Renin, Prorenin and the Putative (Pro)renin Receptor

A.H. Jan Danser, Jaap Deinum

Renin is an aspartic protease that consists of 2 homologous lobes. The cleft in between contains the active site with 2 catalytic aspartic residues. Unlike other aspartic proteases such as pepsin or cathepsin D, renin is monospecific and only cleaves angiotensigen, to generate angiotensin (Ang) I. Ang I is the precursor of the active end-product of the renin-angiotensin system (RAS), Ang II.

Renin has also been called active renin to underline that an enzymatically inactive form of renin exists. In 1971, Lumbers found that amniotic fluid, left at low pH in the cold, acquired renin activity. Later, Skinner described a similar phenomenon in plasma. Acidification was not strictly necessary for this increase in Ang I-generating activity, because incubation at low temperature also increased renin activity, albeit to only 15% of activity after acidification. Soon it was postulated that this inactive, but activatable “big” renin (its molecular weight was 5 kDa higher than that of renin) was the biosynthetic precursor of renin. Hence, it was named prorenin. Only with the cloning of the renin gene in 1984 was prorenin definitively proved to be the precursor of renin. For reasons that are unknown, prorenin circulates in human plasma in excess to renin, sometimes at concentrations that are 100-times higher. Prorenin has also been demonstrated in plasma of cat, dog, cattle, pig, horse, sheep, rabbit, rat, and mouse.

A 43-amino acid N-terminal propeptide explains the absence of enzymatic activity of prorenin. This propeptide covers the enzymatic cleft and obstructs access of angiotensinogen to the active site of renin. (Pro)renin is synthesized as a preprohormone. It contains a signal peptide that directs the protein to the endoplasmic reticulum and ultimately to the exterior of the cell.

Both renin and prorenin can be fractionated into multiple species by isoelectric focusing. This heterogeneity is largely caused by differential glycosylation. Recently, a second product of the renin gene was identified. It is synthesized from a transcript that contains an alternative exon 1. It lacks the signal peptide and part of the prosegment and thus gives rise to a truncated prorenin that remains intracellular and displays enzymatic activity. The latter relates to the fact that a prosegment of insufficient length will not fully cover the enzymatic cleft. Evidence for intracellular angiotensin generation is, however, lacking, and truncated prorenin has also been demonstrated extracellularly.

Prorenin Activation

Prorenin can be activated in 2 ways: proteolytic or nonproteolytic. Proteolytic activation involves actual removal of the propeptide, eg, by (endogenous) kallikrein or (exogenous) trypsin or plasmin. Kallikrein is generated from prekallikrein in plasma after destruction of the natural inhibitors of contact activation, by exposure to low pH or low temperature. An unidentified aspartic protease activates prorenin proteolytically in acidified amniotic fluid. When using trypsin to activate prorenin in vitro, care must be taken to prevent destruction of prorenin, eg, by applying brief trypsin exposure times and by terminating the activation with a trypsin inhibitor. Another, more elegant way of trypsin-induced prorenin activation without destruction is incubation at 4°C with trypsin linked to Sepharose. This is easily removed by centrifugation. Plasmin (purified or recombinant) also activates prorenin, although the presence of endogenous plasmin inhibitors in plasma limits the use of plasmin to prorenin-containing samples other than plasma.

In vivo, proteolytic activation of prorenin occurs in the kidney. Various renal processing enzymes have been proposed, including proconvertase 1 and cathepsin B. No evidence exists for in vivo prorenin activation by kallikrein, even though patients with prekallikrein deficiency (Figure 1) or high-molecular-weight kininogen deficiency have relatively low levels of renin. Bolus infusions of recombinant human prorenin in monkeys did not provide evidence for prorenin–renin conversion in the circulation. Proteolytic prorenin activation, possibly involving a serine protease, has however been demonstrated in isolated cardiac and vascular cells.

Nonproteolytic activation of prorenin is a reversible process. It can best be imagined as an unfolding of the propeptide from the enzymatic cleft (Figure 2). This unfolding consists of at least 2 steps. In the first step, the propeptide moves out of the enzymatic cleft, and in the second step the renin part of the molecule assumes its enzymatically active conformation. Nonproteolytic activation can be induced by exposure to low pH (with an optimum at pH 3.3) and cold, called acid activation and cryoactivation, respectively. Acid activation leads to complete activity of prorenin, cryoactivation to partial (>15%) activity. Note that acidification of plasma will...
destruction the inhibitors of proteases that are capable of proteolytically activating prorenin after restoration of pH.

Nonproteolytically activated prorenin is enzymatically fully active and can be recognized by monoclonal antibodies that are specific for the active site. Remarkably, these antibodies also recognize prorenin after incubation with a renin inhibitor. Application of monoclonal antibodies against the propeptide after prorenin exposure to a renin inhibitor confirmed that, under these conditions, the prosegment was still present. Thus, renin inhibitors, like low pH and cold, are capable of nonproteolytically “activating” prorenin, although of course, because of the presence of the renin inhibitor, this activated prorenin cannot display enzymatic activity (Figure 2).

Kinetic studies of the nonproteolytic activation process have indicated that an equilibrium exists between the closed

---

**Figure 1.** Relationship between the renin/total renin ratio (ordinate, mean±SD) and total renin (=renin+prorenin) (abscissa, geometric mean, 95% CI) in various clinical conditions. Data are from Deinum and Schalekamp.21

**Figure 2.** Proteolytic and non-proteolytic activation of prorenin. A renin inhibitor will increase the amount of nonproteolytically activated prorenin. Such a drug binds to prorenin when it is in its open active conformation. Once bound, the prosegment cannot regain its original “closed” position, and thus prorenin will now be recognized by antibodies directed against the active site, although of course it is incapable of generating angiotensin (Ang) I from angiotensinogen (Aog). Because of the high affinity of the renin inhibitor, prorenin will stay in the “open” conformation, and thus the equilibrium will shift into the direction of the open conformation. Eventually, all prorenin will be in the open conformation.
(inactive) and open (active) forms of prorenin. The inactivation step is highly temperature-dependent and occurs very rapidly at neutral pH and 37°C (Figure 2). Consequently, under physiological conditions only a small percentage (<2%) of prorenin is in the open, active form. Exposure to a renin inhibitor will affect the equilibrium, because such a drug (because of its high affinity for the active site) will prevent inactivation.

**Measurement of Renin and Prorenin**

Nowadays, 2 types of assays exist for measurement of renin. The first one uses the enzymatic activity of renin. For renin researchers, the working horse of renin assays has always been the plasma renin activity (PRA) assay. It is performed by incubating plasma in the presence of inhibitors of Ang I-degrading enzymes. The generated Ang I is an index of renin activity. This generation depends not only on the amount of renin but also on the angiotensinogen concentration in plasma. This concentration is in humans ≈1000 to 1500 nM, ie, close to the Michaelis constant (Km). Care should therefore be taken not to dilute the sample.

To make the assay independent of angiotensinogen concentration (in other words, to measure plasma renin concentration (PRC) rather than PRA), exogenous substrate should be added in saturating quantities. Because human angiotensinogen is not readily available, plasma from nephrectomized sheep might be used instead. The pH optimum of cleavage of sheep angiotensinogen by human renin is 7.4, and the angiotensinogen concentration in the assay is 3 × Km, Under these saturating conditions, Ang I generation is directly proportional to the concentration of renin. In general, PRC correlates well with PRA. However, there are some exceptions, eg, in pregnant women and women on contraceptive pills, who display 2-fold increased angiotensinogen levels, and in subjects with severe heart failure who display diminished angiotensinogen levels. For the same PRC, these individuals will have higher and lower PRA values, respectively.

The second type of renin assay is a direct immunoassay. Three assays are currently marketed, an immunoradiometric assay (IRMA) by Cis Bio, and 2 assays by Nichols Diagnostics, an IRMA and a chemoluminometric assay that runs on an automated platform. All 3 assays use an immobilized capture antibody that binds both renin and prorenin. The second developing antibody is specific for an epitope on the renin–IRMA can then detect and quantify captured prorenin.

An IRMA might be used to demonstrate the rise in renin that will occur during renin inhibition. Theoretically, however, because renin inhibitors activate prorenin nonproteolytically, a significant amount of prorenin might now be detected as renin, thus leading to an overestimation of the renin surge (a measure for the response of the juxtaglomerular apparatus) after renin inhibition. A solution to this problem is to use prorenin-specific assays. Comparison of the results of both assays will reveal the true rise in renin after renin inhibition.

**Regulation of Renin and Prorenin**

Prorenin and renin levels are highly correlated but do not alter in parallel under all circumstances. Acute stimuli of renin will not affect prorenin levels, whereas chronic stimuli will lead to both increased renin and prorenin levels. This suggests that renin is stored as active enzyme and is released immediately on stimulation of the juxtaglomerular apparatus. Prorenin is released constitutively, and no acute responses occur. Chronic stimulation causes more prorenin to be converted to renin, leading to an increased renin/prorenin ratio in plasma (Figure 1). However, some exceptions to this rule exist. A very striking example is diabetes mellitus complicated by retinopathy and nephropathy. In microalbuminuric diabetic subjects, prorenin is increased out of proportion to renin (Figure 1). This increase starts before the occurrence of microalbuminuria, and the prorenin level in conjunction with the glycohemoglobin level may even be used to predict the occurrence of later microalbuminuria. Pregnant women also have high plasma prorenin levels, derived from the ova-
The function of this prorenin is unknown, as is the function of prorenin in amniotic fluid, in which prorenin was discovered. A genetic cause of high plasma prorenin levels has been found in a family with a mutated renin allele. These individuals are phenotypically normal.

Clinical Use of Plasma Renin and Prorenin Measurements

Plasma renin, measured by PRA or IRMA, is useful for the differential diagnosis of hypertension. Suppressed renin levels are compatible with a so-called volume-hypertension in which extracellular volume is increased. This is observed in primary hyperaldosteronism with elevated aldosterone, in licorice abuse with suppressed aldosterone, and in most monogenic hypertensive syndromes, like Liddle syndrome, Gordon syndrome, apparent mineralocorticoid excess syndrome, and glucocorticoid-remediable aldosteronism. Renin measurement is also important to assess mineralocorticoid replacement therapy in adrenogenital syndrome and to assess glucocorticoid suppression therapy in glucocorticoid-remediable aldosteronism.

An older but still propagated idea proposes that renin level may be used to guide therapy in hypertension: low levels suggest volume hypertension which should be treated with diuretics or calcium antagonists, high renin levels suggest a renin-dependent hypertension, which should be treated with β-adrenoceptor blockers, angiotensin-converting enzyme inhibitors, or AT1 receptor antagonists. The observation that increased renin predicts myocardial infarction was recently confirmed. Whether treatment directed at this renin level is warranted remains to be determined in a clinical trial.

As discussed, prorenin may predict diabetic nephropathy. The next step should be to study whether early intervention with RAS blockers in normotensive normoalbuminuric patients with increased prorenin levels prevents the development of nephropathy.

Tissue Renin

Local synthesis of angiotensins at tissue sites, independent of angiotensin generation in the circulation, is now widely accepted. Interference with such locally synthesized Ang II may in fact underlie the beneficial effects of RAS blockers. For a long time it was thought that local angiotensin synthesis, eg, in the heart, depended on locally synthesized renin. Although renin is present in cardiac tissue, there is no convincing evidence for its local synthesis in the heart. First, renin mRNA levels in the heart are low or undetectable. Second, renin activity can no longer be demonstrated in cardiac tissue after a bilateral nephrectomy, nor does the isolated perfused rat Langendorff heart release or contain renin. Third, the cardiac tissue levels of renin closely correlate with the plasma levels of renin, both under normal and pathological conditions. Fourth, cultured neonatal and adult rat cardiac myocytes and fibroblasts do not release renin or its inactive precursor, prorenin, into the medium. Taken together, therefore, it appears that the renin required for cardiac angiotensin generation is taken up from the circulation, ie, is kidney-derived. A similar concept applies to the vascular wall. Local prorenin synthesis does appear to occur in adrenal, eye, ovaries, and testis. This explains why prorenin is still present in plasma of nephrectomized subjects (Figure 1).

An attractive concept is that in tissues not synthesizing renin locally, circulating prorenin, after its local activation, contributes to angiotensin generation. This would not only provide a role for prorenin in vivo, but also explain why tissues, in contrast to plasma, contain predominantly renin. In support of this concept, transgenic rodents with (inducible) prorenin expression in the liver display increased cardiac Ang I levels, cardiac hypertrophy, and/or vascular damage. Importantly, when performing studies in transgenic animals, the species-specificity of the renin–angiotensinogen reaction should be kept in mind. This aspect not only hampers the use of human renin inhibitors in rodents but also may lead to incorrect renin measurements (eg, by measuring Ang I generation under conditions that are suboptimal for the various possible renin–angiotensinogen combinations in the transgenic animal).

Sequestration of Circulating (Pro)Renin: Diffusion or a Receptor-Mediated Process?

Cardiac renin may be localized in blood, in interstitial fluid, and/or on or in cells. The cardiac renin levels per se (expressed per gram wet weight) are too high to be explained based on the amount of (renin-containing and prorenin-containing) blood plasma (~5%) in the heart. Thus, circulating renin and prorenin either diffuse into the interstitial space and/or bind to (pro)renin receptors. Diffusion is supported by studies in a modified version of the isolated perfused rat Langendorff heart, allowing separate collection of coronary effluent and interstitial transudate. During perfusion of this heart preparation with renin, renin was found to diffuse slowly into the interstitial space, reaching steady-state levels that were equal to the renin levels in coronary effluent. Renin measurements in rat cardiac tissue fully support the concept that renin is present in cardiac interstitial fluid in concentrations that are as high as those in blood plasma.

In addition, studies in rat and porcine hearts have shown that part of cardiac renin is membrane-associated. Moreover, isolated perfused hearts of rats transgenic for human angiotensinogen release Ang I during renin (but not prorenin) perfusion and this release continues after stopping the renin perfusion. These data support the idea that circulating renin binds to a cardiac renin-binding protein/receptor, and that bound renin is catalytically active. Prorenin apparently did not bind to this receptor, at least not in a manner that allowed Ang I release into the coronary effluent.

The idea of renin binding is not new. In fact, evidence for renin binding was already obtained 20 years ago, when it was observed that vascular renin disappeared more slowly than circulating renin following a bilateral nephrectomy.

(Pro)Renin Receptors

Currently, 2 (pro)renin receptors have been identified, and the existence of a third receptor has been proposed (Figure 3). In addition, several “(pro)renin-binding proteins” have been investigated, either in membranes prepared from rat tissues or in intracellular com-
partments. Of these (P)RnBPs, only the intracellular RnBP has been cloned and characterized. Although it inhibits renin, it is also identical to the enzyme N-acetyl-β-glucosamine 2-epimerase. Mice lacking RnBP display normal blood pressure and plasma renin activity. Therefore, it is unlikely that this intracellular RnBP is a determinant of renin activity and/or metabolism in vivo.

The mannose-6-phosphate (M6P) receptor binds renin and prorenin with high affinity (Kd ≈ 1 nM) in neonatal rat cardiac myocytes and fibroblasts, as well as in human endothelial cells. This receptor is identical to the insulin-like growth factor II (IGFII) receptor, and as such it contains binding domains for both IGFII and phosphomannosylated (M6P-containing) proteins like renin and prorenin. It does not bind nonglycosylated (pro)renin. After binding, both renin and prorenin are rapidly (within minutes) internalized, and internalized prorenin is proteolytically cleaved to renin (Figure 3). (Pro)renin binding to M6P/IGFII receptors did not result in extracellular or intracellular angiotensin generation. Alternatively, binding of M6P-containing proteins to M6P/IGFII receptors results in activation of second messenger pathways in a G-protein-dependent manner. It is possible that renin and prorenin act as agonists for this receptor.

Using rats with an inducible expression of the ren-2d renin gene restricted to the liver, Peters et al have found that increased synthesis of ren-2d renin was associated not only with high circulating levels of ren-2d prorenin but also with high cardiac levels of ren-2d prorenin. Subsequent studies in isolated adult rat cardiomyocytes revealed that these cells internalized ren-2d prorenin, and not (or very weakly) ren-2d renin. Interestingly, internalization was followed by nonproteolytic activation of prorenin, increasing its enzymatic activity from 0.7% to 3.3%. Because ren-2d prorenin is nonglycosylated, the internalization process cannot be attributed to M6P/IGFII receptors. Currently, no information is available on the identity of the receptor that mediates this internalization.

Nguyen et al and Sealey et al, using radiolabeled (pro)renin, demonstrated high-affinity renin binding sites/receptors (Kd ≈ 1 nM) in human mesangial cells and in membranes prepared from rat tissues, respectively. Renin binding to the mesangial receptor increased 3H-thymidine incorporation (a measure for DNA synthesis) and plasminogen activator inhibitor (PAI)-1 synthesis. The receptor was subsequently cloned from an adult human kidney expression library (GenBank accession number AF 291814). It is a 350-amino-acid protein with a single transmembrane domain which displays >95% identity with the previously identified vascular proton-ATPase membrane sector-associated protein M8. The physiological meaning of this resemblance is currently unknown.

The cloned renin receptor was found to bind prorenin equally well (ie, renin’s active site is not involved in the binding process), and in contrast to the described receptors, cell surface-bound renin and prorenin were neither internalized nor degraded. Importantly, binding of renin to this receptor induced a 4-fold increase of the catalytic efficiency of angiotensinogen conversion to Ang I, and receptor-bound prorenin became fully enzymatically active in a nonproteolytic manner. These data support angiotensin generation on the cell surface, allowing Ang II to bind immediately to AT1 receptors after its synthesis, without leaking into the extracellular space. Furthermore, in the presence of the AT1 receptor antagonist losartan, (pro)renin binding to the (pro)renin receptor resulted in rapid activation of the MAP kinases ERK1 (p44)/ERK2 (p42), thereby demonstrating for the first time Ang II-independent effects of renin and prorenin. Immunohistochemistry and in situ hybridization studies have localized the receptor in vascular smooth muscle cells in human heart and kidney, in glomerular mesangial cells and in distal and collecting tubular cells in the kidney.

Based on experiments with a series of antibodies directed against various parts of the prosegment, Suzuki et al
recently proposed that human prorenin has so-called gate and handle regions for its nonproteolytic activation. According to this concept, the handle region (I11PFLKR15P) interacts with a putative receptor, which then leads to dissociation of the gate region T7PFKR10P from the renin molecule. Because this gate is crucial for refolding and the maintenance of the inactive state, dissociation allows prorenin to display enzymatic activity. In a subsequent in vivo study, these investigators applied a decoy peptide corresponding to the handle region to block nonproteolytic prorenin activation.79 This peptide reduced the renal content of Ang I and II and fully prevented the development of diabetic nephropathy in streptozotocin-induced diabetic rats. Interestingly, there were no effects on the plasma levels of Ang I and II, nor did the decoy peptide affect the tissue levels of Ang I and II in control rats. Thus, these data are the first to confirm that endogenous prorenin contributes to tissue Ang I and II generation in diabetic animals. It is tempting to speculate that this receptor is the above-mentioned (pro)renin receptor, but this remains to be proven. An explanation should also be provided for the lack of prorenin-dependent (renal) Ang I generation in nondiabetic animals.

Summary and Perspectives

After establishing the concept of renin uptake as the underling cause of tissue angiotensin generation, focus is now on the mechanism that mediates this uptake process. Several renin receptors have already been described. Importantly, these receptors also bind prorenin, and such binding results in prorenin activation, either proteolytically or nonproteolytically. Thus, for the first time, a physiological role for prorenin might be established. This is important in view of earlier observations that high renin levels in diabetic subjects are associated with angiotensin generation but also led to activation of renin and prorenin binding to their receptors not only facilitating renin inhibitor absorption but also being a target for cardiovascular-active drugs, at 2.5 A resolution. Science. 1989;243:1346–1351.

2. Lumbers ER. Activation of renin in human amniotic fluid by low pH. Enzymologia. 1971;40:329–336.

3. Skinner SL, Cran EJ, Gibson R, Taylor R, Walters WA, Catt KJ. Angiotensins I and II, active and inactive renin, renin substrate, renin activity, and angiotensinase in human liquor amnii and plasma. Am J Obstet Gynecol. 1975;121:626–630.

4. Hobart PM, Fogliano M, O’Connor BA, Schaef B, Chirgwin JM. Human renin gene: structure and sequence analysis. Proc Natl Acad Sci USA. 1984;81:5026–5030.

5. Danser AHJ, Derkx FHM, Schalekamp MADH. Hense HW, Riegger GAJ, Schunkert H. Determinants of interindividual variation of renin and prorenin concentrations: evidence for a sex dimorphism of (pro)renin levels in humans. J Hypertens. 1998;16:853–862.

6. Katz SA, Opsahl JA, Lunzer MM, Forbis LM, Hirsch AT. Effect of bilateral nephrectomy on active renin, angiotensinogen, and renin glycoform in plasma and myocardium. Hypertension. 1997;30:259–266.

7. Clausmeyer S, Sturzebecher R, Peters J. An alternative transcript of the rat renin gene can result in a truncated renin that is transported into adrenal mitochondria. Circ Res. 1999;84:337–344.

8. van Kats JP, Chai W, Duncker DJ, Schalekamp MADH, Danser AHJ. Adrenal angiotensin. Origin and site of generation. Am J Hypertens. 2005;18:1045–1051.

9. Saris J, van den Eijnden MMD, Lamers MJM, Saxena PR, Schalekamp MADH, Danser AHJ. Prorenin-induced myocyte proliferation: no role for intracellular angiotensin II. Hypertension. 2002;39:573–577.

10. Shingawaga T, Do YS, Baxter J, Hsieh WA. Purification and characterization of human truncated prorenin. Biochemistry. 1992;31:2758–2764.

11. Hirose S, Kim S, Miyazaki H, Park YS, Murakami K. In vitro biosynthesis of human renin and identification of plasma inactive renin as an activation intermediate. J Biol Chem. 1985;260:16400–16405.

12. Derkx FHM, Tan-Tjong HL, Man in ’t Veld AJ, Schalekamp MADH. Activation of inactive plasma renin by plasma and tissue kallikreins. Clin Sci (Lond). 1979;57:351–357.

13. Derkx FHM, Schalekamp MP, Schalekamp MADH. Two-step prorenin-renin conversion. Isolation of an intermediary form of activated prorenin. J Biol Chem. 1987;262:2472–2477.

14. Derkx FHM, Tan-Tjong L, Wenting GJ, Boomsma F, Man in ’t Veld AJ, Schalekamp MADH. Asynchronous changes in prorenin and renin secretion after captopril in patients with renal artery stenosis. Hypertension. 1983:5:244–256.

15. Reudelhuber TL, Ramla D, Chiu L, Mercure C, Seidah NG. Proteolytic processing of human prorenin in renal and non-renal tissues. Kidney Int. 1994;46:1522–1524.

16. Neves FA, Duncan KG, Baxter JD. Cathepsin B is a prorenin processing enzyme. Hypertension. 1996;27:514–517.

17. Lenz T, Sealey JE, Maack T, James GD, Heinrikson RL, Marion D, Laragh JH. Half-life, hemodynamic, renal, and hormonal effects of prorenin in cynomolgus monkeys. Am J Physiol Heart Circ Physiol. 2001;280:H1706–H1715.

18. van den Eijnden MMD, Schalekamp MADH, Saris JJ, de Bruin RJA, de Wit E, Sluiter W, Reudelhuber TL, Schalekamp MADH, Derkx FHM, Danser AHJ. Prorenin accumulation and activation in human endothelial cells. Importance of mannose 6-phosphate receptors. Arterioscler Thromb Vasc Biol. 2001;21:911–916.

19. Derkx FHM, Deinum J, Lipovski M, Verhaar MA, Fischl W, Schalekamp MADH. Nonproteolytic ‘activation’ of prorenin by active site-directed renin inhibitors as demonstrated by renin-specific monoclonal antibody. J Biol Chem. 1992;267:22837–22842.

20. Pitarresi TM, Rabattu S, Heinrikson R, Sealey JE. Reversible croyacti-

References

1. Sielecki AR, Hayakawa K, Fujinaga M, Murphy ME, Fraser M, Mair AK, Carilli CT, Lewicki JA, Baxter JD, James MN. Structure of recombinant human renin, a target for cardiovascular-active drugs, at 2.5 A resolution. Science. 1989;243:1346–1351.

2. Lumbers ER. Activation of renin in human amniotic fluid by low pH. Enzymologia. 1971;40:329–336.

3. Skinner SL, Cran EJ, Gibson R, Taylor R, Walters WA, Catt KJ. Angiotensins I and II, active and inactive renin, renin substrate, renin activity, and angiotensinase in human liquor amnii and plasma. Am J Obstet Gynecol. 1975;121:626–630.
27. de Bruin RA, Bouhuizen A, Diederich S, Perschel FH, Boomsma F, Deijun J. Validation of a new automated renin assay. Clin Chem. 2004;50:2111–2116.

28. Morganti A, Pelizzola D, Mantero F, Gazzano G, Opocher G, Piffanelli A. Immunoradiometric versus enzymatic renin assay: results of the Italian Multicenter Comparative Study. Italian Multicenter Study for Standardization of Renin Measurement. J Hypertens. 1995:13:19–26.

29. Derks FHM, de Bruin RJ, van Goor JM, van den Hoek MJ, Beerendonk CC, Rosmalen F, Haima P, Schalekamp MADH. Clinical validation of renin monoclonal antibody-based sandwich assays of renin and prorenin, and use of renin inhibitor to enhance renin immunoreactivity. Clin Chem. 1996;42:1051–1063.

30. Derks FHM, van den Meiracker AH, Fischli W, Adriaas PJ, Man in’t Veld AJ, van Brummelen P, Schalekamp MADH. Nonparallel effects of renin inhibitor treatment on plasma renin activity and angiotensins I and II in hypertensive subjects. An assay-related artifact. Am J Hypertens. 1991;4:602–609.

31. Jeunemaitre X, Ménard J, Nussberger J, Guyene TT, Brunner HR, Corvol P. Plasma angiotensins, renin, and blood pressure during acute renin inhibition by CDP 38 560A in hypertensive patients. Am J Hypertens. 1989;2:819–827.

32. Toffelmire EB, Slater K, Corvol P, Ménard J, Schambelan M. Response of plasma prorenin and active renin to chronic and acute alterations of renin secretion in normal humans. Studies using a direct immunoradiometric assay. J Clin Invest. 1989;83:679–687.

33. Danser AHJ, van der Doel MA, Demeur J, Derks FHM, Franken AA, Peperkamp E, de Jong PTVM, Schalekamp MADH. Renin, prorenin, and immunoreactive renin in vitreous fluid from eyes with and without diabetic retinopathy. J Clin Endocrinol Metab. 1989;68:160–167.

34. Deijun J, Ronn B, Mathiesen E, Derks FHM, Hop WC, Schalekamp MADH. Increase in serum prorenin precedes onset of microalbuminuria in patients with insulin-dependent diabetes mellitus. Diabetologia. 1999;42:1006–1010.

35. Itoikovitz J, Sealey JE, Glorioso N, Rosenwaks Z. Plasma prorenin response to human chorionic gonadotropin in ovarian-hyperstimulated women: correlation with the number of ovarian follicles and steroid hormone concentrations. Proc Nutl Acad Sci U S A. 1987;84:7285–7289.

36. Derks FHM, Alberda AT, de Jong FH, Zeilmaker FH, Makovitz JW, Derkx FHM, Alberda AT, de Jong FH, Zeilmaker FH, Makovitz JW, Schalekamp MADH. Renin-angiotensin system components in the interstitial fluid of the isolated perfused rat heart. Circ Res. 2000;87:564–573.

37. van den Eijnden MMED, de Bruin RJA, de Wit E, Sluiter W, Deinum J. Partial inhibition by CGP 38 560A in hypertensive patients. Circ Res. 1982;51:120–125.

38. Kantachuvesiri S, Fleming S, Peters J, Peters B, Brooker G, Lammie AG, Lammie AG. Increase in serum prorenin precedes onset of microalbuminuria in hypertensive subjects. An assay-related artifact. J Hypertens. 1995;13:19–26.

39. Van den Eijnden MMED, de Bruin RJA, de Wit E, Sluiter W, Deinum J. Renin-angiotensin system components in the interstitial fluid of the isolated perfused rat heart. Circ Res. 2000;87:564–573.

40. Alderman MH, Madhavan S, Ooi WL, Cohen H, Sealey JE, Laragh JH. Association of the renin-sodium profile with the risk of myocardial infarction in patients with hypertension. N Engl J Med. 1991;324:1098–1104.

41. van Kats JP, Danser AHJ, van Meegen JR, Sassen LM, Verdoud PD, Schalekamp MADH. Angiotensin production by the heart: a quantitative study in pigs with the use of radiolabeled angiotensin infusions. Circulation. 1998:98:73–81.

42. van Kats JP, Schalekamp MADH, Verdoud PD, Duncker DJ, Danser AHJ. Intrarenal angiotensin II: interstitial and cellular levels and site of production. Kidney Int. 2001;60:2311–2317.

43. Danser AHJ, van de Kats JP, Admiraal PJJ, Derkx FHM, Lammie AG, Lammie AG. Increase in serum prorenin precedes onset of microalbuminuria in hypertensive subjects. An assay-related artifact. J Hypertens. 1995;13:19–26.

44. Louden M, Bingham RF, Thurston H, Swales JD. Arterial wall uptake of renin and blood pressure control. Hypertension. 1983;5:629–634.

45. van Kesteren CAM, Danser AHJ, Derkx FHM, Dekkers DHW, Lamers MJM, Saxena PR, Schalekamp MADH. Mannose 6-phosphate receptor-mediated internalization and activation of prorenin by cardiac cells. Hypertension. 1999;33:312–317.

46. de Lannoy LM, Danser AHJ, van Kats JP, Schoemaker RG, Saxena PR, Schalekamp MADH. Renin-angiotensin system components in the interstitial fluid of the isolated perfused rat heart. Local production of angiotensin I. Hypertension. 1997;29:1240–1251.

47. Keller LJ, Opsahl JA, Wernsing SE, Saxena R, Katz SA. Myocardial and plasma renin-angiotensin system dynamics during pressure-induced cardiac hypertrophy. Am J Physiol. 1998;274:R849–R856.

48. van Kesteren CAM, Saris JJ, Dekkers DHW, Lamers MJM, Saxena PR, Schalekamp MADH, Danser AHJ. Cultured neonatal rat cardiac myocytes and fibroblasts do not synthesize renin or angiotensinogen: evidence for stretch-induced cardio-myocyte hypertrophy independent of angiotensin II. Cardiovasc Res. 1999;43:148–156.

49. Katz SA, Opsahl JA, Forbin LM. Myocardial enzymatic activity of renin and cathepsin D before and after bilateral nephrectomy. Basic Res Cardiol. 2001;96:659–668.

50. Hilgers KF, Veecken R, Müller DN, Kohler H, Hartner A, Botkin SR, Stampf C, Schneider RE, Gomez RA. Renin uptake by the endothelium mediates vascular angiotensin formation. Hypertension. 2001;38:243–248.

51. Sealey JE, Goldstein M, Pitarresi T, Kudlak TT, Glorioso N, Fiamengo SA. Laragh JH. Prorenin secretion from human testis: no evidence for secretion of active renin or angiotensinogen. J Clin Endocrinol Metab. 1988;66:974–978.

52. Itoikovitz J, Rubattu S, Levron J, Sealey JE. Highest concentrations of prorenin and human chorionic gonadotropin in gestational sacs during early human pregnancy. J Clin Endocrinol Metab. 1992;75:906–910.

53. Sealey JE, Moon C, Laragh JH, Atlas SA. Plasma prorenin in normal, hypertensive, and anephric subjects and its effect on renin measurements. Circ Res. 1997;80:431–445.

54. Deijun J, Derks FHM, Danser AHJ, Schalekamp MADH. Identification and quantification of renin and prorenin in the bovine eye. Endocrinology. 1999;100:1673–1682.

55. Kantachuvesiri S, Fleming S, Peters J, Peters B, Brooker G, Lammie AG, Lammie AG. Increase in serum prorenin precedes onset of microalbuminuria in patients with insulin-dependent diabetes mellitus. Diabetologia. 1999;42:1006–1010.

56. Itoikovitz J, Sealey JE, Glorioso N, Rosenwaks Z. Plasma prorenin response to human chorionic gonadotropin in ovarian-hyperstimulated women: correlation with the number of ovarian follicles and steroid hormone concentrations. Proc Nutl Acad Sci U S A. 1987;84:7285–7289.

57. Gregg TM, Sealey JE, Kudlak TT, Glorioso N, Fiamengo SA, Laragh JH. Prorenin secretion from human testis: no evidence for secretion of active renin or angiotensinogen. J Clin Endocrinol Metab. 1988;66:974–978.

58. Itoikovitz J, Rubattu S, Levron J, Sealey JE. Highest concentrations of prorenin and human chorionic gonadotropin in gestational sacs during early human pregnancy. J Clin Endocrinol Metab. 1992;75:906–910.

59. Sealey JE, Moon C, Laragh JH, Atlas SA. Plasma prorenin in normal, hypertensive, and anephric subjects and its effect on renin measurements. Circ Res. 1997;80:431–445.

60. Deijun J, Derks FHM, Danser AHJ, Schalekamp MADH. Identification and quantification of renin and prorenin in the bovine eye. Endocrinology. 1999;100:1673–1682.
69. Sealey JE, Catanzaro DF, Lavin TN, Gahnem F, Pitarresi T, Hu LF, Laragh JH. Specific prorenin/renin binding (ProBP). Identification and characterization of a novel membrane site. *Am J Hypertens.* 1996;9:491–502.

70. Takahashi S, Ohsawa T, Miura R, Miyake Y. Purification of high molecular weight (HMW) renin from porcine kidney and direct evidence that the HMW renin is a complex of renin with renin binding protein (RnBP). *J Biochem.* 1983;93:265–274.

71. Takahashi S, Inoue H, Miyake Y. The human gene for renin-binding protein. *J Biol Chem.* 1992;267:13007–13013.

72. Maru I, Ohta Y, Murata K, Tsukada Y. Molecular cloning and identification of N-acyl-D-glucosamine 2-epimerase from porcine kidney as a renin-binding protein. *J Biol Chem.* 1996;271:16294–16299.

73. Schmitz C, Gotthardt M, Hinderlich S, Leheste JR, Gross V, Vorum H, Christensen EI, Takahashi S, Willnow TE. Normal blood pressure and plasma renin activity in mice lacking the renin-binding protein, a cellular renin inhibitor. *J Biol Chem.* 2000;275:15357–15362.

74. Danser AHJ, Saris JJ. Prorenin uptake in the heart: a prerequisite for local angiotensin generation? *J Mol Cell Cardiol.* 2002;34:1463–1472.

75. Groskopf JC, Syu LJ, Saltiel AR, Linzer DIH. Proliferin induces endothelial cell chemotaxis through a G protein-coupled, mitogen-activated protein kinase-dependent pathway. *Endocrinology.* 1997;138:2835–2840.

76. Di Bacco A, Gill G. The secreted glycoprotein CREG inhibits cell growth dependent on the mannose-6-phosphate/insulin-like growth factor II receptor. *Oncogene.* 2003;22:5436–5445.

77. Nguyen G, Delarue F, Berrou J, Rondeau E, Graer JD. Specific receptor binding of renin on human mesangial cells in culture increases plasminogen activator inhibitor-1 antigen. *Kidney Int.* 1996;50:1897–1903.

78. Ludwig J, Kerscher S, Brandt U, Pfeiffer K, Getlaw F, Appes DK, Schägger H. Identification and characterization of a novel 9.2-kDa membrane sector-associated protein of vacuolar proton-ATPase from chromaffin granules. *J Biol Chem.* 1998;273:10939–10947.

79. Ichihara A, Hayashi M, Kaneshiro Y, Suzuki F, Nakagawa T, Tada Y, Koura Y, Nishiyama A, Okada H, Uddin MN, Nabi AH, Ishida Y, Inagami T, Saruta T. Inhibition of diabetic nephropathy by a decoy peptide corresponding to the “handle” region for nonproteolytic activation of prorenin. *J Clin Invest.* 2004;114:1128–1135.

80. Gradman AH, Schmieder RE, Lins RL, Nussberger J, Chiang Y, Bedigian MP. Aliskiren, a novel orally effective renin inhibitor, provides dose-dependent antihypertensive efficacy and placebo-like tolerability in hypertensive patients. *Circulation.* 2005;111:1012–1018.

81. Deinum J, Schalekamp MADH. *Textbook of Hypertension.* Philadelphia: WB Saunders; 1999.