Prorenin, Renin, and Their Receptor
Moving Targets
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In addition to its well-known role in regulating blood pressure and fluid balance, the renin-angiotensin-aldosterone system (RAAS) has continued to fascinate both researchers and clinicians because of the several additional functions that it has been proposed to carry out. Disruption of the production or action of angiotensin II (Ang II) during development, either by gene mutation or by pharmacological blockade, results in tubular agenesis and anemia in the fetus (humans) or offspring (laboratory animals) and is most often lethal. Some of the suspected, but still unproven, roles of angiotensin peptides include effects on energy metabolism, longevity, memory, autoimmune diseases, and a direct, blood pressure–independent effect on tissue damage. This latter role is extremely hard to prove, because the effects of Ang II on blood pressure are hard to separate from its potential direct effects on tissues both in the laboratory and in clinical trials. The most recent developments in RAAS research have led to the suggestion that tissue damage may not be solely attributable to the action of angiotensin peptides but may also involve a direct effect of renin and its protein precursor, prorenin.

The concept that prorenin and renin play a direct role in vascular pathologies is largely built on 4 lines of evidence. First is the finding that elevations of circulating prorenin were associated (and may even precede) diabetic microvascular disease. Second is the report that transgenic rats engineered to have a 400-fold increase in circulating prorenin developed severe cardiac remodeling and renal lesions in the absence of hypertension, raising the possibility that prorenin was not only associated with tissue damage but that it may even be responsible for the damage by a mechanism that did not require Ang II generation. Third is the discovery of the so-called (pro)renin receptor ((P)RR), which binds both prorenin and renin with nanomolar affinity, causes an unfolding of prorenin rendering it capable of contributing to local Ang II generation, and triggers several mitogen-activated protein kinase (MAPK) signaling pathways on prorenin and renin binding (Figure). Because this MAPK stimulation occurs in the presence of RAAS inhibitors, the triggered signaling appears to be independent of the angiotensin-generating enzymatic activity of prorenin and renin. The fourth major line of support came from a series of experiments suggesting that inhibiting the binding of renin and prorenin to (P)RR with a competing peptide called handle region peptide (HRP; later also called prorenin receptor blocker or PRRB; see Figure) reduced cardiac hypertrophy and fibrosis in hypertensive rats without reducing blood pressure, reduced diabetic glomerulosclerosis and proteinuria in rats and in mice in which the Ang II type 1 (AT₁a) receptor had been inactivated, and reduced pathological, but not physiological, retinal neovascularization. Altogether, these data have led to a model in which renin and prorenin, through their interaction with (P)RR, stimulate a signal other than Ang II that promotes cardiac remodeling, microvascular damage, and retinal neovascularization. Not surprisingly, these reports have stirred the hope that new and more effective treatments could be developed to prevent secondary organ damage in hypertension and diabetes mellitus by blocking the action of renin or prorenin on (P)RR. It is also important to revisit the key findings that brought us to this new hope, because these 4 lines of evidence have been severely tested in the last couple of years. Where do we stand today?

A Re-Examination of the Role of Elevated Prorenin

The report in 1996 by Véniant et al that very high circulating prorenin levels in transgenic rats resulted in cardiac and renal lesions in the absence of hypertension has stood as the single most important experimental evidence linking prorenin to pathologies by a novel (non-angiotensin-mediated) mechanism. Although those experiments consisted of overexpressing rat prorenin in rats, in 2008 Peters et al reported a variant of that experiment using an inducible liver promoter to regulate the release of mouse Ren-2 prorenin into the circulation of rats. These investigators were able to achieve a 200-fold increase in circulating prorenin for a period of ≤6 weeks and got significantly different results from those of Véniant et al: as prorenin increased in the cyp1a1ren-2 transgenic rats, so did blood pressure. More strikingly, there was no evidence of the glomerular injury attributed previously to high circulating prorenin levels. Could the differences in these studies be because of the shorter time during which circulating prorenin is increased or the use of mouse prorenin in the study by Peters et al? More recently, Mercure et al again revisited this question by overexpressing mouse (Ren-1) prorenin in mice using a

Received October 29, 2009; first decision November 16, 2009; revision accepted February 8, 2010.
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(Hypertension. 2010;55:1071-1074.)
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Hypertension is available at http://hyper.ahajournals.org

DOI: 10.1161/HYPERTENSIONAHA.108.120279

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constitutive liver promoter resulting in a lifetime 30- to 60-fold increase in circulating prorenin levels. Again, high levels of circulating prorenin were associated with hypertension. Moreover, this hypertension was not evident when prorenin carrying an active site mutation was overexpressed at equivalent levels, and the blood pressure was quickly normalized by angiotensin-converting enzyme inhibitor treatment, suggesting that it was because of increased Ang II production. Like the study by Peters et al,22 however, Mercure et al23 found no evidence for the severe cardiac and renal lesions originally reported by Véniant et al.10 How can these significant differences be explained?

A very recent report suggests that the transgenic rats originally reported by Véniant et al10 may not be so different from the more recently generated models; the same transgenic rats used in the study of Véniant et al10 were later found to be hypertensive by 3 months of age.24 Perhaps more surprisingly, these animals had no evidence of cardiac or renal lesions at 6 months of age,24 although they had ~1000 times the normal prorenin levels. Thus, in spite of somewhat different transgenic approaches, these studies agree on 2 conclusions. First, prorenin, when present at very high concentrations, can lead to hypertension, and although Camp­bell et al24 did not detect increased Ang II in the circulation of their transgenic rats, both Mercure et al23 and Mitchell et al25 demonstrated that the resulting hypertension responds to classic RAAS inhibitors, suggesting that Ang II production is responsible and that it occurs in tissues in these models. The second and perhaps most clinically important conclusion of the more recent studies is that there is no direct relationship between elevated levels of circulating prorenin and renal lesions, although the animal models achieved much greater prorenin levels (30- to 1000-fold) than have been reported in diabetic nephropathy (0.5- to 3.0-fold).8

**(P)RR: A Protein With Another Job and on the Move**

Although Nguyen et al11 originally reported that (P)RR had no homology with any known membrane protein, it has more recently become obvious that the coding sequence of (P)RR is identical to that of ATP6AP2, an accessory protein to a vacuolar H-ATPase (reviewed in Reference 26; Figure). The role of the v-H-ATPase is to acidify intracellular vesicular bodies including endosomes and lysosomes, as well as to acidify urine in the collecting ducts. This latter function is difficult to reconcile with the original report that (P)RR was primarily located in mesangial cells and colocalized with renin.11 Although several subsequent studies have confirmed the presence of (P)RR in mesangial cells, a recent re-examination of its kidney distribution by Advani et al27 revealed that (P)RR is predominantly expressed in the collecting ducts and distal tubules, known locations for the v-H-ATPase.

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**Figure.** Schematic representation showing the 3 proposed roles of (P)RR/ATP6AP2. First, (P)RR exists as a dimer on the cell surface and can bind renin, prorenin, and peptides corresponding with a portion of the prorenin prosegment (HRP, PRRB, and PRAM-1). Binding of renin and prorenin triggers the phosphorylation of MAPKs. Shown in white boxes are the binding affinities of rat renin and human prorenin, as well as the calculated inhibitory constant for HRP (data are from References 12 and 13). The red box in the prorenin prosegment shows the location of the peptide sequence corresponding to HRP. Second, (P)RR may also be cleaved by furin to release a renin/prorenin-binding protein in the circulation. Third, (P)RR is identical to ATP6AP2, a protein that associates with vacuolar-type H+-ATPase. It is not clear whether (P)RR exists as a monomer or dimer in the second and third roles.
(P)RR was found to colocalize with H+-ATPase in the microvilli at the apical surface of intercalated A cells in the collecting ducts, where it presumably contributes to urinary acidification. Other results have also contributed to the view that (P)RR does more than bind to prorenin and renin. (P)RR is ubiquitously expressed in humans, and attempts to inactivate the gene for (P)RR in the mouse result in preimplantation lethality of the embryo. In contrast, inactivation of the renin-angiotensin system components (e.g., angiotensinogen, renin, or angiotensin-converting enzyme) in the mouse does not result in embryonic lethality. Natural mutations in (P)RR/ATP6AP2 in humans lead to mental retardation and epilepsy, and the human mutation has been shown recently to act in a dominant-negative fashion to redirect (P)RR/ATP6AP2 in neuronal cells. All of these findings are compatible with (P)RR/ATP6AP2 acting as a critical component of the v-H+-ATPase. As such, it is probably time to acknowledge that (P)RR and ATP6AP2 are, in fact, the same protein and that the small amount of ATP6AP2 that makes it to the cell surface might bind renin and prorenin. Two mysteries remain. First, how (or perhaps, more appropriately, where) do prorenin and renin, which circulate at picomolar levels, achieve the necessary concentrations to bind (P)RR/ATP6AP2, which has nanomolar affinity for these ligands? Added to this conundrum is the recent discovery that (P)RR can also be cleaved in the secretory pathway to release a renin-binding form into the circulation (Figure). The biological function of such a soluble (P)RR is unknown. The second remaining mystery is how renin/prorenin binding to ATP6AP2 triggers MAPK signaling, because neither the ATP6AP2 nor the v-H+-ATPase is a signaling receptor.

**Binding of Prorenin, Renin, and HRP to (P)RR**

Several experiments have now suggested that the HRP/PRRB peptide, derived from the prosegment of prorenin, could have therapeutic applications (see above). Although most of the positive studies have come from Ichihara and collaborators, other groups have more recently reported variable findings with these peptides. Susac et al repeated the test of HRP (they call it PRAM-1) in spontaneously hypertensive rats. Although peptide infusion did not reduce ventricular fibrosis in spontaneously hypertensive rats as reported previously, PRAM-1 infusion considerably reduced serum creatinine, left ventricular mass, and cardiac fibrosis and improved cardiac function without affecting blood pressure in 16-week-old spontaneously hypertensive rats fed a high-salt diet for 8 weeks, leading them to suggest that prorenin might directly mediate some of the salt-induced tissue damage/remodeling. In contrast, this same group has reported that all of the negative effects of a high-salt diet on renal blood flow, urinary albumin excretion, and glomerular filtration rate in the spontaneously hypertensive rats disappeared if the animals were treated with an angiotensin receptor blocker, implicating Ang II in the kidney pathology. Matavelli et al also recently revisited the role of PRRB (HRP) in diabetic nephropathy. They found that both an angiotensin receptor blocker (valsartan) and PRRB reduced urinary albumin and renal inflammatory markers in diabetic rats, whereas only the angiotensin receptor blocker reduced (P)RR expression in these kidneys. Could this explain the apparent treatment overlap in these 2 approaches? In fact, the complete dissociation between AT1 receptor action and HRP/PRRB has never been satisfactorily proven. HRP was found to be effective in reversing diabetic renal damage in mice with inactivation of the AT1 receptor, leading to the suggestion that the signaling mechanism of (P)RR was independent of Ang II. However, mice express 2 AT1 receptors (AT1a and AT1b), and the presence of the AT1b receptor in AT1a-deficient mice still leaves open the possible contribution of Ang II signaling in these pathologies. Notably, HRP infusion was not effective in preventing nephrosclerosis in the Goldblatt model of hypertension and did not reduce blood pressure in mice with elevated circulating prorenin. Thus, the prorenin prosegment-derived peptides (HRP, PRRB, and PRAM-1) have a mode of action that is still difficult to discern from whole animal studies. What have cell culture models revealed?

There have also been some conflicting data concerning the interaction of prorenin, renin, and HRP (or PRR) to (P)RR. Although active renin has been reported to bind to (P)RR with an affinity of 20 nM, renin does not bind to smooth muscle cells isolated from transgenic rats overexpressing (P)RR in that cell type. In contrast, prorenin does bind to these cells, although the amount of enzymatic activation that occurs on its binding now appears to be much lower (2-fold over the basal 1% to 2%) than originally reported (≤40%). In addition, it has been reported that HRP displaces both renin and prorenin from (P)RR with an inhibition constant of ~7 nM, recent reports have not been able to confirm that HRP blocks either prorenin binding or signaling through (P)RR in cultured cells. It is, in fact, difficult to conceive how HRP, a peptide analogous to a portion of the prorenin prosegment, can compete for binding of renin, which does not contain the prosegment (Figure). For this schema to function, (P)RR would have to have 2 independent, high-affinity binding sites for renin and prorenin, both of which are blocked by HRP binding.

As is often the case in science, what began as a simple and compelling explanation for the role of prorenin in diabetic microvascular disease has evolved into a much more complex relationship between prorenin, (P)RR, and HRP-related peptides. Animal models no longer support a direct link between elevated circulating prorenin and tissue damage/remodeling; (P)RR is actually ATP6AP2 with another role to fulfill, and it is no longer clear how HRP-related peptides function. The more recent research has, indeed, raised a number of new questions. Why are HRP-related peptides beneficial in certain, but not all, animal models of disease? How is prorenin contributing to angiotensin peptide generation in tissues without contributing to active renin levels? Does this involve an unfolding of prorenin mediated by (P)RR/ATP6AP2? Could the binding of prorenin to ATP6AP2 somehow affect the function of the v-H+-ATPase? How does this binding link with MAPK signaling? Finally, it will be important to better understand the significant overlap between HRP-related peptide function and classic RAAS inhibition in the treatment and prevention of secondary tissue damage to know if the hope of new therapeutic strategies has, in fact, been realized.

**Sources of Funding**

This work was funded by a grant from the Canadian Institutes of Health Research (MOP-89803) to T.L.R.
Disclosures

None.

References


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Hypertension. 2010;55:1071-1074; originally published online March 8, 2010;
doi: 10.1161/HYPERTENSIONAHA.108.120279
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/55/5/1071

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