The (Pro)Renin Receptor: A New Kid in Town

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Summary: Renin inhibitors are now available in therapeutic doses and it is accepted that they decrease blood pressure as efficiently as the classic inhibitors of the renin-angiotensin system (RAS): angiotensin converting enzyme inhibitors and angiotensin II–receptor blockers (ARBs). One major issue will be to know how, beyond the normalization of blood pressure, renin inhibitors (RIs) will compare with angiotensin converting enzyme inhibitors and ARBs for their ability to protect the organs against the tissue damage associated with overactivation of the RAS. The mechanism(s) of tissue protection may involve the inhibition of a direct cellular effect of renin and prorenin mediated by the (pro)renin receptor ([P]RR). This review updates the recent findings on (P)RR; its role in hypertension, cardiac fibrosis, diabetic nephropathy, and retinopathy; and the effects of a putative (P)RR antagonist.

Keywords: Renin-angiotensin system, renin, prorenin, and (pro)renin receptor

The renin-angiotensin system (RAS) is becoming more and more complex. In 3 decades, the classic intravascular system aimed at the generation of angiotensin II (Ang II), considered a unique biologically active peptide, has been enriched with new enzymes, such as angiotensin converting enzyme 2 and chymase, and new receptors such as for angiotensin IV and for (pro)renin ([pro]renin refers to renin and prorenin collectively). These discoveries will affect our comprehension of the RAS profoundly, but they also may help to improve the use of RAS blockers, which are among the most widely used drugs. Several recent reviews have discussed (pro)renin binding proteins, but this review focuses on the specific receptor of (pro)renin, called the (pro)renin receptor ([P]RR). We mainly discuss the role of (P)RR in prorenin activation and the effects of the putative (P)RR antagonist in diabetic nephropathy, cardiac fibrosis, and endotoxin-induced uveitis.

THE (P)RR

The (P)RR receptor is a 350-amino acid protein with no homology with any known protein. The primary structure analysis showed the existence of the following: (1) a signal peptide, which is indicative of a secreted protein; (2) a large ectodomain responsible for renin and prorenin binding; (3) a single transmembrane domain; and (4) a short cytoplasmic domain involved in the intracellular signaling.

Renin and prorenin bind equally well to (P)RR and their binding has 2 fundamental effects: (1) binding increases renin catalytic activity, remarkably, allowing prorenin to become active; and (2) binding triggers the activation of mitogen-activated protein kinases p44/p42 or extracellular regulated kinases 1/2 (ERK1/2).

Increased Catalytic Activity of Receptor-Bound Renin and Prorenin

One fundamental property of (P)RR is that its existence now provides a functional role for prorenin, the proenzyme inactive form of renin. This functional role for prorenin was suspected long ago. Prorenin is released constitutively from the kidney and its concentration in plasma is 10-fold higher than the concentration of renin. Unlike other proteases, prorenin
does not undergo self-activation and the unique maturation site of prorenin into renin is the myoepithelioid cell of the juxtaglomerular apparatus where the pro-segment of renin is cleaved-off by a still unknown pro-convertase. This gain of activity of receptor-bound prorenin is attributed to a conformational change, provoking the loosening of the pro-segment and making the enzymatic cleft accessible to angiotensinogen. This phenomenon is reversible by eluting prorenin from the receptor and is called nonproteolytic activation, in contrast to the proteolytic activation of prorenin caused by cleavage of the pro-segment to produce the mature renin, which is an irreversible process. The increased catalytic activity by a nonproteolytic activation of receptor-bound prorenin was confirmed using recombinant rat (P)RR and prorenin.

The crystal structure of renin was established some time ago, but there is no crystal structure of prorenin, and therefore of the pro-segment, available so far. However, when comparing the pro-segment of prorenin with the pro-segment of pepsinogen, another aspartate protease, one can identify 2 leucine residues in the sequence R<sup>10</sup>ILL<sup>13</sup>KKMPSV<sup>19</sup> of the pro-segment. The pro-segment of prorenin acts as a trapdoor that covers the active site. In this trapdoor, leucine 13 (L<sup>13</sup>), which is extremely conserved among aspartyl-protease proenzymes and among species, plays a crucial role in keeping the trapdoor closed (Daniel Bur, personal communication). Mutation of L<sup>13</sup> results in an unstable pro-segment and spontaneously active prorenin. By using an antibody to this region that likely reacted with the central L<sup>13</sup>, Suzuki et al obtained a nonproteolytic activation of prorenin and called this region the handle region (HR), using an analogy for the handle of a picnic basket.

By using the HR peptide, Ichihara et al subsequently showed that infusion of HR peptide was able to inhibit cardiac and renal damage induced by hypertension and diabetes, and to provide protection from uveitis induced by lipopolysaccharide. In all these studies the investigators showed an increase of nonproteolytic activation of prorenin by immunohistochemistry using an antibody that recognizes specifically the nonproteolytically activated prorenin and not the native prorenin or mature renin.

In diabetic rats, HR peptide infusion normalized renal angiotensin I and angiotensin II levels that retuned to values comparable with those of control animals, whereas plasma angiotensins were not modified. The proteinuria was normalized and glomerulosclerosis was inhibited completely.

In stroke-prone spontaneously hypertensive rats (SHRsp), HR peptide infusion was able to prevent cardiac fibrosis as a result of increased expression of cardiac (P)RR. Furthermore, HR peptide also could prevent renal damage and normalized transforming growth factor-β (TGF-β1) in SHRsp.

But the most convincing argument of the pathogenic role of prorenin nonproteolytic activation and of (P)RR activation came from the study of the protective effect of HR peptide in diabetic angiotensin II type 1a receptor-deficient mice. Not only did HR peptide attenuate the development of proteinuria and glomerulosclerosis, but it also inhibited the increased ERK1/2 of p38 and of c-Jun N-terminal kinase (JnK) activation in the diabetic kidney, confirming once more that (P)RR can exert a pathologic role independently of Ang II generation. Most importantly, the renal protection achieved with HR peptide was superior to that of an angiotensin converting enzyme inhibitor in diabetic wild-type animals.

To summarize, the results of these experimental studies have raised 2 interesting hypotheses: (1) that prorenin was the main ligand of (P)RR in vivo and (2) that HR peptide may act as an antagonist of (P)RR.

However, many questions still remain unanswered.

**What is the Exact Mechanism of Action of HR Peptide?**

Although the effect of HR peptide was impressive in vivo, its mechanism of action was never shown. Indeed, it was reported that HR peptide may block the binding of prorenin to Chinese hamster ovary (CHO) cells overexpressing (P)RR, however, no data were shown. In addition, there was a mention that HR peptide...
behaves similar to a partial agonist of (P)RR and was able to activate ERK1/2, and this is in complete contradiction with the report that HR peptide infusion inhibited ERK1/2, p38, and JnK activation in the kidney of diabetic mice. Much work is needed and data should be provided that show the binding of HR peptide to the (P)RR and the inhibition of (pro)renin binding by HR peptide before the HR peptide is called a (P)RR antagonist.

**What Would be the Effect of HR Peptide on Situations Associated With Increased Renin?**

Recent reports of rats treated with angiotensin converting inhibitor and on a low-salt diet suggested that the receptor was down-regulated in vivo by high concentrations of renin, although the mechanism of this negative feedback was not established. However, this observation supports the in vitro demonstration of (P)RR gene expression down-regulation by renin via the activation of the transcription factor promyelocytic zinc finger (PLZF). Therefore, if HR peptide behaves similar to an agonist, even a partial one as mentioned by Ichihara et al, one would expect a down-regulation of the receptor during HR peptide infusion, but this was not the case in any of the models described by this group. Moreover, if (P)RR binds renin and prorenin, then why does HR peptide infusion inhibit all the deleterious effects of increased renin and prorenin? Because it was postulated that the HR peptide mimics the domain of prorenin supposedly involved in the interaction with (P)RR, one would expect a competitive inhibition of prorenin binding, not of renin, and therefore only a partial protection by HR peptide infusion and not a complete inhibition of tissue damages such in SHRsp rats.

**GENETIC ALTERATIONS OF THE (P)RR**

The gene of the (P)RR is called ATP6ap2 and is located on the X chromosome in the locus p11.4. The genetic alterations of the (P)RR gene gave unexpected results. Overexpression of (P)RR is Associated With High Blood Pressure and Increased Cyclooxygenase-2 Expression

Transgenic rats overexpressing the human (P)RR in smooth muscle tissue have high blood pressure and increased plasma aldosterone, whereas ubiquitous overexpression induced increased cyclooxygenase-2 expression in the renal cortex. Because there was neither change in plasma renin activity nor in tissue angiotensin II content in the animals, it is likely that the phenotype was dependent on the overexpression of the receptor but independent of Ang II.

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cells was not possible but appeared to be incompatible with their incorporation into the blastocyst (Michael Bader, personal communication), and \((P)RR/ATP6ap2\) gene ablation in the zebrafish provoked the death of the fish before the end of the embryogenesis.\(^{29}\)

Remarkably, a mutation in the \((P)RR/ATP6ap2\) gene is associated with mental retardation and epilepsy and, surprisingly, no cardiovascular or renal dysfunctions were observed in these patients.\(^{30}\) However the patients were young at the time of examination and we cannot exclude that the dysfunctions will appear later in life.

The impossibility of a total ablation of the \((P)RR/ATP6ap2\) gene and the mental retardation associated to \((P)RR/ATP6ap2\) gene mutation point to an essential role of \((P)RR\) in cell survival and in the central nervous system development. To add another degree of complexity to the \((P)RR\), the gene of \((P)RR\) has many different names when performing a search for the nucleotide sequence homology because the protein was cloned from different sources. However, there is only one single gene named \(ATP6ap2\)\(^{31}\) for proton adenosine triphosphatase (ATPase) accessory protein 2 because a truncated form of \((P)RR\) initially was described to co-precipitate with a vacuolar proton-ATPase (V-ATPase).\(^{32}\) Altogether, these data suggest that the \((P)RR\) is a complex molecule and that its gene might originate from the fusion of 2 genes, one ancestral gene coding for the C-terminus transmembrane and cytoplasmic regions and responsible for conserved cellular functions, and one gene coding for the N-terminus ectodomain conserved in vertebrates and responsible for (pro)renin binding and signaling.\(^{33}\)

**CONCLUSIONS**

Now that the receptor is becoming a reality, one of the major issues is to show its physiologic role and to study its involvement in cardiovascular and renal pathologies. These studies are fundamental and deserve careful examination because the question of a potential therapeutic benefit of a \((P)RR\) antagonist depends on their results.

The results of recent studies have suggested that in tissues the major effector is prorenin and that renin would have a negligible role. If this is indeed the case, then we should ask whether renin inhibitors that now are available for therapeutic use would be able to inhibit receptor-bound nonproteolytically activated prorenin and therefore could be beneficial for tissue protection. Nevertheless, there are renin inhibitors known to modify the structures of renin and of prorenin drastically\(^{34}\) and it would be of interest to know whether these inhibitors also can modify (pro)renin and receptor interaction.

**REFERENCES**


