Critical Review of Prorenin and (Pro)renin Receptor Research

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The renin-angiotensin system (RAS) plays an important role in cardiovascular and renal physiology and disease, and the benefits of angiotensin-converting enzyme inhibitor, angiotensin type 1 receptor blocker, and renin inhibitor therapies are mediated in part by their modification of the levels and actions of angiotensin peptides. Despite its long history, the RAS remains an active area of research. Contrary to the classical view of the RAS as an endocrine system whereby kidney-secreted renin acts on circulating angiotensinogen to produce angiotensin peptides in the circulation, it is now recognized that tissues are the main sites of angiotensin peptide formation by the action of plasma- (kidney-) derived renin on plasma-derived and locally synthesized angiotensinogen. A receptor for prorenin and renin was recently identified that is hereby referred to as the (pro)renin receptor. Prorenin and (pro)renin receptor research offers the exciting possibility of a new paradigm for the RAS whereby renin and prorenin binding to the (pro)renin receptor not only target and facilitate angiotensin generation but also lead to activation of (pro)renin receptor signal transduction pathways distinct from angiotensin II receptor signals. By revealing novel potential mechanisms of disease pathogenesis, this new paradigm offers the possibility of new therapies that may be more effective than those currently available. It also suggests that stimulation of the (pro)renin receptor by the increased renin and prorenin levels that accompany angiotensin-converting enzyme inhibitor, angiotensin type 1 receptor blocker, and renin inhibitor therapies may attenuate the benefits these therapies. Recent reviews and editorials have discussed this research. The purpose of this brief review is to critically assess the evidence for this new paradigm from the perspective of disease pathogenesis and to advocate caution in its interpretation. The available evidence presents contradictions and difficulties of interpretation that preclude any conclusion about the reality of this new paradigm.

Renin and Prorenin

Renin is an aspartyl protease synthesized as an inactive zymogen, prorenin. Renin has a bilobed structure, with each lobe contributing 1 of the 2 essential aspartyl residues of its active site. Prorenin has an amino-terminal prosequence that is thought to fold over the cleft between the 2 lobes of the enzyme, thereby preventing access to the active site by its substrate, angiotensinogen. Pure prorenin has a low intrinsic activity of <3% of the activity of fully activated renin, and this intrinsic activity is attributed to partial unfolding of the prosegment. Human renin and prorenin are glycosylated, and a variable proportion of renin and prorenin has mannose-6-phosphate (M6P) residues and binds to the M6P–insulin-like growth factor II receptor. Basal renin levels are 1 pmol/L in humans, and prorenin levels are 10-fold higher than renin levels (Table S1, available online at http://hyper.ahajournals.org).

Renal juxtaglomerular cells are the only known sites of renin production, and the kidney produces both renin and prorenin. A number of extrarenal tissues, including the adrenal, ovary, testis, placenta, and retina, produce prorenin. The importance of extrarenal sites to prorenin production is indicated by plasma prorenin levels in anephric subjects that are approximately half the levels in normal subjects (Table S1). In certain body fluids, such as ovarian follicular fluid and amniotic fluid, and vitreous fluid of diabetic subjects with proliferative retinopathy, prorenin concentrations can approach 100 times the levels found in plasma. The association between plasma prorenin levels and diabetic complications, including diabetic retinopathy, led to the proposal that prorenin may have a pathogenic role in diabetes.

Does Prorenin in Biological Fluids Have a Role?

There have been many attempts to show a role for prorenin in biological fluids. The very low level of angiotensin peptides in anephric subjects, despite plasma prorenin levels approximately half of normal, is consistent with an intrinsic activity of <2% for prorenin, and indicates only a small contribution by prorenin to angiotensin peptide formation in humans. Prorenin administration to experimental animals does not increase blood pressure, and there is no evidence for its activation in biological fluids.

One report cited in support of a biological role for prorenin in vivo is that of Véniant et al. These authors developed a transgenic rat in which rat prorenin expression was targeted to the liver. Male transgenic rats had 400-fold elevation of...
plasma prorenin levels, whereas female transgenic rats showed a 3-fold elevation. Neither male nor female transgenic rats showed alteration in the plasma level of renin or blood pressure. Male, but not female, transgenic rats had cardiac hypertrophy, with an ≈30% increase in the heart weight:body weight ratio, and histological analyses revealed severe renal lesions and hypertrophic cardiomyocytes. Quite different results were reported by Prescott et al22 for mice with human prorenin expression targeted to the liver and human angiotensinogen expression targeted to the heart. Mice with plasma levels of human prorenin 20-fold higher than the prorenin level of control mice had reduced blood pressure, and there was no abnormality of heart size or fibrosis, although cardiac angiotensin I levels were increased in these mice. Véniant et al21 did not measure angiotensin levels in their prorenin transgenic rats, but they observed a marked suppression of renin activity in the kidneys of these rats that may have been because of a local increase in angiotensin levels. Thus, both studies21,22 provide evidence for local angiotensin formation that may have been because of the intrinsic activity of the elevated prorenin levels. The intrinsic activity of the 400-fold increase in prorenin levels may have increased local angiotensin levels sufficiently to cause the cardiac hypertrophy and renal lesions in the prorenin transgenic rats. It is also possible the different phenotypes of these 2 prorenin transgenic models represent species specificity in the effects of prorenin.

Renin-Binding Proteins
Evidence that tissues are the main sites of angiotensin formation by a process that involves sequestration of renin led to a search for the binding proteins responsible. Several binding sites for renin and prorenin were described on membranes from different tissues.9,23,24 One renin-binding protein identified in tissue homogenates is identical to N-acyl-D-glucosamine-2-epimerase, a cytosolic protein that inhibits renin in vitro but is not considered to participate in the RAS in vivo.5 The M6P/insulin-like growth factor II receptor binds renin and prorenin with a \( K_d \) of ≈1 nmol/L. This receptor binds only glycosylated proteins with M6P residues. Prorenin binding results in internalization of the M6PR/insulin-like growth factor II–prorenin receptor complex and rapid activation of prorenin but does not result in angiotensin generation. The M6PR/insulin-like growth factor II receptors are considered to serve as clearance mechanisms for renin and prorenin. None of these renin-binding proteins has been shown to contribute to angiotensin formation in tissues.

The (Pro)renin Receptor
Nguyen et al5 reported saturable binding of radiolabeled renin to cultured human mesangial cells, with a dissociation constant \( K_d \) of 0.4 nmol/L, and dissociation of renin was slow, with a half-time of 4 hours. These authors cloned and sequenced the (pro)renin receptor from a human kidney cDNA library, and binding of radiolabeled renin to stably transfected mesangial cells showed a \( K_d \) of 5.0 to 7.8 nmol/L.5 Based on the observed molecular weight of the cross-linked renin-receptor complex, Nguyen et al5 proposed that the (pro)renin receptor exists as a dimer, which was confirmed by Schefe et al.7 Nurun et al26 subsequently cloned and expressed the rat (pro)renin receptor in COS-7 cells and observed binding of rat prorenin to the receptor with a \( K_d \) of 0.89 nmol/L.26 In parallel experiments, human prorenin bound to the human (pro)renin receptor expressed in COS-7 cells with a \( K_d \) of 1.8 nmol/L. Feldt et al27 detected binding of radiolabeled human renin and prorenin to human U937 monocytes that was not reduced by the renin inhibitor aliskiren. By contrast, Batenburg et al28 reported binding of human prorenin, but not renin, to the human (pro)renin receptor expressed by smooth muscle cells from rats transgenic for this receptor; the \( K_d \) for prorenin binding was 5.9 nmol/L.

The (pro)renin receptor gene is expressed widely, with high expression of its mRNA in human brain, heart, placenta, and, to a lesser extent, in liver, pancreas, and kidney, as well as weak expression in lung and skeletal muscle.5 It is also expressed in the human retina.29 Hybridization in situ demonstrated (pro)renin mRNA expression in glomeruli, tubules, and vessels of rat kidney.30,31 Immunocytochemical studies showed that the main site of expression of the (pro)renin receptor in the kidney of rats and humans was the distal renal tubule, and it was also identified within the mesangial area of glomeruli, in vascular smooth muscle cells of renal and coronary vessels, in neonatal rat cardiomyocytes, and in human monocytes.27

Nguyen et al5 reported that binding to the (pro)renin receptor increased the catalytic efficiency of renin and caused activation of prorenin. Nurun et al5 also found the binding of rat and human prorenins to their respective receptors caused 30% to 40% activation, but with no change in catalytic efficiency, and internalization of receptor-bound prorenin did not occur.26 Batenburg et al28 found a similar activation of human prorenin bound to the human (pro)renin receptor expressed by smooth muscle cells from rats transgenic for this receptor, although <5% of the added prorenin actually bound to cells, and the increase in renin activity represented only 1.5% of the prorenin added to the cells.

A critical issue in the interpretation of (pro)renin receptor binding studies is whether the affinity of the receptor is sufficient for binding of the picomolar levels of renin and prorenin that occur in vivo. Given the affinity of the (pro)renin receptor for renin, Nguyen et al5 acknowledged that it cannot be considered as a binding protein responsible for retaining renin in tissues because only ≈1% of soluble renin is expected to bind to the receptor. Sites of renin synthesis, such as the kidneys, ovaries, testes, adrenal glands, and retina, may have higher prorenin concentrations, but whether these concentrations are sufficient to bind to the (pro)renin receptor is unknown. Notably, the (pro)renin receptor in heart and vasculature is expected to have minimal occupancy by renin or prorenin.

Binding of Renin and Prorenin to the (Pro)renin Receptor Initiates Signal Transduction
Binding of renin and prorenin to the (pro)renin receptor initiates signal transduction mechanisms that are independent of participation by angiotensin peptides. Human renin (16 to
100 nmol/L) increased tritiated thymidine incorporation by human mesangial cells, and human prorenin (2 nmol/L) stimulated proliferation of human vascular smooth muscle cells. Renin (10 nmol/L) induced phosphorylation of the renin receptor, and both renin and prorenin induced phosphorylation of extracellular signal-related protein kinase (ERK) 1 and ERK2, increased mitogen-activated protein kinase (MAPK) activity, and induced phosphorylation of heat shock protein 27 in different cell types but did not affect intracellular Ca²⁺ or cAMP. The increase in ERK 1/2 and heat shock protein 27 phosphorylation was prevented by MAPK kinase inhibition and was independent of angiotensin II signal transduction and epidermal growth factor receptor phosphorylation. Similar MAPK activation was produced by glycosylated and nonglycosylated renin. Recombinant human and rat renins also stimulated transforming growth factor (TGF)-β1 and plasminogen activator inhibitor-1 production by human and rat mesangial cells, and the renin-induced stimulation of plasminogen activator inhibitor-1, fibronectin, and collagen production by rat mesangial cells was blocked by a TGF-β antibody. Small-inhibiting RNA targeted to (pro)renin receptor mRNA prevented stimulation of TGF-β1 mRNA by renin and stimulation of ERK 1/2 phosphorylation by prorenin, thereby demonstrating the participation of the (pro)renin receptor in these responses. Renin- and prorenin-induced activation of ERK 1/2 and MAPK and stimulation of heat shock protein 27 phosphorylation were not reduced by renin inhibitors. Microarray gene transcription profiling of myocytes stimulated with prorenin detected 260 regulated genes. The lowest concentrations of renin and prorenin shown to stimulate signal transduction included 100 pmol/L of human renin for stimulation of TGF-β1 production by human mesangial cells, and 20 pmol/L of human prorenin for stimulation of ERK 1/2 phosphorylation in human vascular smooth muscle cells, and 1 pmol/L of rat renin for stimulation of ERK 1/2 phosphorylation in rat mesangial cells.

Schefè et al analyzed the signal transduction mechanisms of the (pro)renin receptor in human cervical carcinoma (Hela-S3) and transformed human embryonic kidney (HEK293) cells stimulated with 10 nmol/L of renin. They found evidence for a novel signal transduction cascade involving direct physical interaction of the (pro)renin receptor with the transcription factor promyelocytic zinc finger protein and observed a short negative feedback loop whereby renin stimulation caused promyelocytic zinc finger translocation to the nucleus and partial repression of (pro)renin receptor transcription.

Several of these studies of signal transduction by the (pro)renin receptor used nanomolar concentrations of renin and prorenin that were several orders of magnitude higher than circulating levels. Although the discrepancy between the picomolar concentrations of renin and prorenin in biological fluids and the nanomolar affinity of the (pro)renin receptor means that the (pro)renin receptor is unlikely to be responsible for retaining renin or prorenin in tissues, this argument does not apply to the activation of signal transduction, where low receptor occupancy may produce receptor activation. The stimulation of signal transduction by 1 pmol/L of rat renin and 20 pmol/L of human prorenin suggests that basal renin and prorenin levels may activate this transduction pathway in vivo. However, it remains to be established whether any of these renin- and prorenin-induced signal transduction mechanisms operate in vivo. It will be of interest to learn whether the short negative feedback loop, whereby renin stimulation caused promyelocytic zinc finger translocation to the nucleus and repression of (pro)renin receptor transcription, operates in vivo. If such a mechanism were to operate in vivo, it may protect against any harmful effect of (pro)renin receptor stimulation by the increased renin levels that accompany RAS inhibitor therapies. In support of such a possibility, renal (pro)renin receptor mRNA levels were reduced by 18% in rats administered a vasopeptidase inhibitor, and were 30% lower in double transgenic rats expressing the human renin and angiotensinogen genes than in normal Sprague-Dawley rats. However, evidence against the operation of this short-loop feedback repression of (pro)renin receptor transcription in vivo was the lack of suppression of (pro)renin receptor mRNA levels in either the clipped or unclipped kidney of 2-kidney, 1-clip Goldblatt rats.

The (Pro)renin Receptor Is a Multidomain, Multifunction Protein With Multiple Identities The (pro)renin receptor is a 350–amino acid single membrane domain protein with a large nonglycosylated and highly hydrophobic amino-terminal domain, considered responsible for the binding of renin and prorenin, and a short cytoplasmic tail of ~20 amino acids. Analysis of the sequence of the (pro)renin receptor reveals that it has multiple identities. It is identical to ATPase, H⁺ transporting, lysosomal accessory protein 2 (ATP6AP2), and endoplasmic reticulum–localized type I transmembrane adaptor precursor (CAPER), indicating essential intracellular roles for this protein. ATP6AP2 associates with vacuolar ATPases that exert several cellular functions, such as neurotransmitter uptake and storage, endocytosis, and receptor recycling. CAPER associates with phosphatase of regenerating liver-1, which is involved in the regulation of cellular proliferation and transformation and exhibits a cell cycle–dependent subcellular localization, being localized to the endoplasmic reticulum in resting cells and to centrosomes and the spindle apparatus in mitotic cells. Immunocytochemical studies confirmed the predominantly intracellular perinuclear location of the (pro)renin receptor, with only a minor portion on the cell surface and colocalization with the endoplasmic reticulum was shown to be because of a C-terminal atypical retention motif. Evidence that the (pro)renin receptor/ATP6AP2/CAPER protein has a role in the brain is the report of a family with X-linked mental retardation and epilepsy, because of a silent mutation in exon 4 of the (pro)renin receptor gene, producing partial exon skipping and partial deletion of exon 4.

Genetic Models of (Pro)renin Receptor Expression The (pro)renin receptor gene knockout mouse is reported to be embryonic lethal, suggesting an essential function of the protein in development, although these studies do not reveal whether renin or prorenin binding or some other property of
the protein is essential. In agreement with these findings, Amsterdam et al.\(^4^1\) reported that insertional mutation of the (pro)renin receptor gene in zebrafish led to marked changes in the gut and nervous system and was lethal.

Human (pro)renin receptor transgenic rats with the transgene ubiquitously expressed in all of the tissues examined had normal blood pressure, urinary sodium excretion, plasma renin activity, and plasma and renal angiotensin II levels. They did, however, have increased renal cyclooxygenase-2 expression\(^2^8\) and developed proteinuria and glomerulosclerosis with aging, accompanied by MAPK activation and increased TGF-β1 expression in the kidney.\(^4^3\) A different phenotype was seen in human (pro)renin receptor transgenic rats with transgene expression targeted to smooth muscle tissue.\(^4^4\) These rats were fertile and developed normally, and after 6 months of age they developed elevated blood pressure and heart rate. Plasma renin and kidney function were normal, but plasma aldosterone levels were elevated.\(^4^4\) Despite high expression of the transgene,\(^4^4\) vascular smooth muscle cells from these rats showed only a small increase in prorenin binding.\(^2^8\)

The phenotype of overexpressing transgenic animals is difficult to interpret, because the unregulated expression of a protein may exceed any pathophysiological relevance. The different phenotypes of the 2 (pro)renin receptor transgenic models may relate to differences in the targeting and magnitude of transgene expression.\(^4^2–4^4\) A further difficulty with interpretation of the phenotype of (pro)renin receptor transgenic rats is the unknown contribution of the different domains of the (pro)renin receptor/ATP6AP2/CAPER protein to the phenotype. Any contribution by renin or prorenin to the phenotype requires their binding to, and activation of, the human (pro)renin receptor. In support of this possibility, recombinant rat prorenin stimulated ERK 1/2 phosphorylation in human (pro)renin receptor–expressing COS-7 cells, although there was no activation of rat prorenin.\(^4^3\) It is, however, unknown whether endogenous levels of rat renin and prorenin were sufficient to trigger signaling by the human (pro)renin receptor in these transgenic models.

**Handle Region Peptide and the Decoy Hypothesis**

Based on studies of the binding of prorenin to monoclonal antibodies, Suzuki et al.\(^4^5\) proposed that a segment of the prorenin prosegment, called the handle region, participates in the binding of prorenin to its receptor. They further proposed that synthetic handle region peptides (HRP), corresponding with amino acids 10 to 19 of the prorenin prosegment, would interfere with prorenin binding (the decoy hypothesis). In support of this hypothesis, they showed that rat HRP and human HRP blocked the binding of their respective prorenins to their respective recombinant (pro)renin receptors expressed by COS-7 cells, with a \(K_i\) of 6.6 nmol/L.\(^2^6\) Moreover, human HRP prevented prorenin-induced activation of ERK 1/2 in COS-7 cells expressing the human (pro)renin receptor.\(^4^3\)

Ichihara et al.\(^4^6\) tested the decoy hypothesis in vivo by administering HRP to various experimental models of disease. Rat HRP completely prevented the development of diabetic nephropathy in heminephrectomized streptozotocin-diabetic rats, without affecting hyperglycemia; caused regression of established diabetic nephropathy in these rats;\(^3^7\) attenuated the development and progression of proteinuria and glomerulosclerosis and reduced renal angiotensin levels in stroke-prone spontaneously hypertensive rats without an effect on blood pressure;\(^3^1\) and attenuated cardiac fibrosis in spontaneously hypertensive rats fed a high-salt diet.\(^4^8\) Mouse HRP and rat HRP also attenuated various pathologies in mice,\(^4^9–5^1\) and human HRP prevented the proteinuria and glomerulosclerosis that developed in human (pro)renin receptor transgenic rats.\(^5^3\)

By contrast, other laboratories were unable to confirm the decoy hypothesis. Neither rat nor human HRP at 1 μmol/L affected prorenin binding, prorenin activation, or renin binding by vascular smooth muscle cells from rats transgenic for the human (pro)renin receptor.\(^2^8\) Human HRP at 1 μmol/L did not affect binding of radiolabeled renin or prorenin\(^2^7\) or renin- and prorenin-induced ERK 1/2 phosphorylation in human U937 monocytes.\(^2^7,5^3\) Attempts to reproduce the effects of HRP in vivo were also unsuccessful. Administration of a combination of rat and human HRP (each at 3.6 μg/kg per day) had no effect on blood pressure, premature mortality, or renal damage in double transgenic rats expressing the human renin and angiotensinogen genes, and a 30-fold higher dose was also without effect.\(^3^5\) Furthermore, rat HRP (3.6 μg/kg per day) did not affect blood pressure, cardiac hypertrophy, or renal damage in 2-kidney, 1-clip Goldblatt rats.\(^3^6\) Rat HRP had no effect on myocardial collagen content, systemic hemodynamics, left ventricular function, or coronary or renal hemodynamics in spontaneously hypertensive rats, although left ventricular mass was reduced by 8%.\(^5^2\)

The major differences between laboratories in the reported effects of HRP are unexplained. A key difficulty with interpretation of the HRP experiments is uncertainty in whether the doses of HRP administered were sufficient to influence binding of prorenin to the (pro)renin receptor and whether interference with prorenin binding was the mechanism of the reported effects of HRP. A small peptide such as HRP is expected to be rapidly metabolized and cleared, but the plasma levels of HRP achieved in these studies were not reported. By comparison, administration of angiotensin II to 200-g rats by SC minipump at 40 ng/min (288 μg/kg per day) produced plasma angiotensin II levels of \(\approx 150\) pmol/L.\(^5^3\) It is, therefore, unlikely that an SC infusion rate of 1.8 to 3.6 μg/kg per day,\(^3^1\) or even a 30-fold higher dose,\(^3^5\) would achieve the nanomolar HRP concentrations required to inhibit prorenin binding to its receptor. Ichihara and colleagues\(^3^1,5^3\) described the localization of fluorescent-labeled HRP to renal glomeruli and tubular lumen after 28 days of administration. However, there is uncertainty regarding whether the fluorescent material identified represented intact HRP associated with the (pro)renin receptor.

**Future Directions**

Despite extensive research effort and significant advances in the understanding of renin, prorenin, and the (pro)renin receptor, there are important gaps in the evidence for a new paradigm for the RAS involving prorenin and the (pro)renin receptor. The (pro)renin receptor/ATP6AP2/CAPER protein...
has essential roles in development, the brain, and other areas of biology, but it remains to be established whether any function of this protein in vivo involves renin or prorenin. Critical to a new paradigm for the RAS involving prorenin and the (pro)renin receptor is the need to demonstrate a role for prorenin in biological fluids. The low levels of angiotensin peptides in anephric subjects and the lack of effect of prorenin administration indicate that prorenin is unlikely to play a role in vivo, apart from its low intrinsic activity. The cardiac hypertrophy and renal lesions of male prorenin transgenic rats should be interpreted with caution, because the 400-fold increase in prorenin levels was 2 orders of magnitude greater than that which occurs in physiological or pathological states, and the phenotype may have been because of the intrinsic activity of the markedly elevated prorenin levels. It is, therefore, important to determine whether RAS inhibition corrects the phenotype of these rats.

New experimental approaches are required to investigate a potential role for prorenin in biological fluids that involves the (pro)renin receptor and is unrelated to angiotensin II. Genetic approaches could target either prorenin or its receptor. One approach targeting prorenin might be to administer, or to create transgenic animals overexpressing, a form of prorenin with mutation of the 2 aspartyl residues of its active site so that it is unable to contribute to angiotensin formation but is still able to bind to the (pro)renin receptor and initiate signal transduction. Genetic approaches to the (pro)renin receptor/ATP6AP2/CAPER protein could target its interactions with renin and prorenin by modifying specific amino acid residues of the (pro)renin receptor that contribute to renin and prorenin binding, thereby abolishing renin and prorenin binding without change in its other functions.

There is need for clarification of the mechanism of the reported effects of HRP. The binding of renin, prorenin, and HRP with nanomolar affinities suggests that the (pro)renin receptor has 2 separate binding domains, 1 binding renin and 1 binding HRP and the prorenin prosequence. As noted by Nguyen, it is difficult to understand how the reported effects of HRP could be so profound if HRP does not influence the binding of renin to the (pro)renin receptor, given that the (pro)renin receptor would remain exposed to stimulation by renin during HRP administration. It is also difficult to understand how HRP could be so effective in preventing diabetic glomerulosclerosis through a nonangiotensin II–mediated mechanism, given the clinical success of RAS inhibitors in slowing diabetic nephropathy. If the new paradigm for the RAS involving prorenin and the (pro)renin receptor is to provide more effective therapies, it will be necessary to develop better inhibitors of the (pro)renin receptor that target renin and prorenin binding or its signal transduction mechanisms.

In conclusion, many fundamental questions remain to be answered concerning the role of prorenin and the (pro)renin receptor in vivo. Recent prorenin and (pro)renin receptor research has provided exciting possibilities, but more research is necessary to show which possibilities are real.

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