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Review

(Pro)renin receptor as a therapeutic target for the treatment of cardiovascular diseases?

Yuan Sun[a,b], A.H. Jan Danser[b], Xifeng Lu[a,*]

[a] AstraZeneca-Shenzhen University Joint Institute of Nephrology, Department of Physiology, Shenzhen University Health Science Center, Shenzhen University, Shenzhen, China
[b] Erasmus Medical Center, Department of Internal Medicine, Division of Pharmacology and Vascular Medicine, Rotterdam, The Netherlands

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ABSTRACT

The discovery of the (pro)renin receptor [(P)RR] 15 years ago stimulated ideas on prorenin being more than renin's inactive precursor. Indeed, binding of prorenin to the (P)RR induces a conformational change in the prorenin molecule, allowing it to display angiotensin-generating activity, and additionally results in intracellular signaling in an angiotensin-independent manner. However, the prorenin levels required to observe these angiotensin-dependent and −independent effects of the (P)RR are many orders above its *in vivo* concentrations, both under normal and pathological conditions. Given this requirement, the idea that the (P)RR has a function within the renin-angiotensin system (RAS) is now being abandoned. Instead, research is now focused on the (P)RR as an accessory protein of vacuolar H^+-ATPase (V-ATPase), potentially determining its integrity. Acting as an adaptor between Frizzled co-receptor LRP6 and V-ATPase, the (P)RR appears to be indispensable for Wnt/β-catenin signaling, thus explaining why (P)RR deletion (unlike renin deletion) is lethal even when restricted to specific cells, such as cardiomyocytes, podocytes and smooth muscle cells. Furthermore, recent studies suggest that the (P)RR may play important roles in lipoprotein metabolism and overall energy metabolism. In this review, we summarize the controversial RAS-related effects of the (P)RR, and critically review the novel non-RAS-related functions of the (P)RR, ending with a discussion on the potential of targeting the (P)RR to treat cardiovascular diseases.

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1. Introduction

The renin-angiotensin system (RAS) is a key system regulating blood pressure and controlling body fluid homeostasis. Renin is formed by cleavage of the N-terminal prosegment from its non-active precursor prorenin exclusively in the juxtaglomerular cells of the kidney and secreted into the circulation in a controlled manner. Active renin catalyzes the conversion of angiotensinogen to angiotensin (Ang) I, which is then further converted by angiotensin-converting enzyme (ACE) to Ang II, the main effector molecule of the RAS. Unlike renin, prorenin is constantly secreted into the circulation by the kidney and other organs, such as the eye, reproductive organs and adrenal gland [1]. Plasma prorenin levels are in excess of plasma renin levels under physiological conditions, and can be up to 100-fold higher under pathological conditions such as diabetes mellitus [2–4]. Since proteolytic activation of prorenin

* Corresponding author at: Department of Physiology, Shenzhen University Health Science Center, room 517, Shenzhen University, Nanhai Avenue 3688, Shenzhen, China.
E-mail addresses: x.lu@szu.edu.cn, lxfstart@vip.163.com (X. Lu).

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has never been demonstrated outside the juxtaglomerular cells, it seemed unlikely that prorenin would somehow contribute to angiotensin generation at tissue sites. Consequently, prorenin's function remained solely as the inactive precursor of renin until the discovery the (pro)renin receptor [(P)RR] fifteen years ago. Upon binding to the (P)RR, prorenin is activated in a non-proteolytic manner. In addition, binding of renin and prorenin triggers intracellular signaling pathways independent from the generation of Ang II. This indicates that the (pro)renin-(P)RR interaction could be a potential target for treating cardiovascular and renal complications, and it soon drew great attention. Now, more than a decade later, it is still not clear how and to what degree the (P)RR contributes to end-organ damage and whether this involves local RAS activation. Yet, novel and Ang II-independent functions of the (P)RR have been identified, as an accessory protein of vacuolar H^+-ATPase (V-ATPase), with important roles in Wnt signaling, low-density lipoprotein (LDL) clearance, and glucose metabolism. In this review, we summarize the latest findings on the novel functions of the (P)RR and discuss the pharmacological potential of targeting the (P)RR for the treatment of cardiovascular diseases.

2. (P)RR and the RAS

The (P)RR is a 350-amino acid protein with a single transmembrane domain, encoded by the *ATP6AP2* gene located on the X chromosome, which is highly conserved in vertebrates [5]. The C-terminal fragment (CTF) of the (P)RR is identical to the 8.9 kDa accessory protein of V-ATPase [6], and the N-terminal domain (NTD) of the (P)RR can bind both renin and prorenin [7], denoted together as (pro)renin. The (P)RR induces a conformational change in prorenin upon binding, resulting in full exposure of the catalytic cleft with the prosegment still being present [7–10]. In addition, the (P)RR also directly stimulates intracellular signaling networks, including extracellular signal-regulated kinases 1/2 (Erk1/2) activation in vascular smooth muscle cells, meningeal cells, monocytes, collecting duct cells, endothelial cells and adipocytes, and phosphatidylinositol 3-kinase/Akt (PI3K/Akt) activation in HEK293T cells [11–19] (Fig. 1). Activation of these signaling pathways can subsequently result in upregulation of profibrotic genes including transforming growth factor-β1, plasminogen-activator inhibitor 1 (PAI-1), fibronectin and collagen-1 [12,20,21]. As a consequence, the (P)RR might directly (i.e., independent from Ang II formation) promote tissue damage. If true, it would be a novel target to prevent cardiovascular and renal complications. In agreement with this concept, aging transgenic rats overexpressing the human (P)RR develop proteinuria and glomerulosclerosis [22] in the absence of hypertension, and ACE inhibition, although capable of lowering Ang II, did not prevent this renal damage.

A peptidic antagonist (handle region peptide, HRP) has been designed based on the concept that the prosegment of prorenin contains a "handle region" that binds to the (P)RR [23]. HRP should thus theoretically occupy the binding pocket of the (P)RR, preventing prorenin binding and activation. Initially, studies reported that HRP indeed prevented nephropathy and retinopathy in streptozotocin-induced diabetic human (P)RR transgenic rats [24–26] and reduced ocular inflammation, cardiac hypertrophy and cardiac fibrosis in other pathological animal models [27–30], as reviewed elsewhere [31]. However, later studies demonstrated no or even detrimental effects of HRP in hypertensive and diabetic models [30,32–36]. In addition, *in vitro* studies showed that HRP does not prevent (pro)renin-induced signaling, not even when applied at micromolar concentrations, and that HRP might actually behave as a partial agonist of (P)RR [14,30,37,38]. Even more confusing, new transgenic mice models with enhanced (P)RR expression show no alterations in blood pressure, and no damage in the heart or kidney [39,40].

Contrasting data on the (P)RR-prorenin interaction in the brain have also been reported. Given the low, if not absent, renin levels in the brain, prorenin should be the exclusive agonist of the (P)RR. Indeed, the brain (P)RR has been suggested to play an important role in prorenin-stimulated signaling pathways associated with the pathogenesis of neurogenic hypertension [41]. Inhibiting (P)RR expression in the brain using shRNA decreased blood pressure and vasomotor sympathetic tone, possibly through a reduction in Ang II type 1 receptor expression [42]. Moreover, neuron-specific (P)RR KO mice, showing a normal cardiovascular phenotype, were resistant to salt-dependent hypertension [43]. Conceptually in line with these studies, intracerebroventricular infusion of the newly developed HRP-like (P)RR peptidic inhibitor PRO20 attenuated DOCA-salt induced hypertension [44]. Interestingly, Ang II upregulates the expression of brain (P)RR by increasing cAMP response element-binding protein binding to the promoter of the (P)RR [45]. The (P)RR is expressed in hypothalamic magnocellular neurosecretory cells and in the parasympathetic paraventricular nucleus, and prorenin stimulates the neuronal activities in these areas via both Ang II-dependent and −independent effects [46]. Nevertheless, recently, we were unable to detect prorenin in the brain, nor did brain (pro)renin levels increase following the induction of neurogenic hypertension with DOCA-salt [47]. Taken together these studies suggest that the (P)RR-prorenin interaction in the brain is highly unlikely, and that the central effects of putative (P)RR antagonists like HRP and PRO20 occur independently of the RAS [44]. Importantly, this does not exclude the possibility that the brain (P)RR plays a role in blood pressure regulation via a non RAS-mediated pathway. In fact, given the above findings, it is highly likely that there is a link between the (P)RR and neurogenic hypertension. To solve these discrepancies, a detailed pharmacological characterization of PRO20 is still anxiously awaited.

Cleavage of the NTD of the (P)RR by proteases such as furin and ADAM19 (a disintegrin and metalloproteinase 19) is followed by its secretion into the circulation [48,49]. The secreted form of the (P)RR is denoted as s(P)RR [soluble (P)RR]. Initially, s(P)RR was thought to be able to bind and activate prorenin in the circulation, resulting in chronic RAS overactivation. However, this hypothesis was incorrect [50]. In fact, to what degree the (pro)renin-(P)RR interaction truly occurs *in vivo* remains questionable until today. As reviewed elsewhere, to observe angiotensin-dependent and −independent effects in *in vitro* experiments, the required prorenin levels are 1000-fold and 10,000-fold, respectively, above its normal physiological (i.e., picomolar) concentrations [31]. Such elevations are unlikely to ever occur *in vivo*, even under pathophysiological conditions, and have also never been achieved in transgenic animal models. Although the collecting duct is believed to be a prorenin-synthesizing site which abundantly expresses the (P)RR, also in collecting duct cells 10 nmol/L prorenin was required to activate Erk1/2 [51]. This implies that even at sites where prorenin-(P)RR interaction is theoretically feasible, it may not happen easily *in vivo*. In agreement with this concept, a recent study using kidney-specific (P)RR knockout mice observed that renal Ang II production, sodium handling and angiotensin-dependent blood pressure regulation were not affected by (P)RR abolishment [52]. Finally, unlike other RAS components, deletion of (P)RR in mice is lethal, even when (P)RR deletion is restricted to cardiomyocytes [53], podocytes [54,55] or nephron progenitor cells [56]. Taken together, the (pro)renin-(P)RR interaction is unlikely to occur in normal physiology, and the (P)RR is more likely to play an important role beyond the RAS.