

## Renin, Prorenin and the Putative (Pro)renin Receptor

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**R**enin is an aspartic protease that consists of 2 homologous lobes. The cleft in between contains the active site with 2 catalytic aspartic residues.<sup>1</sup> Unlike other aspartic proteases such as pepsin or cathepsin D, renin is monospecific and only cleaves angiotensinogen, 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.<sup>2</sup> Later, Skinner described a similar phenomenon in plasma.<sup>3</sup> 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.<sup>4</sup> For reasons that are unknown, prorenin circulates in human plasma in excess to renin, sometimes at concentrations that are 100-times higher.<sup>5</sup> 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 prohormone. 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.<sup>6</sup> Recently, a second product of the renin gene was identified.<sup>7</sup> 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,<sup>8,9</sup> and truncated prorenin has also been demonstrated extracellularly.<sup>10,11</sup>

### 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.<sup>12</sup> An unidentified aspartic protease activates prorenin proteolytically in acidified amniotic fluid.<sup>13</sup> 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.<sup>14</sup> 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.<sup>15,16</sup> 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.<sup>17</sup> Proteolytic prorenin activation, possibly involving a serine protease, has however been demonstrated in isolated cardiac and vascular cells.<sup>18,19</sup>

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.<sup>20</sup> 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.<sup>21,22</sup> Acid activation leads to complete activity of prorenin, cryoactivation to partial ( $\approx 15\%$ ) activity. Note that acidification of plasma will

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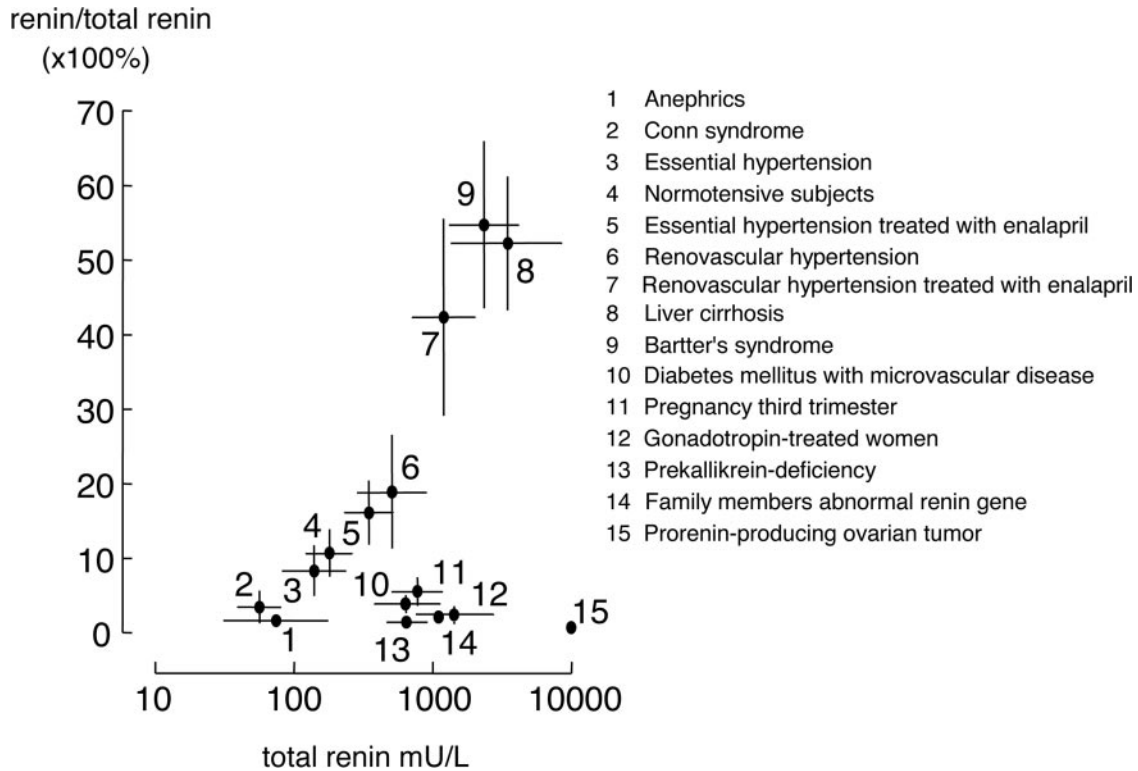
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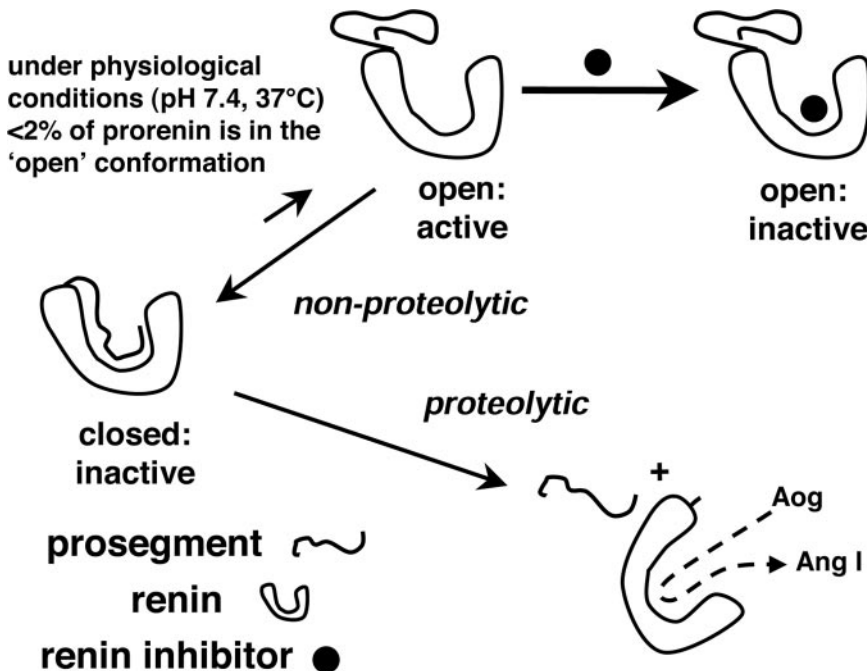
**Figure 1.** Relationship between the renin/total renin ratio (*ordinate*, mean±SD) and total renin (=renin+prorenin) (*abscissa*, geometric mean, 95% CI) in various clinical conditions. Data are from Deinum and Schalekamp.<sup>61</sup>

destruct 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 recognized prorenin after incubation with a renin inhibitor.<sup>20</sup> Application of monoclonal antibodies against the propeptide after prorenin exposure to a renin

inhibitor confirmed that, under these conditions, the prosegment was still present.<sup>23</sup> 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 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).<sup>24</sup> 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  $\approx 1000$  to 1500 nM, ie, close to the Michaelis constant ( $K_m$ ). 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.<sup>14</sup> The pH optimum of cleavage of sheep angiotensinogen by human renin is 7.4, and the angiotensinogen concentration in the assay is  $\approx 3 \times K_m$ . 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.<sup>25</sup> 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,<sup>26</sup> and 2 assays by Nichols Diagnostics,<sup>24,27</sup> 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 renin and is labeled by either radioactive iodine for the IRMA or acridinium for the chemoluminometric assay.

The results of direct immunoassays of renin are identical to those of the enzymatic renin concentration assays (with added angiotensinogen) provided they have been calibrated with the same standard. The WHO has kept a reference preparation since 1974, consisting of a partially purified kidney renin that is defined by its enzymatic activity and therefore expressed in units per liter. Correlation between PRA and renin immunoassays is usually good<sup>27</sup> and for clinical purposes both assays may be used. The disadvantage of the PRA assay is the large interlaboratory variation.<sup>28</sup>

All renin assays may overestimate renin because of the presence of cryoactivated prorenin. Samples should therefore

never be left on ice for prolonged periods of time. The Nichols IRMA suffered from overestimation of renin through comasurement of prorenin, but this was solved by shorter incubation at higher temperature.<sup>24</sup>

Prorenin can be measured indirectly by performing a renin assay after converting prorenin to renin (proteolytic or non-proteolytic).<sup>20,23,29</sup> The results of this assay will reflect total renin levels, ie, the levels of prorenin plus renin. Subtracting the renin level from the total renin level is then a measure of prorenin. Direct prorenin assays are not commercially available. We developed a prorenin assay that uses exposure of the propeptide through preincubation with a renin inhibitor.<sup>18,19</sup> An immobilized antibody that is specific for an epitope on the propeptide traps this conformationally changed prorenin by its exposed propeptide. The same renin-specific labeled antibody that is used for the renin-IRMA can then detect and quantify captured prorenin.

A panel of these assays is mandatory when comparing the RAS response to renin inhibition versus the responses of the system to angiotensin-converting enzyme inhibition or AT<sub>1</sub> receptor blockade. A PRA assay yields information on the achieved degree of renin inhibition, but should be adapted because the angiotensinase inhibitors that are added in the normal assay displace the renin inhibitor from plasma proteins and thus falsely lead to a high degree of inhibition *in vitro*.<sup>30,31</sup> This can be overcome by incubation with antiserum to Ang I (“antibody-trapping assay”) instead of angiotensinase inhibitors. The antiserum traps the generated Ang I and thus protects it against degradation.

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,<sup>20</sup> 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.<sup>5</sup> Acute stimuli of renin will not affect prorenin levels, whereas chronic stimuli will lead to both increased renin and prorenin levels.<sup>32</sup> 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.<sup>33</sup> 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.<sup>34</sup> Pregnant women also have high plasma prorenin levels, derived from the ova-

ries.<sup>35,36</sup> 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.<sup>37</sup> 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  $\beta$ -adrenoceptor blockers, angiotensin-converting enzyme inhibitors, or AT<sub>1</sub> receptor antagonists.<sup>38</sup> The observation that increased renin predicts myocardial infarction was recently confirmed.<sup>39,40</sup> Whether treatment directed at this renin level is warranted remains to be determined in a clinical trial.

As discussed, prorenin may predict diabetic nephropathy.<sup>34</sup> 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.<sup>41,42</sup> 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,<sup>6,43</sup> there is no convincing evidence for its local synthesis in the heart. First, renin mRNA levels in the heart are low or undetectable.<sup>44</sup> Second, renin activity can no longer be demonstrated in cardiac tissue after a bilateral nephrectomy,<sup>6,43</sup> nor does the isolated perfused rat Langendorff heart release or contain renin.<sup>45,46</sup> Third, the cardiac tissue levels of renin closely correlate with the plasma levels of renin, both under normal and pathological conditions.<sup>25,43,47</sup> Fourth, cultured neonatal and adult rat cardiac myocytes and fibroblasts do not release renin or its inactive precursor, prorenin, into the medium.<sup>48,49</sup> 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.<sup>50</sup> Local prorenin synthesis does appear to

occur in adrenal, eye, ovaries, and testis.<sup>7,33,51,52</sup> This explains why prorenin is still present in plasma of nephrectomized subjects (Figure 1).<sup>53</sup>

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.<sup>25,43,54</sup> 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.<sup>55–57</sup> 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).<sup>58</sup>

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

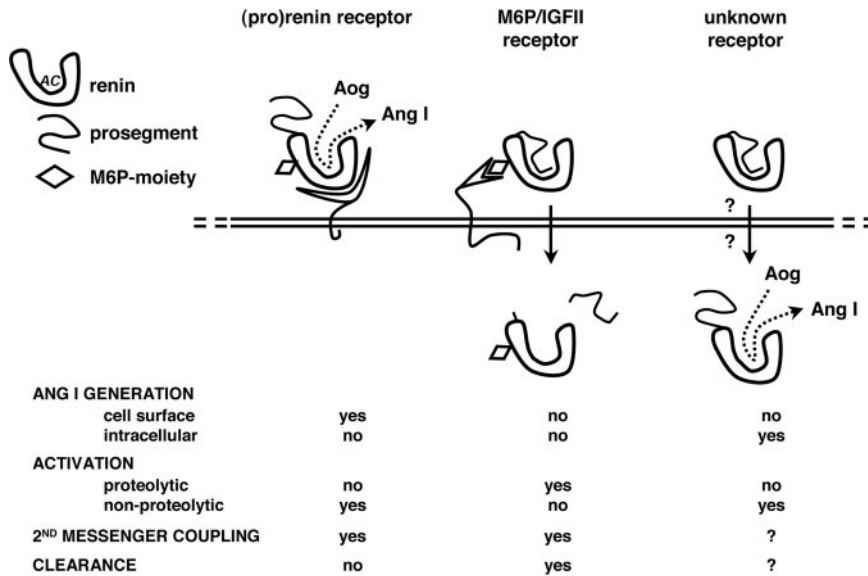
Cardiac renin may be localized in blood, in interstitial fluid, and/or on or in cells. The cardiac renin levels per se (expressed per gram wet weight) are too high to be explained based on the amount of (renin-containing and prorenin-containing) blood plasma ( $\approx 5\%$ ) in the heart.<sup>6,43</sup> Thus, circulating renin and prorenin either diffuse into the interstitial space<sup>59</sup> 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.<sup>46</sup> 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.<sup>6,60</sup>

In addition, studies in rat and porcine hearts have shown that part of cardiac renin is membrane-associated.<sup>43,61</sup> 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.<sup>62,63</sup> 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.<sup>64</sup>

### (Pro)Renin Receptors

Currently, 2 (pro)renin receptors have been identified,<sup>18,65–67</sup> and the existence of a third receptor has been proposed (Figure 3).<sup>68</sup> In addition, several “(pro)renin-binding proteins” [(P)RnBP] have been investigated, either in membranes prepared from rat tissues<sup>61,69</sup> or in intracellular com-



**Figure 3.** Current status of (pro)renin receptors, prorenin internalization and prorenin-induced effects. The (pro)renin receptor cloned by Nguyen et al<sup>67</sup> facilitates cell surface angiotensin (Ang) generation from angiotensinogen (Aog), mannose 6-phosphate/insulin-like growth factor II (M6P/IGFII) receptor-induced internalisation of M6P-containing prorenin results in prorenin clearance,<sup>18,19</sup> and an unknown mechanism allows nonglycosylated (ie, non-M6P-containing) prorenin to internalize and to subsequently generate Ang I intracellularly.<sup>68</sup> AC indicates active center.

partments.<sup>70</sup> Of these (P)RnBPs, only the intracellular RnBP has been cloned and characterized.<sup>71</sup> Although it inhibits renin, it is also identical to the enzyme N-acyl-D-glucosamine 2 epimerase.<sup>72</sup> Mice lacking RnBP display normal blood pressure and plasma renin activity.<sup>73</sup> 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 ( $K_d \approx 1$  nM) in neonatal rat cardiac myocytes and fibroblasts,<sup>18,65</sup> as well as in human endothelial cells.<sup>19,66</sup> 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.<sup>74</sup> It does not bind nonglycosylated (pro)renin.<sup>18,19</sup> After binding, both renin and prorenin are rapidly (within minutes) internalized, and internalized prorenin is proteolytically cleaved to renin (Figure 3).<sup>18,19</sup> (Pro)renin binding to M6P/IGFII receptors did not result in extracellular or intracellular angiotensin generation,<sup>9,19</sup> and (prorenin-derived) intracellular renin was found to be degraded slowly (within hours).<sup>18,19</sup> Thus, M6P/IGFII receptors most likely serve as clearance receptors for both renin and prorenin, thereby determining the extracellular levels of (pro)renin. Alternatively, because binding of M6P-containing proteins to M6P/IGFII receptors results in activation of second messenger pathways in a G-protein-dependent manner,<sup>75,76</sup> it is possible that renin and prorenin act as agonists for this receptor.

Using rats with an inducible expression of the *ren-2<sup>d</sup>* renin gene restricted to the liver, Peters et al<sup>68</sup> have found that increased synthesis of *ren-2<sup>d</sup>* renin was associated not only with high circulating levels of *ren-2<sup>d</sup>* prorenin but also with high cardiac levels of *ren-2<sup>d</sup>* (pro)renin. Subsequent studies in isolated adult rat cardiomyocytes revealed that these cells internalized *ren-2<sup>d</sup>* prorenin, and not (or very weakly) *ren-2<sup>d</sup>* renin. Interestingly, internalization was followed by nonproteolytic activation of prorenin, increasing its enzymatic activity from 0.7% to 3.3%.<sup>68</sup> Because *ren-2<sup>d</sup>* prorenin is nonglycosylated, the internalization process cannot be attrib-

uted 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 ( $K_d \approx 1$  nM) in human mesangial cells and in membranes prepared from rat tissues, respectively.<sup>69,77</sup> Renin binding to the mesangial receptor increased <sup>3</sup>H-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).<sup>67</sup> It is a 350-amino-acid protein with a single transmembrane domain which displays >95% identity with the previously identified vacuolar proton-ATPase membrane sector-associated protein M8-9.<sup>78</sup> 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 AT<sub>1</sub> receptors after its synthesis, without leaking into the extracellular space.<sup>9</sup> Furthermore, in the presence of the AT<sub>1</sub> 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.<sup>67</sup>

Based on experiments with a series of antibodies directed against various parts of the prosegment, Suzuki et al<sup>22</sup>

recently proposed that human prorenin has so-called gate and handle regions for its nonproteolytic activation. According to this concept, the handle region ( $^{11}\text{PFLKR}^{15\text{P}}$ ) interacts with a putative receptor, which then leads to dissociation of the gate region  $\text{T}^{\text{7P}}\text{FKR}^{10\text{P}}$  from the renin molecule. Because this gate region 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.<sup>79</sup> 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 via a mechanism involving binding of its handle region to a receptor. 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 underlying 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 prorenin levels in diabetic subjects are an indication of microvascular complications. Unexpectedly, renin and prorenin binding to their receptors not only facilitated angiotensin generation but also led to activation of second messenger pathways, thereby implying that renin and prorenin may act as agonists independently of Ang II generation. Now that renin inhibitors will soon be clinically available,<sup>80</sup> it will be of the greatest interest to investigate how these drugs affect these mechanisms in comparison with other RAS blockers. Tools that are available are prorenin-specific assays, transgenic rats with vascular (pro)renin receptor expression, and/or (hepatic) prorenin expression, and peptides that block prorenin activation at tissue sites. Eventually a new class of drugs might emerge, the renin receptor blockers, which selectively block angiotensin generation at tissue sites and/or renin receptor-mediated effects.

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