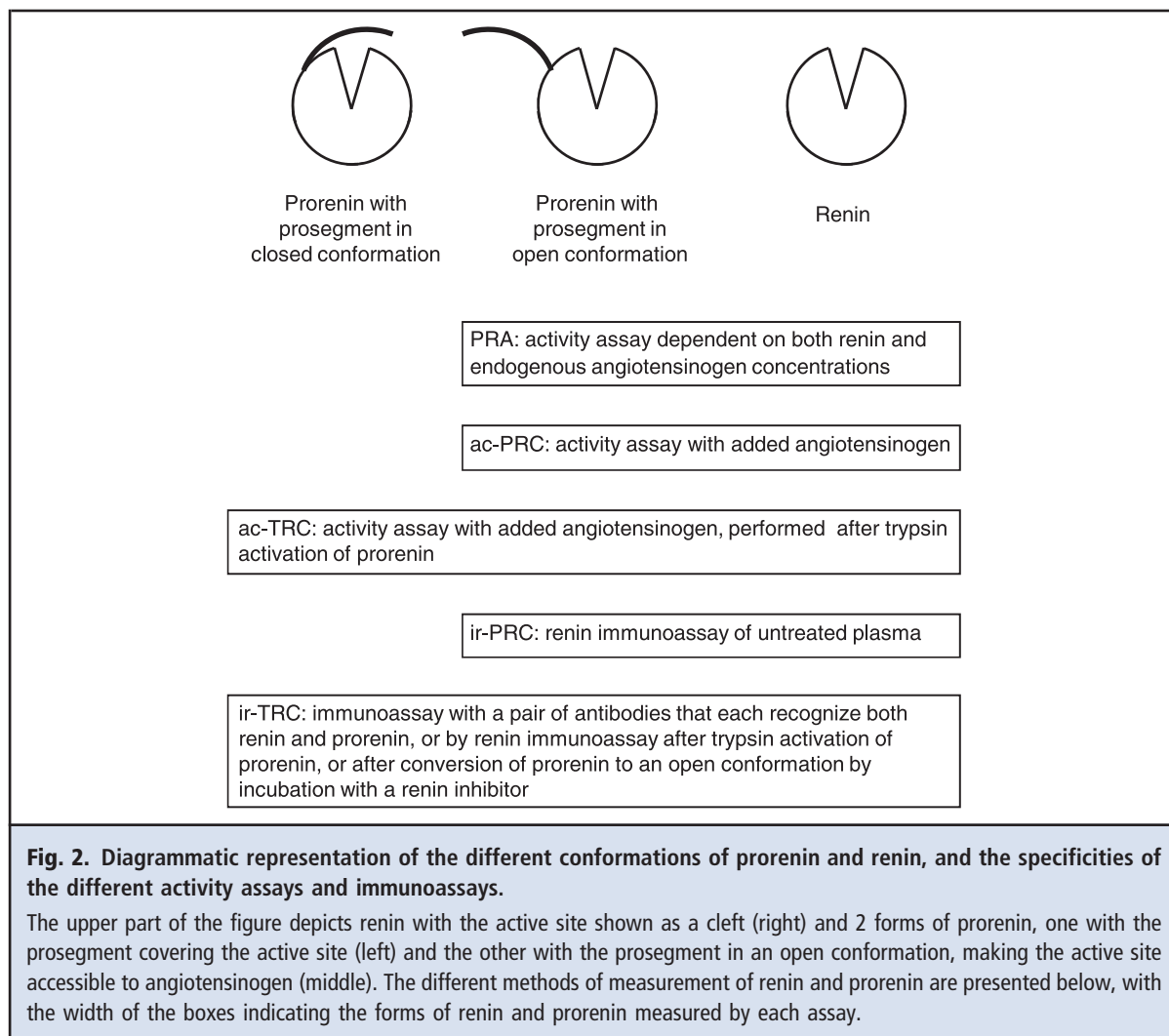
Renin inhibitors bind to the active sites of both renin and the open form of prorenin. Importantly for immunoassay measurement of renin concentrations during renin inhibitor therapy, binding of the renin inhibitor to the active site of prorenin molecules with an open conformation prevents refolding of the prosegment, thereby increasing the amount of prorenin recognized by the renin immunoassay (1, 10). Concentrations of a renin inhibitor, aliskiren, in the therapeutic range (60–600 nmol/L) (11) may increase the amount of prorenin that the renin immunoassay recognizes as renin if appropriate precautions are not taken (10). Conversely, the ability of renin inhibitors to promote the conversion of prorenin to an open conformation has been exploited for the measurement of total renin (renin plus prorenin) by renin



immunoassay (12–14). Incubating plasma with a renin inhibitor at 4 °C for 24–48 h causes complete conversion of prorenin to the open conformation, thereby permitting the renin immunoassay to measure all renin forms.

Measurement of renin by activity assay or immunoassay requires special precautions to avoid unfolding and cleavage of the prorenin prosegment. Both cooling and low pH promote unfolding of the prorenin prosegment (15–17), whereas refolding of the prosegment is promoted at 37 °C and physiological pH (14, 17–19). Cryoactivation of plasma prorenin occurs when plasma is cooled to between –5 °C and 4 °C. This activation is attributed to unfolding of the prosegment and its subsequent cleavage by plasma proteases (15); 12 h at 0 °C can cause approximately 5% activation of recombinant prorenin (19). Sealey et al. (20) advised that blood collected for renin measurement can be safely kept in an unopened EDTA Vacutainer (BD Medical Systems) for up to 24 h at ambient temperature before centrifugation. There are, however, several reasons why blood samples should be promptly centrifuged and the plasma quickly frozen. Leaving blood at ambient temperature may cause unfolding of the prorenin prosegment and lead to overestimation of renin activity and concentration. Spontaneous activation of prorenin occurs within 8 h at room temperature (19).

Incubation of plasma at 22 °C for 24 h during a renin immunoassay measures approximately 5% of the plasma prorenin as renin, owing to the unfolding of the prosegment (21), and the measured renin concentration may be twice that measured by an immunoassay with a 6-h incubation at 37 °C (10). The renin immunoassay may be more susceptible to unfolding of the prorenin prosegment than the activity assay. This is because prorenin may refold during the incubation at 37 °C in the activity assay, whereas refolding is less likely to occur with the immunoassay because the antibodies rapidly lock the prorenin in an open conformation before refolding can occur. A second reason for promptly centrifuging blood samples is the wide variation in ambient temperature that occurs by season. Renin will consume angiotensinogen and produce angiotensin I (Ang I) at ambient temperature; therefore, when renin is measured by activity assay, the Ang I produced at ambient temperature will need to be subtracted from the Ang I generated during the subsequent incubation at 37 °C. Patients undergoing renin inhibitor therapy are a third reason why blood samples should be promptly centrifuged: to minimize renin inhibitor promotion of prorenin conversion to an open conformation that is recognized by the renin immunoassay (10, 12–14). Blood should be centrifuged within 30 min of collection, preferably within 10 min, and the



plasma sample should be rapidly frozen if it is not assayed immediately. Care must be taken to ensure that frozen samples do not thaw during storage or during transport to the laboratory. Frozen plasma should be rapidly thawed before assay, and only once. These precautions are especially important for plasma samples with a reduced renin/prorenin ratio, such as those from patients with primary aldosteronism, pregnancy, or diabetes (3, 8, 21–23) (Table 2).

Measurement of PRA

PRA refers to the production of Ang I due to the enzymatic activity of plasma renin acting on endogenous plasma angiotensinogen; the PRA is linearly related to the plasma Ang II concentration (24, 25). Plasma angiotensinogen concentrations are typically in the first-order range of the renin enzymatic reaction, and the

amount of Ang I produced by a constant amount of renin is linearly related to the angiotensinogen concentration (22, 26, 27). Thus, the PRA is as dependent on the angiotensinogen concentration as it is on the renin concentration. In interpreting the PRA, the clinician needs to be aware of not only conditions that affect the renin concentration but also those that affect the angiotensinogen concentration. Plasma angiotensinogen concentrations are increased during pregnancy, glucocorticoid excess, and estrogen administration (22, 28–30) and are decreased when angiotensinogen production is reduced in liver disease (21, 30). Renin secretion is subject to tonic negative-feedback inhibition by Ang II (Fig. 1), and one consequence of a change in angiotensinogen concentration is usually a compensatory, reciprocal change in renin concentration that restores the PRA toward the typical range of concentrations (28). Independent of hepatic produc-

Table 2. PRA, PRC, and TRC in humans during physiological and pathologic states.^a

| Individuals | Renin | | Renin plus prorenin | Reference |
|---|------------------------------|----------------------------|----------------------------|--|
| | PRA, $\mu\text{g/L per h}^b$ | ir-PRC, mIU/L ^c | ir-TRC, mIU/L ^c | |
| Healthy, sodium replete | 1.5 (0.7–2.2) | 23 (3–116) | 202 (123–344) | Campbell et al. (7), Nussberger et al. (11), Deinum et al. (21), Iervasi et al. (51), Trenkel et al. (56), Diederich et al. (58), Maillard et al. (72) |
| Plus enalapril, 20 mg/day ^d | 29 (6–52) | 625 (365–885) | | Nussberger et al. (11) |
| Plus valsartan, 320 mg/day ^d | 20 (14–26) | | | Maillard et al. (72) |
| Plus aliskiren, 640 mg/day ^d | | 1048 (752–1344) | | Nussberger et al. (11) |
| Anephric | | <2 | 92 (15–169) | Campbell et al. (7) |
| Primary aldosteronism | | 2.8 (0.8–11.7) | 56 (44–68) | Deinum et al. (21), Unger et al. (57), Diederich et al. (58) |
| Renovascular hypertension | | 86 (58–140) | 411 (385–445) | Deinum et al. (21) |
| Plus enalapril | | 496 (128–1188) | 1171 (383–1923) | Deinum et al. (21) |
| Diabetes (insulin dependent) | | 37 (24–54) | 678 (381–859) | Deinum et al. (21) |
| Pregnancy | | 53 (30–121) | 658 (426–1348) | Deinum et al. (21) |
| Liver cirrhosis | | 1847 (528–7436) | 3569 (807–4605) | Deinum et al. (21) |

^a Data are presented as the mean (95% CI).
^b PRA measured at pH 7.4 with the antibody-trapping assay [Jeunemaitre et al. (39), Derckx et al. (40), Poulsen et al. (41), Millar et al. (42), Nussberger et al. (43)].
^c Based on a conversion factor of 1000 IU = 0.6 mg renin [Simon et al. (71)]; 1 mIU/L = 0.6 ng/L.
^d Maximum renin responses during chronic (>1 week) therapy with enalapril, valsartan, and aliskiren.

tion of angiotensinogen, high renin concentrations may reduce angiotensinogen concentrations by cleaving a large proportion of plasma angiotensinogen, and low angiotensinogen concentrations are particularly likely to occur when high renin concentrations are associated with decreased hepatic angiotensinogen production, such as occurs in adrenal insufficiency and heart failure (31, 32). Renin concentrations may be sufficiently increased to decrease angiotensinogen concentrations during sodium deficiency and during therapy with angiotensin-converting enzyme inhibitors and with type 1 Ang II receptor blockers (33, 34).

In the classic method of PRA measurement, inhibitors of angiotensinase and angiotensin-converting enzyme are added to plasma to prevent degradation of Ang I and its conversion to Ang II during incubation at 37 °C (27, 35–37). The rate of Ang I production in the PRA assay depends on the pH and the extent of plasma dilution in the assay, and differences in methodology between laboratories make comparisons difficult. The duration of incubation can be adjusted according to the renin concentration and thus the rate of Ang I production. The incubation time needs to be limited when the renin concentration is high to prevent excessive consumption of angiotensinogen and the consequent nonlinearity of Ang I production. Conversely, the incubation time can be extended for several hours

to allow sufficient Ang I formation to allow precise measurement of low PRA values (27, 38). The classic method of PRA assay, however, is unsuitable for precisely evaluating the magnitude of renin inhibition by renin inhibitor therapy. Renin inhibitors show high binding to plasma proteins (1), and peptidase inhibitors in the PRA assay displace protein-bound renin inhibitor that can then inhibit any uninhibited plasma renin. As a consequence, the displaced renin inhibitor causes the PRA assay to overestimate the extent of renin inhibition (39, 40). Displacement of protein-bound renin inhibitor can be reduced with the antibody-capture method of PRA assay (39–43), in which excess Ang I antibody is added to protect Ang I as it is produced, thereby avoiding the need for peptidase inhibition.

Measurement of Renin, Total Renin, and Prorenin Concentration by Activity Assay

PLASMA RENIN CONCENTRATION MEASURED BY ACTIVITY ASSAY (ac-PRC)

An alternative approach to measuring renin activity that avoids the influence of variation in plasma angiotensinogen concentration is to add exogenous angiotensinogen to the plasma before incubation. Plasma from nephrectomized sheep is ideal for this purpose

because human renin has a much higher affinity for and shows a much greater reaction velocity with sheep angiotensinogen than human angiotensinogen. This addition permits a much higher rate of Ang I production (26, 44). An advantage of the use of exogenous angiotensinogen is that the ac-PRC can be calibrated against a renin calibrator, such as the International Reference Preparation of human renin (45), and the concentration can be expressed in international units per liter. The antibody-capture method of activity assay can be used to measure ac-PRC.

TOTAL RENIN CONCENTRATION MEASURED BY ACTIVITY ASSAY (ac-TRC)

Measurement of the total renin concentration (renin plus prorenin) by activity assay (ac-TRC) can be made after prorenin has been activated by conversion to renin (Fig. 2). Methods for converting prorenin to renin include acidification to pH 3.3 or a brief incubation with immobilized or soluble trypsin at 4 °C (35, 46, 47). Because angiotensinogen is destroyed by acidification to pH 3.3, nephrectomized sheep plasma can be used as a source of angiotensinogen for ac-TRC measurement when prorenin is activated by this method (35). Endogenous angiotensinogen is not destroyed during trypsin activation of prorenin, and ac-TRC can be measured by monitoring Ang I production from endogenous angiotensinogen, although the rate of Ang I production is influenced by the endogenous angiotensinogen concentration, as discussed above for the PRA assay. Use of exogenous angiotensinogen allows calibration of the ac-TRC method against the International Reference Preparation of human renin (45), and concentrations can be expressed in international units per liter. The antibody-capture method of activity assay can be used to measure ac-TRC.

PLASMA PRORENIN CONCENTRATION MEASURED BY ACTIVITY ASSAY (ac-PRORENIN)

ac-prorenin is calculated by subtracting the ac-PRC value from the ac-TRC value; concentrations are expressed in international units per liter.

Measurement of Renin, Total Renin, and Prorenin Concentration by Immunoassay

Both renin and total renin immunoassays can be described as sandwich assays, because the protein being measured is “sandwiched” between 2 different antibodies: a capture antibody that is immobilized on a bead or other surface, and a detection antibody that may be radioactive or have a chemiluminescent label. The same capture antibody can be used for both the prorenin and renin immunoassays because it binds to a region common to the prorenin and renin molecules

that is distant from the active site and the prosegment. The detection antibody for the total renin immunoassay binds to a separate region common to the prorenin and renin molecules that is also distant from the active site and the prosegment. By contrast, the detection antibody for the renin immunoassay binds to a region adjacent to the active site that is exposed when the prosegment is absent (as for renin) or in an open conformation (as for open prorenin) but that is masked when the prosegment is in the closed conformation (Fig. 2).

Sandwich immunoassays offer particular advantages with respect to specificity and limit of detection: Measurement of prorenin and renin molecules requires recognition by 2 different monoclonal antibodies, and the presence of both capture and detection antibodies in excess ensures rapid and complete detection. Table 3 lists different renin and total renin immunoassays.

PLASMA RENIN CONCENTRATION MEASURED BY IMMUNOASSAY (ir-PRC)

The ir-PRC is the sum of the concentrations of renin and open prorenin (Fig. 2). As discussed earlier, it is essential to prevent inadvertent conversion of plasma prorenin from a closed conformation to an open conformation during sample preparation and during the ir-PRC assay itself (1, 10). Important determinants of the extent of prorenin conversion from a closed to an open conformation during the assay are temperature and assay time. Deinum et al. showed that renin immunoassays performed at 22 °C for 24 h measured approximately 5% of the plasma prorenin as renin, owing to the unfolding of the prosegment (21); however, these investigators did not detect any unfolding of the prosegment when the ir-PRC assay was performed at 37 °C for 6 h (21). In contrast to the difficulty of comparing PRA measurements made in different laboratories (47), the use of well-characterized renin calibrators in immunoassays allows direct comparison of ir-PRC measurements from different laboratories. The ir-PRC can be expressed in international units per liter, picograms per milliliter, or picomoles per liter.

It is important to note that the PRA and ir-PRC assays give different information, not only for patients who are undergoing renin inhibitor therapy but also for patients who are not receiving such therapy. Whereas the ir-PRC assay measures only immunoreactive renin (and open prorenin), Ang I production by the PRA assay is influenced by both renin (and open prorenin) and angiotensinogen concentrations. Although a close correlation between the results of PRA and ir-PRC assays was reported for samples from homogeneous populations of healthy volunteers (6) and hypertensive patients (48), this correlation was not as

Table 3. Monoclonal antibody combinations for renin and prorenin immunoassays.^a

| Assay specificity | Capture antibody | Detection antibody | Manufacturer | References |
|--|------------------|--------------------|--|--|
| Renin (and prorenin with an open conformation) | 3E8 | 4G1 | Cisbio International, Bio-Rad Laboratories, ERIA, Diagnostic Pasteur | Nussberger et al. (6), Dessi-Fulgheri et al. (49), Simon et al. (63), Menard et al. (73), Morganti et al. (74) |
| | R3-36-16 | R1-20-5 | Nichols Institute/Diagnostics Systems Laboratories | Deinum et al. (14) |
| | 12-12 | 11-6 | DiaSorin | DiaSorin (53) |
| Total renin (renin plus prorenin, closed and open conformations) | 4E1 | 3E8 | | Simon et al. (63) |
| | 3E8 | R3-36-16 | | Nussberger et al. (6) |
| | R3-27-6 | R3-36-16 | | Derkx et al. (23), Thatcher et al. (62), Nielsen et al. (64), Heusser et al. (75) |
| | R3-36-16 | R3-27-5 | | Deinum et al. (14) |
| | R3-36-16 | R1-20-5 | | Derkx et al. (13), Deinum et al. (14), Deinum et al. (21) |
| | 3E8 | 4G1 | | Derkx et al. (12) |

^a Total renin immunoassays may be performed without activation of untreated plasma through the use of antibodies that recognize both renin and prorenin [Nussberger et al. (6), Deinum et al. (14), Derkx et al. (23), Thatcher et al. (62), Simon et al. (63), Nielsen et al. (64), Heusser et al. (75)], by renin immunoassay after trypsin activation of prorenin [Derkx et al. (13), Derkx et al. (23), Simon et al. (63), Nielsen et al. (64)], or after activation of prorenin to an open conformation by incubation with a renin inhibitor [Derkx et al. (12), Derkx et al. (13), Deinum et al. (14), Deinum et al. (21)].

close for low PRA and ir-PRC values (6, 49) and across different patient groups with different angiotensinogen concentrations (50, 51). Because of variation in the methods of PRA measurement and the dependence of the PRA on plasma angiotensinogen concentration, it is not appropriate to quote a factor for converting PRA to ac-PRC or ir-PRC. Any conversion factor would be specific to the laboratory and would be applicable only to plasma samples with “normal” angiotensinogen concentrations.

Whereas the ac-PRC assay can measure renin concentrations well below the reference interval (44), the limit of detection for the ir-PRC assay is at the lower limit of the reference interval for the ir-PRC (Table 2). The functional sensitivity of the ir-PRC assay (i.e., the minimum concentration that can be measured from assay to assay with a CV of <20%) was reported to be approximately 4 mIU/L (13, 21). Ferrari et al. reported an intraassay CV of <12% for ir-PRC values in the interval of 3–320 ng/L (5–533 mIU/L), with a detection limit of 1 ng/L (1.7 mIU/L) (48). The ir-PRC assay loses precision, however, when renin concentrations are below the functional sensitivity of the assay: A QC survey found between-laboratory CVs of 46%–47% for plasma samples with renin concentrations <2 ng/L (<3.3 mIU/L) (52). Of note, however, is that the ir-PRC and PRA assays had similar between-laboratory CVs for plasma samples with low ir-PRC and PRA val-

ues, and the ir-PRC assay had a lower CV than the PRA assay for plasma samples with typical or increased values (50). A new renin immunoassay developed by DiaSorin reportedly has a functional sensitivity of <2 mIU/L, but information on the performance of this immunoassay in clinical laboratories is still limited (53).

One of the main clinical indications for renin measurement is a suspicion of primary aldosteronism. There has been debate about the suitability of ir-PRC assays for measuring the low renin concentrations that occur in primary aldosteronism (38, 54, 55). Several reports have claimed that PRA and ir-PRC assays perform similarly in screening for primary aldosteronism (48, 56–58), and Gordon concluded that the ir-PRC assay can probably be substituted for the PRA assay in screening for this disease (59). Clinicians should be aware, however, that the immunoassay may be associated with a false-positive aldosterone/renin ratio in individuals with increased plasma angiotensinogen concentrations, such as those taking an estrogen-containing agent (oral contraceptives, for example) (60). As mentioned above, increased angiotensinogen concentrations are usually associated with a compensatory suppression of renin concentrations (28). In such instances, the PRA is usually close to typical, and the aldosterone/PRA ratio is a more reliable indicator of primary aldosteronism than the aldosterone/ir-PRC ratio (60). Recently, the Endo-

crine Society (US), cosponsored by the European and International Societies of Endocrinology and Hypertension, published guidelines for the diagnosis and management of patients with primary aldosteronism (61). The guidelines recommend that renin immunoassays be able to measure concentrations as low as 1.2 ng/L (2 mIU/L).

TOTAL RENIN CONCENTRATION MEASURED BY IMMUNOASSAY (ir-TRC)

The ir-TRC can be measured by total renin immunoassay of untreated plasma (Table 1, Fig. 2). Alternatively, activation of prorenin by trypsin or by conversion to the open conformation via incubation with a renin inhibitor (Table 1 and Fig. 2) allows measurement of total renin by either renin immunoassay or total renin immunoassay.

Table 3 presents a list of ir-TRC assays, although none of these assays are available commercially. Thatcher et al. found an ir-TRC assay of untreated plasma detected only 41% of total plasma renin, compared with that obtained with the same assay after acid activation of plasma samples, whereas this ir-TRC produced identical results before and after acid activation of amniotic fluid samples (62). Simon et al. also found ir-TRC assays to underestimate the TRC in untreated plasma, compared with both the ac-TRC assay and the renin immunoassay of trypsin-activated prorenin (63). These findings of Thatcher et al. (62) and Simon et al. (63) suggest that the prosegment may interfere with the detection of plasma prorenin—but not amniotic fluid prorenin—by one or both of the antibodies used in the ir-TRC assay. Although other workers did not find underestimation of the ir-TRC in assays of untreated plasma (23, 64), the ir-TRC of plasma may best be measured by an ir-TRC assay or a renin immunoassay after prorenin activation or conversion to an open conformation by incubation with a renin inhibitor. The ir-TRC can be expressed in international units per liter, picograms per milliliter, or picomoles per liter.

PLASMA PRORENIN CONCENTRATION MEASURED BY IMMUNOASSAY (ir-PRORENIN)

The ir-prorenin is calculated by subtracting the ir-PRC value from the ir-TRC value and can be expressed in international units per liter, picograms per milliliter, or picomoles per liter.

Assessment of Renin Inhibition

The inhibition of renin *in vivo* can be assessed in a number of ways. One approach is to measure the decrease in the plasma concentrations of Ang I and Ang II (11). Another approach is to measure the residual en-

zymatic activity of plasma renin with the antibody-capture method of the PRA or ac-PRC assay (11, 39). Given the increased ir-PRC values that accompany renin inhibition, one approach to estimating the degree of renin inhibition is to calculate the PRA/ir-PRC ratio or the ac-PRC/ir-PRC ratio, for which the PRA and the ac-PRC are measured by the antibody-capture method (65, 66). The PRA/ir-PRC ratio has been referred to as the Ang I–production rate (65), whereas the ac-PRC/ir-PRC ratio has been referred to as the renin-specific activity (67). The PRA/ir-PRC and ac-PRC/ir-PRC ratios more accurately estimate the proportion of renin that is inhibited by a renin inhibitor than the activity assays alone, because PRA and ac-PRC are expressed as a ratio to a quantity that represents the total number of renin molecules, given that the ir-PRC assay measures both active and inhibited renin. Although antibody-capture methods of activity assay measure the degree of inhibition of renin activity in plasma, they do not measure the full impact of the renin inhibitor on angiotensin concentrations *in vivo*, which can only be assessed by measuring plasma Ang I and Ang II concentrations.

The increase in the ir-PRC provides an indirect measure of renin inhibition in patients receiving renin inhibitor therapy, but it may overestimate the increase in renin concentrations due to the renin inhibitor promoting prorenin conversion to an open conformation (1, 2, 10). Different commercial renin immunoassays produce different estimates of the ir-PRC in patients receiving renin inhibitor therapy because of differences in the amount of prorenin that adopts an open conformation during the assay procedure (1, 10). When interpreting plasma ir-PRC values in patients receiving renin inhibitor therapy, it is necessary to consider the type of assay, the manufacturer, whether the assay is automated or manual, and the time and temperature of incubation. It is also necessary to consider whether appropriate precautions were taken during the collection and centrifugation of blood samples, in the storage of plasma, and during any freeze-thaw cycles. Unfortunately, this information is usually not provided in published reports. It is also necessary to consider the dose of renin inhibitor and whether the inhibitor concentrations achieved in the blood are likely to promote conversion of prorenin to an open conformation (1, 10). Although precautions may be taken to prevent conversion of prorenin to an open conformation during the ir-PRC assay, it is unknown to what extent renin inhibitors bind to prorenin *in vivo* and promote unfolding of the prosegment (1).

Calibrators

It is not possible to use a renin calibrator for the enzymatic PRA assay because Ang I production in this assay

is determined by both the renin and angiotensinogen concentrations in the plasma. The results of the PRA assay are expressed in nanograms or picomoles Ang I per milliliter of plasma per hour of incubation; thus, results are determined by incubation conditions, including pH and extent of dilution. Different laboratories and commercial suppliers of PRA assay kits may adopt different modifications of the assay, making comparisons between laboratories difficult (47). Ang I can be obtained from commercial sources, with the amount expressed as the net peptide content. It is therefore important that reports of PRA measurement provide details of assay pH, degree of plasma dilution, incubation time, minimum detectable amount of Ang I, and details about the Ang I used for the calibration curve of the Ang I RIA.

In contrast to the PRA assay, the measurement of renin, total renin, and prorenin by activity assay in the presence of exogenous angiotensinogen can be expressed in international units per liter, and activities are calculated from the activity of the International Reference Preparation of human renin incubated under the same conditions. The International Reference Preparation of human renin (68/356) is a crude preparation of human kidney renin (approximately 0.1% pure) that Dr. E. Haas and Dr. H. Goldblatt prepared via an 8-step procedure (68) that converts all prorenin to renin (45). The international unit corresponds to the Goldblatt unit (GU), which is defined as the amount of renin that, when injected intravenously into a nonanesthetized trained dog, raises the mean direct systemic (femoral) blood pressure by 30 mmHg in about 2 min (69).

The renin calibrators used in commercial immunoassays have been calibrated against the International Reference Preparation of human renin. Pure recombinant renin and prorenin may be obtained from Dr. Walter Fischli (Actelion Pharmaceuticals, Allschwil, Switzerland). Recombinant renin requires calibration against the International Reference Preparation of human renin. Derkx et al. reported that 1400 IU ac-TRC was equivalent to 1 mg renin (13). A similar conversion factor of 1500 IU/mg protein was reported by Poorman et al. for recombinant human renin (70). The Nichols Institute calibrated its renin immunoassay against the International Reference Preparation (68/356) of human renin by means of a conversion factor of 1000 IU being equivalent to 0.6 mg pure renin (1667 IU/mg), as described by Simon et al. (71).

Iervasi et al. used the same conversion factor (51). It is important that reports of renin immunoassays include details of the type of assay, its manufacturer, whether the assay is automated or manual, the time and temperature of incubation, and the source and details of the calibrator used in the assay.

Conclusion

Measurement of plasma renin is important for the clinical assessment of hypertensive patients. Activity assays and immunoassays provide different, although complementary, information about plasma renin, which clinicians and clinical chemists need to understand if they are to correctly interpret these assays. This necessity is no more evident than for renin measurement in cases of patients receiving renin inhibitor therapy, in which activity assays and immunoassays may show opposite changes in renin activity and concentration. Careful attention to the precautions necessary for collecting and processing of blood samples and for performing these assays should ensure that the information these assays provide is accurate.

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