HHS Public Access

Author manuscript

Pharmacol Ther. Author manuscript; available in PMC 2017 August 01.

Published in final edited form as:

Pharmacol Ther. 2016 August ; 164: 126–134. doi:10.1016/j.pharmthera.2016.04.006.

The critical role of the central nervous system (pro)renin receptor in regulating systemic blood pressure

Quanbin Xu[1], **Dane D. Jensen**[1], **Hua Peng**[2], and **Yumei Feng**[1]

[1]Departments of Pharmacology and Physiology & Cell Biology, Center for Cardiovascular Research, University of Nevada School of Medicine, Reno, NV, USA

[2]Department of Pediatrics, Union Hospital, Tongji Medical College, Huangzhong University of Sciences and Technology, Wuhan, China

Abstract

The systemic renin–angiotensin system (RAS) has long been recognized as a critically important system in blood pressure (BP) regulation. However, extensive evidence has shown that a majority of RAS components are also present in many tissues and play indispensable roles in BP regulation. Here, we review evidence that RAS components, notably including the newly identified (pro)renin receptor (PRR), are present in the brain and are essential for the central regulation of BP. Binding of the PRR to its ligand, prorenin or renin, increases BP and promotes progression of cardiovascular diseases in an angiotensin II-dependent and -independent manner, establishing the PRR a promising antihypertensive drug target. We also review the existing PRR blockers, including handle region peptide and PRO20, and propose a rationale for blocking prorenin/PRR activation as a therapeutic approach that does not affect the actions of the PRR in vacuolar H^+-ATPase and development. Finally, we summarize categories of currently available antihypertensive drugs and consider future perspectives.

Keywords

Hypertension; (Pro)renin receptor; Prorenin; Brain; Antihypertensive drugs; (Pro)renin receptor antagonists

Correspondence To: Yumei Feng, MD, PhD, FAHA, Assistant Professor, Departments of Pharmacology and Physiology & Cell Biology, University of Nevada School of Medicine, 1664 N. Virginia Street, Reno, Nevada, 89557, Phone: (775)-784-4116, yumeifeng@medicine.nevada.edu.

Disclosures

The authors declare no conflicts of interest, financial or otherwise.

1. Introduction

1.1 Endocrine/systemic renin-angiotensin systems

The renin–angiotensin system (RAS) is one of the most important systems in blood pressure (BP) regulation. The RAS has been the target of active research interest since the discovery of renin more than 100 years ago (Tigerstedt R & PG., 1898), reflecting its importance in the pathogenesis of hypertension and other cardiovascular diseases (Ellis, et al., 2012; Hsueh & Wyne, 2011; Re, 2004; Te Riet, van Esch, Roks, van den Meiracker, & Danser, 2015; Vijayaraghavan & Deedwania, 2011). In the traditional view of the systemic RAS, active renin is generated by the removal of a prosegment from prorenin, a precursor of renin, in the juxtaglomerular cells of the kidney. Active renin is released into the circulation and cleaves its only known substrate, angiotensinogen (AGT), which is primarily synthesized in the liver, generating the decapeptide, angiotensin (Ang) I. Ang I is further converted into Ang II by angiotensin-converting enzyme (ACE), which is predominantly expressed on endothelial cells of the pulmonary circulation and the kidney (Cushman & Cheung, 1971; Ryan, Ryan, Whitaker, & Chiu, 1976). Ang II, the major bioactive peptide of the RAS, binds to G-protein-coupled Ang II receptors, including AT_1R and AT_2R (Vinturache & Smith, 2014), and contributes to BP regulation as well as the pathogenesis of hypertension by regulating sympathetic activity, vasoconstriction, sodium retention, thirst, and aldosterone synthesis and secretion from the adrenal cortex.

1.2 Tissue/local renin-angiotensin systems

There is considerable evidence that a majority of RAS components are also present in many tissues and exert indispensable roles in BP regulation (Bader, 2010). Renin production is the rate-limiting step for systemic RAS activity. However, the kidney secretes both renin and prorenin in response to a decrease in renal perfusion and sodium chloride concentration (Hsueh & Baxter, 1991). Although renin is produced only in the renal juxtaglomerular apparatus, prorenin is synthesized in many tissues apart from the kidney, including the adrenal gland, ovary, testis, placenta, retina, and the brain (Bader, et al., 2001; Danser, et al., 1989; Hsueh & Baxter, 1991; von Lutterotti, Catanzaro, Sealey, & Laragh, 1994). Renin is produced from prorenin by cleavage of a 43-amino-acid N-terminal prosegment. This can be accomplished by enzymes such as glandular kallikrein 13, cathepsin D, and pepsin *in vitro* (Morris, 1978) (Kim, et al., 1991). However, more recent studies in cultured HEK-293 cells suggest that renin production might not require a specific enzyme, but rather is mediated by general hydrolysis in lysosome-like granules of juxtaglomerular cells (Schmid, Oelbe, Saftig, Schwake, & Schweda, 2013; Xa, Lacombe, Mercure, Lazure, & Reudelhuber, 2014). The prosegment prevents the exposed renin catalytic site from interacting with AGT, as reflected in the fact that prorenin has only 3% of the intrinsic activity of fully active renin (Lenz, et al., 1991). These findings have suggested the concept that prorenin is an inactive biosynthetic precursor of renin (Hsueh & Baxter, 1991). However, this concept cannot explain why some tissues that only produce prorenin, such as the brain, have a significant amount of Ang II (Hermann, McDonald, Unger, Lang, & Ganten, 1984; Hirose, Naruse, Ohtsuki, & Inagami, 1981). In fact, more recent evidence has shown that prorenin can be activated independently of conventional enzymes or lysosome-like granules through

association with a membrane protein termed the (pro)renin receptor (PRR), also called APT6AP2 (Nguyen, et al., 2002).

The PRR is a 350-amino-acid protein composed of a large extracellular domain (ECD; ~310 amino acids), a single transmembrane domain (TMD; ~20 amino acids), and an intracellular domain (ICD; ~19 amino acids). Under physiological conditions, the PRR is expressed at high levels in the heart, brain and placenta, and at low level in the kidney and liver (Nguyen, et al., 2002). It exists as a homodimer, formed through interactions involving its ECD and ICD domains (Suzuki-Nakagawa, et al., 2014; Zhang, Gao, & Michael Garavito, 2011). The discovery of the PRR revealed a new RAS regulatory mechanism.

The discovery of the PRR revealed a new RAS regulatory mechanism. The PRR binds and increases the enzymatic activity of renin and prorenin (Nguyen, et al., 2002), functioning as a tissue-originating activator of prorenin that increases the activity of prorenin to a level comparable to that of free, active renin (Nguyen, et al., 2002). The association of prorenin with the PRR is mediated by both the prosegment and mature fragment of renin (Nabi, et al., 2009a; Nabi & Suzuki, 2009). These interacting regions form the basis for the development of peptides, including the handle region peptide (R^{10P} IFLKR^{15P}), the decoy peptide (R^{10P}IFLKRMPSI^{19P}), the hinge region peptide (S^{149P}QGVLKEDVF^{158P}) and the PRO20 peptide (L^{1P}PTRTATFERIPLKKMPSVR^{20P}), which abrogate PRR-prorenin interactions by competitively binding to the PRR (W. Li, Sullivan, et al., 2014; Nabi, et al., 2009a; Nabi & Suzuki, 2009). The involvement of the prosegment in PRR binding not only increases the binding affinity, but also enables prorenin to adopt a conformation suitable for recognition of the substrate AGT (Morales, Watier, & Bocskei, 2012; Nabi, et al., 2009b). The conformational change in prorenin upon PRR binding increases the activity of prorenin to a level 3- to 4-fold higher than that of renin (Nabi, et al., 2009b), suggesting that prorenin exerts its function mainly at the level of tissues where the PRR is expressed. However, this leaves an interesting evolutionary question: how did juxtaglomerular cells of the kidney retain the ability to cleave prorenin to renin, while most other tissues in the body use prorenin and probably the PRR as the regulator of the RAS?

Unlike renin, which is secreted by juxtaglomerular cells but functions systematically, the PRR appears to be a bona fide local player, which serves at the level of the tissue where it is produced. In the kidney, the PRR is mainly expressed in cells of the collecting ducts and in the distal nephron (Advani, et al., 2009), where it may complex with vacuolar H^+-ATPase to regulate proton transport (Advani, et al., 2009; Daryadel, et al., 2016). Expression of the PRR is regulated by changes in sodium concentration induced by the cGMP-dependent protein kinase (PKG) pathway in the kidney (Huang & Siragy, 2011; Quadri & Siragy, 2016; Rong, et al., 2015). In addition, PRR expression levels are regulated by Ang II through CREB (cAMP response element binding protein) in the central nervous system (CNS) during hypertension (W. Li, et al., 2015), and a cyclooxygenase (COX)-2–dependent pathway in the kidney (Wang, et al., 2014). Increased PRR expression may further promote the production of Ang II, ultimately resulting in positive feedback regulation of the receptor itself and the development of hypertension (Wang, et al., 2014; T. Yang, 2015).

The PRR is involved in the development of diabetic nephropathy through enhancement of renal production of the inflammatory factors tumor necrosis factor (TNF)-α and interleukin (IL)-1β (Matavelli, Huang, & Siragy, 2010). In addition, the PRR is also an important regulator in smooth muscle cells, including arterial smooth muscle. A transgenic mouse in which the PRR is overexpressed in smooth muscle cells is prone to the development of hypertension in association with increased heart rate (Burckle, et al., 2006). *In vitro* assays have shown that expression of the PRR in smooth muscle cells contributes to cell migration by regulating cytoskeletal reorganization, small GTPase activation, and $pp125^{FAK}$ cleavage (Greco, et al., 2012). These findings suggest that expression of the PRR in smooth muscle may contribute to arterial integrity (Kurauchi-Mito, et al., 2014). Notably, knockout of the PRR in smooth muscle cells in mice results in nonatherogenic sclerosis in the abdominal aorta, but leaves BP unaffected (Kurauchi-Mito, et al., 2014). This suggests that the PRR serves other essential functions unrelated to the RAS that mask its role in hypertension. Actually, the PRR has been identified as an essential regulator of *Xenopus* development through its role in the Wnt signaling pathway. Here, the PRR mediates activation of the Wnt receptor, LRP6, by recruiting V-ATPase, resulting in β-catenin activation (Cruciat, et al., 2010). Following up on this, Li and Siragy provided evidence showing that the PRR may also contribute to high-glucose–induced podocyte injury through the PRR-Wnt-β-catenin-snail signaling pathway (C. Li & Siragy, 2014).

Upon binding to prorenin, the PRR engages intracellular signaling networks independent of Ang II. These include the mitogen-activated protein kinases (MAPKs), p38 and ERK1/2 (extracellular signal-regulated kinases 1 and 2, p40/42), and their downstream targets, such as heat shock protein 27, tumor growth factor (TGF)-β, c-Jun N-terminal kinase (JNK) and NADPH oxidase, resulting in enhanced production of pro-inflammatory cytokines and expression of promyelocytic zinc finger (PLZF) protein (Feng, 2015). In cultured rat inner medullary cells, binding of prorenin to the PRR can promote ERK1/2 activation and COX-2 upregulation in an Ang II-independent manner (Gonzalez, Luffman, Bourgeois, Vio, & Prieto, 2012). Over-activation of the PRR contributes to diabetic nephropathy, as evidenced by the fact that PRR blockade abolishes MAPK activation and reverses the progression of nephropathy (Ichihara, et al., 2004; Ichihara, et al., 2006; Takahashi, et al., 2007). Consistent with this, treatment with high levels of glucose increases PRR levels via protein kinase C (PKC)-Raf-ERK and PKC-JNK-c-Jun signaling pathways (Huang & Siragy, 2010). This renal-protective effect of PRR blockade also involves the TGF-β1 signaling cascade (Huang, Matavelli, & Siragy, 2011). These findings suggest that over-activation of the PRR contributes to diabetic nephropathy through RAS-independent signaling pathways. In addition, renin induces tyrosine or serine phosphorylation of the PRR *in vitro* independent of Ang II (Nguyen, et al., 2002). Whether these phosphorylation events directly link the PRR to activation of MAPK or other Ang II-independent signal transduction pathways is currently unknown.

2. The PRR is a master regulator of BP in the brain RAS

The existence of a blood-brain barrier (BBB) between the brain and the circulation prevents circulating RAS components, including renin and Ang II, from reaching most brain area, except for the circumventricular organs (CVOs), such as the subfornical organ (SFO), the

vascular organ of lamina terminalis (OVLT) and area posterma (AP), under physiological conditions (Bader, 2010). It is now well accepted that the brain has its own local RAS, in part because the majority of RAS components are known to be expressed in the brain. Both AT_1R and AT_2R are abundantly expressed in the CNS (Allen, Zhuo, & Mendelsohn, 2000; Bunnemann, et al., 1992; Johren, Inagami, & Saavedra, 1995; Lenkei, Palkovits, Corvol, & Llorens-Cortes, 1996; Zhuo, et al., 1998). AT_1R is expressed in brain areas involved in BP and fluid homeostasis, including the paraventricular nucleus (PVN) and the supraoptic nucleus of the hypothalamus, the rostral ventral lateral medulla (RVLM), the nucleus tractus of solitarii (NTS), the SFO, and the AP. The presence of AT_1R in the SFO is indispensable for Ang II-induced hypertension (Sakai, et al., 2007). AT_2R is expressed at a relatively lower level in the lateral septum, several thalamic nuclei, the subthalamic nucleus, the locus coeruleus, and the inferior olive nucleus (Zhuo, et al., 1998). AT_2R exerts an antagonistic effect on AT_1R upon binding to their common ligand, Ang II, and thus appears to function as a negative regulator of BP (Z. Li, et al., 2003). The opposite function of these two receptors suggests the existence of a fine regulatory network, but the exact mechanisms underlying its operation are not yet clear. ACE is also prominently expressed throughout the brain (Zhuo, et al., 1998), enabling Ang II to be produced locally. Transgenic mice with increased Ang II generation in the brain develop hypertension (Morimoto, et al., 2001; Morimoto, Cassell, & Sigmund, 2002), highlighting the physiological relevance of this local Ang II production. ACE2 (Tipnis, et al., 2000), a mono-carboxypeptidase sharing 42% homology with ACE, further cleaves Ang II to Ang-(1–7). Ang-(1–7) has an effect opposite that of Ang II in that it stimulates nitric oxide (NO) release, improves baroreceptor reflexes sensitivity, and promotes vasodilation (Rabelo, Alenina, & Bader, 2011). Consistent with this, overexpression of human ACE2 in neurons or specifically in the RVLM or SFO causes a reduction in BP in Ang II-induced hypertensive mice and spontaneously hypertensive rats (SHR) (Feng, et al., 2008; Xia, et al., 2015; Yamazato, Yamazato, Sun, Diez-Freire, & Raizada, 2007). AGT in the brain is synthesized and secreted from astroglial cells (Campbell, Bouhnik, Menard, & Corvol, 1984; Deschepper, Bouhnik, & Ganong, 1986; Sernia & Mowchanuk, 1983) and neurons (G. Yang, Gray, Sigmund, & Cassell, 1999), and its levels are high in cerebrospinal fluid (Schelling, Ganten, Sponer, Unger, & Ganten, 1980). In contrast, whether renin and prorenin in the brain are sufficient to convert AGT into bioactive angiotensin peptides has remained controversial for decades owing to the undetectable levels of active renin in the CNS (Cuadra, Shan, Sumners, & Raizada, 2010). Although enzymes such as tonin and cathepsins are abundant in the CNS (Lomez, Araujo, Bader, Pesquero, & Pesquero, 2002) (Klickstein, Kaempfer, & Wintroub, 1982), there is no solid evidence to show that the enzymatic pathway for Ang II generation by tonin functions under physiological conditions.

Our recent studies showed that the PRR is a master regulator of central BP that contributes to the majority of Ang II generation in the brain (W. Li, Peng, et al., 2014; W. Li, Peng, Seth, & Feng, 2012). In the CNS, PRR mRNA and protein are expressed mainly in neurons and to a lesser extent in astrocytes. In cardiovascular regulatory nuclei of the brain, including the SFO, PVN, RVLM, NTS and AP, the PRR is expressed exclusively in neurons, and not astrocytes, as shown in Figure 1. However, in the lateral cortex of the brain, some PRR immunostaining is detectable in astrocytes (W. Li, Peng, Cao, et al., 2012; W. Li, Peng, Seth,

et al., 2012; Shan, Cuadra, Sumners, & Raizada, 2008). There is also evidence for expression of PRR protein in microglial cells, and it has been shown that prorenin promotes the release of pro-inflammatory factors in cultured microglial cells through activation of the NF-κB signaling pathway (Shi, et al., 2014). However, the functional importance of the PRR in microglial cells has not yet been clearly established.

Evidence for the importance of the PRR in the central regulation of BP comes from studies of PRR knockdown in the mouse brain (W. Li, Peng, Cao, et al., 2012). Knockdown of the PRR in the SFO of the human renin and AGT transgenic hypertensive mouse model by intracerebroventricular (ICV) delivery of short-hairpin RNA was shown to significantly reduce PRR expression level, leading to decreased BP and cardiac and vasomotor sympathetic tone, and improved baroreceptor reflex sensitivity (W. Li, Peng, Cao, et al., 2012). These changes were associated with a concomitant decrease in AT_1R expression and vasopressin levels in the SFO and PVN, respectively (W. Li, Peng, Cao, et al., 2012). In a study by Raizada and colleagues (Shan, et al., 2010), knockdown of the PRR in the supraoptic nucleus of SHR model animals was found to reduce BP and cause a decrease in heart rate and plasma vasopressin. To further test the hypothesis that PRR-mediated, non-proteolytic activation of prorenin is the main source of Ang II in the brain, we generated a neuron-specific PRR (ATP6AP2)-knockout mouse model using the Cre-LoxP system. Importantly, PRR knockout in neurons significantly decreased the elevated BP and Ang II formation induced by ICV infusion of prorenin (W. Li, Peng, et al., 2014). Furthermore, PRR knockout in neurons prevented the development of deoxycorticosterone acetate (DOCA)-salt–induced hypertension as well as activation of cardiac and vasomotor sympathetic tone (W. Li, Peng, et al., 2014). In summary, most RAS components are found in the CNS. Instead of active renin, prorenin, activated by binding to PRR, might be the key angiotensinogenase in the CNS. As we previously reported, the neuronal PRR is present in the cell membrane as well as the cytoplasm (W. Li, Peng, et al., 2014). Accordingly, we propose that the PRR acts through two pathways of Ang II formation—intracellular and extracellular—to serve as a master regulator of the brain RAS and sympathetic activity in neurons, as shown in Figure 2.

3. The development of PRR antagonists

Despite the commercial availability of dozens of antihypertensive drugs, efforts to control BP in hypertensive patients still faces many challenges. In approximately 20–30% of hypertensive patients, BP is not controlled by existing drugs (Calhoun, et al., 2008), and hypertension-related cardiovascular diseases are still the number one cause of morbidity and mortality in the USA (Kochanek, Murphy, & Xu, 2015). A major category of antihypertensive drugs encompasses those that target the RAS, including ACE-inhibitors, AT_1R blockers, and renin inhibitors (Figure 3). These drugs function at different steps in the RAS cascade, providing important treatment options for patients (Feig, Roy, & Cody, 2010). However, long-term administration of ACE-inhibitors or AT_1R blockers causes elevation of circulating active renin levels and enhanced production of Ang I and Ang II, with subsequent return of aldosterone secretion to pre-treatment levels (Pitt, 1995; Riccioni, 2013). The development of renin antagonists has been a priority for more than 50 years since the initial discovery that renin production is the rate-limiting step in the RAS signaling

cascade. Importantly, the discovery of aliskiren, the first non-peptide, oral renin inhibitor with long-term effectiveness, has improved treatment efficacy by reducing the activity of rebound elevation of renin and Ang II levels (van den Meiracker & Jan Danser, 2007). Aliskiren exerts its effects by blocking the association of renin with its substrate angiotensinogen, but not with the PRR. Alisikiren also decreases PRR protein levels in glomeruli, tubules and cortical vessels, exerting an additional renal-protective role in hypertension (Feldman, et al., 2008). Nevertheless, aliskiren does increase renin levels (Feldman, et al., 2008), suggesting activation of a feedback network to the RAS. Consistent with these findings, clinical data show that aliskiren works better when combined with other antihypertensive drugs (Azizi, et al., 2004; O'Brien, et al., 2007; Siragy, 2011). Evidence that the PRR—the newly discovered RAS component that mediates both Ang II formation and Ang II-independent intracellular signaling—plays a significant role in the development of hypertension and cardiovascular end-organ damage through prorenin (Ichihara, Itoh, & Inagami, 2008) suggests that the PRR might be a promising new target for the treatment of hypertension. The development and experimental applications of PRR antagonists are summarized below.

3.1. Handle region peptides

The first PRR blocker was a 10-amino-acid decoy peptide with a sequence corresponding to the handle region of the prosegment of prorenin. Both the 10-aminoacid decoy peptide and the entire handle region peptide (HRP) bind the PRR, but the latter has a lower binding affinity (Nurun, et al., 2007). HRP, initially developed by Ichihara and coworkers (Ichihara, et al., 2004; Suzuki, et al., 2003), inhibits the conformational change and non-proteolytic activation of prorenin that occurs upon binding to the PRR (Morales, et al., 2012). HRP has been shown to attenuate the development of diabetic nephropathy in streptozotocin-induced diabetic rats and decrease the levels of Ang I and Ang II, but it has no effect on other components of the RAS (Ichihara, et al., 2004). HRP was also shown to exert a renal-protective effect in PRR transgenic rats. Over-expression of the human PRR induces slowly progressing, Ang II-independent glomerulosclerosis in aged rats, an effect that is significantly decreased by chronic infusion of HRP (Kaneshiro, et al., 2007). Overall, these data indicate that HRP has a greater beneficial effect than ACE-inhibitors in terms of alleviating proteinuria and glomerulosclerosis in experimental animal models of diabetes and essential hypertension (Ichihara, Sakoda, Kurauchi-Mito, Kaneshiro, & Itoh, 2008). However, findings from other independent laboratories have cast doubt on the efficacy of HRP. In a human renin and AGT double-transgenic rat model, HRP failed to reduce BP, attenuate albuminuria, or reduce cystatin C and neutrophil gelatinase-associated lipocalin levels (Feldt, Maschke, Dechend, Luft, & Muller, 2008). Similarly, HRP does not show a protective effect in the clipped kidney, Goldblatt hypertensive rat model (Krebs, et al., 2008). Moreover, in diabetic TGR(mREN2)27 rats, a well-established nephropathy model characterized by high prorenin levels, HRP was shown to counteract the beneficial effects of aliskiren in the kidney, induce hyperkalemia, and increase plasma plasminogen activator-inhibitor 1, renal COX-2, and cardiac collagen content (te Riet, et al., 2014). A recent study showed that HRP may actually have an agonistic effect upon binding to the PRR, increasing phosphorylation of ERK1/2 in the retina (Wilkinson-Berka, Heine, et al., 2010). These

findings suggest that HRP may not be stable and has pleiotropic regulatory effects on PPR activity.

3.2. PRO20

Our laboratory recently developed a PRR peptide antagonist, termed PRO20 (W. Li, Sullivan, et al., 2014), which is derived from the first 20 amino acids of the prorenin prosegment. The design of PRO20 was based on previous reports that the physical structure of prorenin presents multiple possible binding sites for the PRR (Morales, et al., 2012). PRO20 contains most of the previously reported PRR binding sites in the prosegment of prorenin. In addition, the N-terminus of PRO20 is in close proximity to a previously identified PRR-binding domain in both renin and prorenin ($S^{149P}QGVLKEDVF^{158P}$), revealed by the three-dimensional crystal structure of prorenin (Morales, et al., 2012). We propose that PRO20 mimics part of the three-dimensional conformation of the PRR-binding region of prorenin and acts as a competitive antagonist (Figure 4). This conclusion is supported by results of our fluorescent receptor-binding assays, which showed that FITC-labeled PRO20 binding to mouse or human PRR is completely blocked by co-incubation with unlabeled mouse prorenin (W. Li, Sullivan, et al., 2014). PRO20 dose-dependently binds to mouse and human brain tissue with dissociation constants (K_d) of about 4.2 and 1.8 nmol/L, respectively. An *in vitro* functional study has shown that PRO20 inhibits prorenin (4 nmol/L)-induced calcium influx in neuronal cells with a half-maximal inhibitory concentration (IC_{50}) of 81 nmol/L (W. Li, Sullivan, et al., 2014). More importantly, acute ICV infusion of PRO20 was shown to attenuate prorenin-induced hypertension and reduce BP in DOCA-salt–induced and genetic hypertensive mice, whereas chronic ICV infusion of PRO20 attenuated the development of DOCA-salt–induced hypertension and decreased brain Ang II formation (W. Li, Sullivan, et al., 2014). Similar effects were also observed in an Ang II-induced rat hypertension model (Wang, et al., 2015). Recently, PRO20 was shown to block prorenin-PRR–induced ENaC (epithelial sodium channel) activation, which is triggered by a reactive oxygen species (ROS) signaling pathway (Lu, et al., 2015). Unique features of PRO20 compared with HRP revealed by our studies include (1) the prominent ability of PRO20 to reduce BP and Ang II formation in hypertensive mice; (2) direct competition of PRO20 with prorenin to prevent binding to the PRR; and (3) the ability of PRO20 to prevent prorenin-induced ERK1/2 phosphorylation in mouse Neuro-2A cells.

3.3. Selectively blocking prorenin/PRR activation: a new therapeutic strategy for treatment of hypertension and related cardiovascular complications

In addition to the role of the PRR in mediating Ang II formation and activation of Ang II-independent signaling by prorenin, the PRR also plays important roles in cellular homoeostasis through vacuolar H^+-ATPase or canonical and non-canonical Wnt signaling pathways (Danser, 2009; Daryadel, et al., 2016; Ichihara, 2012). The latter has been shown to be critical for embryonic development, as evidenced by the embryonic lethality of PRR gene deletion in mouse and zebrafish models (Danser, 2015). Despite the discovery of the PRR as a novel tissue Ang II-formation pathway, especially in the CNS (W. Li, Sullivan, et al., 2014), and the establishment of its importance in tissue fibrosis, inflammation and end-organ damage (Balakumar & Jagadeesh, 2010; Wilkinson-Berka, Miller, & Binger, 2010), the involvement of the PRR in embryonic development and autophagy has dampened

enthusiasm on the part of the scientific community and pharmaceutical companies for exploring the PRR as a pharmaceutical target. This lack of enthusiasm stems from an overly generalized understanding of the complex PRR signaling pathways. Figure 4 provides a summary of currently known PRR signaling pathways. It is clear that the function of the PRR in mediating Wnt signaling is independent of prorenin or renin (Sihn, Rousselle, Vilianovitch, Burckle, & Bader, 2010). The role of the PRR in facilitating vacuolar H+-ATPase activity in autophagy also does not require prorenin or renin (Cruciat, et al., 2010; Daryadel, et al., 2016). Importantly, however, PRR-mediated activation of MAPKs, including p38, ERK1/2 and JNK signaling pathways, and their downstream activation of plasminogen activator inhibitor-1 (PAI-1), TGF-β, and cyclooxygenase 2 (COX2) transcriptional regulation requires prorenin binding to the PRR (Danser, 2015; Feng, 2015; W. Li, Peng, Seth, et al., 2012). PLZF is a repressive auto-regulatory signaling molecule that is also induced by the binding of prorenin to the PRR (Schefe, Unger, & Funke-Kaiser, 2008). The pathophysiological effects of the PRR are mostly manifested upon over-activation of the PRR by prorenin. Accordingly, we propose that blocking prorenin binding to the PRR (Figure 4) will prevent activation of specific signaling pathways, such as MAPK cascades and their downstream signaling pathways, without affecting the physiological function of PRR in vacuolar H^+-ATPase and Wnt signaling pathways. This conclusion is supported by previous studies showing that HRP and PRO20 do not produce overt adverse effects in multiple experimental animal models (Ichihara, et al., 2006; W. Li, Sullivan, et al., 2014; Muller, et al., 2008; Wang, et al., 2015). In contrast, deletion of the PRR gene, globally or in specific tissues, will certainly result in embryonic lethality because of the essential role of the PRR in H^+-ATPase and Wnt pathways (Danser, 2015).

4. Challenges and future directions

A total of 81 antihypertensive drugs have been approved for hypertension therapy, most of which are ACE-inhibitors, AT_1R blockers, adrenergic receptor blockers (α/β blocker), or calcium channel blockers (Figure 5A). Despite the vast array of available drugs, approximately 20–30% of patients are considered to have resistant hypertension, defined as BP that is not controlled using three or more types of antihypertensive agents (Calhoun, et al., 2008). This creates a need for the development of new categories of antihypertensive drugs. The development of antihypertensive drugs is still a very active field. However, drugs developed based on novel targets are rare; most ongoing development efforts are focused on existing targets, alone or in combination (Figure 5B). The discovery and elucidation of the PRR as a key component of central BP regulation paves the way for the development of potential new antihypertensive drugs. The success of aliskiren suggests that drugs that act upstream of the RAS have advantages over traditional antihypertensive drugs, such as better pharmacokinetics, greater potency (allowing lower drug doses), and better efficacy with fewer side effects (Morganti & Lonati, 2011). These advantages make developing new PRR inhibitors that act upstream of the RAS an attractive proposition. The development of PRR antagonists such as HRP and PRO20 has provided pharmacological tools for studying the physiological and pathophysiological significance of the PRR beyond genetic knockdown models, and suggest the potential of PRR antagonism as a novel approach for treating hypertension and other cardiovascular diseases. However, current antagonists of the PRR are

all peptide-derived, and many challenges that hamper their development into viable drugs remain. For example, the higher molecular weight of peptides limits their ability to traverse the BBB, and the relative instability of peptides in serum makes it difficult to achieve a good pharmacokinetic profile. Strategies for delivering drugs across the BBB have been explored extensively. One such example is the use of transmembrane peptides, such as the HIV tat sequence (Wadia & Dowdy, 2005), that directly penetrate the cell membrane. Another strategy is to exploit receptor-mediated transcytosis, for example by employing a chimeric engineered antibody against the transferrin receptor, a fusion protein containing transferrin, and cholera toxin B (Jain & Jain, 2015). Despite their theoretical promise, the efficiency of such engineered chimeric molecules in traversing the BBB remains inadequate for therapeutic purposes. A breakthrough in BBB-traversing brain-delivery technology is clearly needed.

In summary, the PRR is an emerging, key component of the RAS that mediates both Ang II formation and Ang II-independent signaling. Prorenin, acting via the PRR, plays pivotal roles in regulating BP and cardiovascular end-organ damage. Although many challenges for the development of PRR antagonists remain, accumulating data indicate that the PRR is an important target for hypertension treatment and suggest that PRR antagonists may represent a new category of antihypertensive drugs.

Acknowledgments

Sources of Funding

The work was supported in part by a grant from the National Institutes of Health (NHLBI/R01HL122770) to Y. Feng.

Abbreviations

RAS	renin–angiotensin system
BP	blood pressure
PRR	(pro)renin receptor
AGT	angiotensinogen
Ang I	angiotensin I
Ang II	angiotensin II
ACE	angiotensin-converting enzyme
AT_1R	angiotensin II type 1 receptor
AT_2R	angiotensin II type 2 receptor
MAPKs	mitogen-activated protein kinases
ERK1/2	extracellular signal-regulated kinases 1 and 2
BBB	blood-brain barrier

SFO	subfornical organ
AP	area posterma
PVN	paraventricular nucleus of the hypothalamus
RVLM	rostral ventral lateral medulla
NTS	the nucleus tractus of solitarii
CNS	central nervous system
ICV	intracerebroventricular
DOCA	deoxycorticosterone acetate
PAI-1	plasminogen activator inhibitor-1
COX2	cyclooxygenase 2
NOX4	NADPH oxidase 4
LPR6	low-density lipoprotein receptor-related protein 6
LPR6	low-density lipoprotein receptor-related protein 6

References

Advani A, Kelly DJ, Cox AJ, White KE, Advani SL, Thai K, Connelly KA, Yuen D, Trogadis J, Herzenberg AM, Kuliszewski MA, Leong-Poi H, Gilbert RE. The (Pro)renin receptor: site-specific and functional linkage to the vacuolar H+-ATPase in the kidney. Hypertension. 2009; 54:261–269. [PubMed: 19546380]

Allen AM, Zhuo J, Mendelsohn FA. Localization and function of angiotensin AT1 receptors. Am J Hypertens. 2000; 13:31S–38S. [PubMed: 10678286]

Azizi M, Menard J, Bissery A, Guyenne TT, Bura-Riviere A, Vaidyanathan S, Camisasca RP. Pharmacologic demonstration of the synergistic effects of a combination of the renin inhibitor aliskiren and the AT1 receptor antagonist valsartan on the angiotensin II-renin feedback interruption. J Am Soc Nephrol. 2004; 15:3126–3133. [PubMed: 15579516]

Bader M. Tissue renin-angiotensin-aldosterone systems: Targets for pharmacological therapy. Annu Rev Pharmacol Toxicol. 2010; 50:439–465. [PubMed: 20055710]

Bader M, Peters J, Baltatu O, Muller DN, Luft FC, Ganten D. Tissue renin-angiotensin systems: new insights from experimental animal models in hypertension research. J Mol Med (Berl). 2001; 79:76–102. [PubMed: 11357942]

Balakumar P, Jagadeesh G. Cardiovascular and renal pathologic implications of prorenin, renin, and the (pro)renin receptor: promising young players from the old renin-angiotensin-aldosterone system. J Cardiovasc Pharmacol. 2010; 56:570–579. [PubMed: 21108514]

Bunnemann B, Iwai N, Metzger R, Fuxe K, Inagami T, Ganten D. The distribution of angiotensin II AT1 receptor subtype mRNA in the rat brain. Neurosci Lett. 1992; 142:155–158. [PubMed: 1280791]

Burckle CA, Jan Danser AH, Muller DN, Garrelds IM, Gasc JM, Popova E, Plehm R, Peters J, Bader M, Nguyen G. Elevated blood pressure and heart rate in human renin receptor transgenic rats. Hypertension. 2006; 47:552–556. [PubMed: 16401765]

Calhoun DA, Jones D, Textor S, Goff DC, Murphy TP, Toto RD, White A, Cushman WC, White W, Sica D, Ferdinand K, Giles TD, Falkner B, Carey RM. Resistant Hypertension: Diagnosis, Evaluation, and Treatment: A Scientific Statement From the American Heart Association

Professional Education Committee of the Council for High Blood Pressure Research. Circulation. 2008; 117:e510–e526. [PubMed: 18574054]

Campbell DJ, Bouhnik J, Menard J, Corvol P. Identity of angiotensinogen precursors of rat brain and liver. Nature. 1984; 308:206–208. [PubMed: 6199679]

Cruciat CM, Ohkawara B, Acebron SP, Karaulanov E, Reinhard C, Ingelfinger D, Boutros M, Niehrs C. Requirement of prorenin receptor and vacuolar H+-ATPase-mediated acidification for Wnt signaling. Science. 2010; 327:459–463. [PubMed: 20093472]

Cuadra AE, Shan Z, Sumners C, Raizada MK. A current view of brain renin-angiotensin system: Is the (pro)renin receptor the missing link? Pharmacol Ther. 2010; 125:27–38. [PubMed: 19723538]

Cushman DW, Cheung HS. Concentrations of angiotensin-converting enzyme in tissues of the rat. Biochim Biophys Acta. 1971; 250:261–265. [PubMed: 4334856]

Danser AH. (Pro)renin receptor and vacuolar H+-ATPase. Hypertension. 2009; 54:219–221. [PubMed: 19546373]

Danser AH. The Role of the (Pro)renin Receptor in Hypertensive Disease. Am J Hypertens. 2015; 28:1187–1196. [PubMed: 25890829]

Danser AH, van den Dorpel MA, Deinum J, Derkx FH, Franken AA, Peperkamp E, de Jong PT, Schalekamp MA. Renin, prorenin, and immunoreactive renin in vitreous fluid from eyes with and without diabetic retinopathy. J Clin Endocrinol Metab. 1989; 68:160–167. [PubMed: 2642484]

Daryadel A, Bourgeois S, Figueiredo MF, Gomes Moreira A, Kampik NB, Oberli L, Mohebbi N, Lu X, Meima ME, Danser AH, Wagner CA. Colocalization of the (Pro)renin Receptor/Atp6ap2 with H+-ATPases in Mouse Kidney but Prorenin Does Not Acutely Regulate Intercalated Cell H+-ATPase Activity. PLoS One. 2016; 11:e0147831. [PubMed: 26824839]

Deschepper CF, Bouhnik J, Ganong WF. Colocalization of angiotensinogen and glial fibrillary acidic protein in astrocytes in rat brain. Brain Res. 1986; 374:195–198. [PubMed: 3521789]

Ellis KL, Palmer BR, Frampton CM, Troughton RW, Doughty RN, Whalley GA, Ellis CJ, Pilbrow AP, Skelton L, Yandle TG, Richards AM, Cameron VA. Genetic variation in the renin-angiotensin-aldosterone system is associated with cardiovascular risk factors and early mortality in established coronary heart disease. J Hum Hypertens. 2012; 27:237–244. [PubMed: 22739771]

Feig PU, Roy S, Cody RJ. Antihypertensive drug development: current challenges and future opportunities. J Am Soc Hypertens. 2010; 4:163–173. [PubMed: 20537610]

Feldman DL, Jin L, Xuan H, Contrepas A, Zhou Y, Webb RL, Mueller DN, Feldt S, Cumin F, Maniara W, Persohn E, Schuetz H, Jan Danser AH, Nguyen G. Effects of aliskiren on blood pressure, albuminuria, and (pro)renin receptor expression in diabetic TG(mRen-2)27 rats. Hypertension. 2008; 52:130–136. [PubMed: 18490518]

Feldt S, Maschke U, Dechend R, Luft FC, Muller DN. The putative (pro)renin receptor blocker HRP fails to prevent (pro)renin signaling. J Am Soc Nephrol. 2008; 19:743–748. [PubMed: 18235083]

Feng Y. ANG II-independent prorenin/(pro)renin receptor signaling pathways in the central nervous system. Am J Physiol Heart Circ Physiol. 2015; 309:H731–733. [PubMed: 26209058]

Feng Y, Yue X, Xia H, Bindom SM, Hickman PJ, Filipeanu CM, Wu G, Lazartigues E. Angiotensin-converting enzyme 2 overexpression in the subfornical organ prevents the angiotensin II-mediated pressor and drinking responses and is associated with angiotensin II type 1 receptor downregulation. Circ Res. 2008; 102:729–736. [PubMed: 18258853]

Gonzalez AA, Luffman C, Bourgeois CR, Vio CP, Prieto MC. Angiotensin II-independent upregulation of cyclooxygenase-2 by activation of the (Pro)renin receptor in rat renal inner medullary cells. Hypertension. 2012; 61:443–449. [PubMed: 23184385]

Greco CM, Camera M, Facchinetti L, Brambilla M, Pellegrino S, Gelmi ML, Tremoli E, Corsini A, Ferri N. Chemotactic effect of prorenin on human aortic smooth muscle cells: a novel function of the (pro)renin receptor. Cardiovasc Res. 2012; 95:366–374. [PubMed: 22721990]

Hermann K, McDonald W, Unger T, Lang RE, Ganten D. Angiotensin biosynthesis and concentrations in brain of normotensive and hypertensive rats. J Physiol (Paris). 1984; 79:471–480. [PubMed: 6100310]

Hirose S, Naruse M, Ohtsuki K, Inagami T. Totally inactive renin zymogen and different forms of active renin in hog brain tissues. J Biol Chem. 1981; 256:5572–5576. [PubMed: 7016855]

Hsueh WA, Baxter JD. Human prorenin. Hypertension. 1991; 17:469–477. [PubMed: 2013474]

Hsueh WA, Wyne K. Renin-Angiotensin-aldosterone system in diabetes and hypertension. J Clin Hypertens (Greenwich). 2011; 13:224–237. [PubMed: 21466617]

Huang J, Matavelli LC, Siragy HM. Renal (pro)renin receptor contributes to development of diabetic kidney disease through transforming growth factor-beta1-connective tissue growth factor signalling cascade. Clin Exp Pharmacol Physiol. 2011; 38:215–221. [PubMed: 21265872]

Huang J, Siragy HM. Regulation of (pro)renin receptor expression by glucose-induced mitogen-activated protein kinase, nuclear factor-kappaB, and activator protein-1 signaling pathways. Endocrinology. 2010; 151:3317–3325. [PubMed: 20444941]

Huang J, Siragy HM. Sodium depletion enhances renal expression of (pro)renin receptor via cyclic GMP-protein kinase G signaling pathway. Hypertension. 2011; 59:317–323. [PubMed: 22203739]

Ichihara A. (Pro)renin receptor and vacuolar H(+)-ATPase. Keio J Med. 2012; 61:73–78. [PubMed: 23018489]

Ichihara A, Hayashi M, Kaneshiro Y, Suzuki F, Nakagawa T, Tada Y, Koura Y, Nishiyama A, Okada H, Uddin MN, Nabi AH, Ishida Y, Inagami T, Saruta T. Inhibition of diabetic nephropathy by a decoy peptide corresponding to the “handle” region for nonproteolytic activation of prorenin. J Clin Invest. 2004; 114:1128–1135. [PubMed: 15489960]

Ichihara A, Itoh H, Inagami T. Critical roles of (pro)renin receptor-bound prorenin in diabetes and hypertension: sallies into therapeutic approach. J Am Soc Hypertens. 2008; 2:15–19. [PubMed: 20409880]

Ichihara A, Sakoda M, Kurauchi-Mito A, Kaneshiro Y, Itoh H. Involvement of (pro)renin receptor in the glomerular filtration barrier. J Mol Med (Berl). 2008; 86:629–635. [PubMed: 18335185]

Ichihara A, Suzuki F, Nakagawa T, Kaneshiro Y, Takemitsu T, Sakoda M, Nabi AH, Nishiyama A, Sugaya T, Hayashi M, Inagami T. Prorenin receptor blockade inhibits development of glomerulosclerosis in diabetic angiotensin II type 1a receptor-deficient mice. J Am Soc Nephrol. 2006; 17:1950–1961. [PubMed: 16738017]

Jain A, Jain SK. Ligand-Appended BBB-Targeted Nanocarriers (LABTNs). Crit Rev Ther Drug Carrier Syst. 2015; 32:149–180. [PubMed: 25955883]

Johren O, Inagami T, Saavedra JM. AT1A, AT1B, and AT2 angiotensin II receptor subtype gene expression in rat brain. Neuroreport. 1995; 6:2549–2552. [PubMed: 8741760]

Kaneshiro Y, Ichihara A, Sakoda M, Takemitsu T, Nabi AH, Uddin MN, Nakagawa T, Nishiyama A, Suzuki F, Inagami T, Itoh H. Slowly progressive, angiotensin II-independent glomerulosclerosis in human (pro)renin receptor-transgenic rats. J Am Soc Nephrol. 2007; 18:1789–1795. [PubMed: 17494887]

Kim WS, Nakayama K, Nakagawa T, Kawamura Y, Haraguchi K, Murakami K. Mouse submandibular gland prorenin-converting enzyme is a member of glandular kallikrein family. J Biol Chem. 1991; 266:19283–19287. [PubMed: 1918045]

Klickstein LB, Kaempfer CE, Wintroub BU. The granulocyte-angiotensin system. Angiotensin I-converting activity of cathepsin G. J Biol Chem. 1982; 257:15042–15046. [PubMed: 6294088]

Kochanek KD, Murphy SL, Xu J. Deaths: Final Data for 2011. Natl Vital Stat Rep. 2015; 63:1–120.

Krebs C, Weber M, Steinmetz O, Meyer-Schwesinger C, Stahl R, Danser AH, Garrelds I, van Goor H, Nguyen G, Muller D, Wenzel U. Effect of (pro)renin receptor inhibition by a decoy peptide on renal damage in the clipped kidney of Goldblatt rats. Kidney Int. 2008; 74:823–824. [PubMed: 18756297]

Kurauchi-Mito A, Ichihara A, Bokuda K, Sakoda M, Kinouchi K, Yaguchi T, Yamada T, Sun-Wada GH, Wada Y, Itoh H. Significant roles of the (pro)renin receptor in integrity of vascular smooth muscle cells. Hypertens Res. 2014; 37:830–835. [PubMed: 24830537]

Lenkei Z, Palkovits M, Corvol P, Llorens-Cortes C. Distribution of angiotensin II type-2 receptor (AT2) mRNA expression in the adult rat brain. J Comp Neurol. 1996; 373:322–339. [PubMed: 8889931]

Lenz T, Sealey JE, Maack T, James GD, Heinrikson RL, Marion D, Laragh JH. Half-life, hemodynamic, renal, and hormonal effects of prorenin in cynomolgus monkeys. Am J Physiol. 1991; 260:R804–810. [PubMed: 2012251]

Li C, Siragy HM. High glucose induces podocyte injury via enhanced (pro)renin receptor-Wnt-beta-catenin-snail signaling pathway. PLoS One. 2014; 9:e89233. [PubMed: 24533170]

Li W, Liu J, Hammond SL, Tjalkens RB, Saifudeen Z, Feng Y. Angiotensin II regulates brain (pro)renin receptor expression through activation of cAMP response element-binding protein. Am J Physiol Regul Integr Comp Physiol. 2015; 309:R138–147. [PubMed: 25994957]

Li W, Peng H, Cao T, Sato R, McDaniels SJ, Kobori H, Navar LG, Feng Y. Brain-targeted (pro)renin receptor knockdown attenuates angiotensin II-dependent hypertension. Hypertension. 2012; 59:1188–1194. [PubMed: 22526255]

Li W, Peng H, Mehaffey EP, Kimball CD, Grobe JL, van Gool JM, Sullivan MN, Earley S, Danser AH, Ichihara A, Feng Y. Neuron-specific (pro)renin receptor knockout prevents the development of salt-sensitive hypertension. Hypertension. 2014; 63:316–323. [PubMed: 24246383]

Li W, Peng H, Seth DM, Feng Y. The Prorenin and (Pro)renin Receptor: New Players in the Brain Renin-Angiotensin System? Int J Hypertens. 2012; 2012:290635. [PubMed: 23316344]

Li W, Sullivan MN, Zhang S, Worker CJ, Xiong Z, Speth RC, Feng Y. Intracerebroventricular infusion of the (Pro)renin receptor antagonist PRO20 attenuates deoxycorticosterone acetate-salt-induced hypertension. Hypertension. 2014; 65:352–361. [PubMed: 25421983]

Li Z, Iwai M, Wu L, Shiuchi T, Jinno T, Cui TX, Horiuchi M. Role of AT2 receptor in the brain in regulation of blood pressure and water intake. Am J Physiol Heart Circ Physiol. 2003; 284:H116–121. [PubMed: 12388241]

Lomez ES, Araujo RC, Bader M, Pesquero JB, Pesquero JL. Tonin and kallikrein in the brain of transgenic rat line expressing human tissue kallikrein. Hypertension. 2002; 39:229–232. [PubMed: 11847189]

Lu X, Wang F, Liu M, Yang KT, Nau A, Kohan DE, Reese VR, Richardson RS, Yang T. Activation of ENaC in Collecting Duct Cells by Prorenin and Its Receptor PRR: Involvement of Nox4-Derived Hydrogen Peroxide. Am J Physiol Renal Physiol. 2015 ajprenal 00492 02015.

Matavelli LC, Huang J, Siragy HM. (Pro)renin receptor contributes to diabetic nephropathy by enhancing renal inflammation. Clin Exp Pharmacol Physiol. 2010; 37:277–282. [PubMed: 19769609]

Morales R, Watier Y, Bocskei Z. Human prorenin structure sheds light on a novel mechanism of its autoinhibition and on its non-proteolytic activation by the (pro)renin receptor. J Mol Biol. 2012; 421:100–111. [PubMed: 22575890]

Morganti A, Lonati C. Aliskiren: the first direct renin inhibitor available for clinical use. J Nephrol. 2011; 24:541–549. [PubMed: 21786226]

Morimoto S, Cassell MD, Beltz TG, Johnson AK, Davisson RL, Sigmund CD. Elevated blood pressure in transgenic mice with brain-specific expression of human angiotensinogen driven by the glial fibrillary acidic protein promoter. Circ Res. 2001; 89:365–372. [PubMed: 11509454]

Morimoto S, Cassell MD, Sigmund CD. Glia- and neuron-specific expression of the renin-angiotensin system in brain alters blood pressure, water intake, and salt preference. J Biol Chem. 2002; 277:33235–33241. [PubMed: 12080069]

Morris BJ. Activation of human inactive (“pro-”) renin by cathepsin D and pepsin. J Clin Endocrinol Metab. 1978; 46:153–157. [PubMed: 376540]

Muller DN, Klanke B, Feldt S, Cordasic N, Hartner A, Schmieder RE, Luft FC, Hilgers KF. (Pro)renin receptor peptide inhibitor “handle-region” peptide does not affect hypertensive nephrosclerosis in Goldblatt rats. Hypertension. 2008; 51:676–681. [PubMed: 18212268]

Nabi AH, Biswas KB, Nakagawa T, Ichihara A, Inagami T, Suzuki F. ‘Decoy peptide’ region (RIFLKRMPSI) of prorenin prosegment plays a crucial role in prorenin binding to the (pro)renin receptor. Int J Mol Med. 2009a; 24:83–89. [PubMed: 19513539]

Nabi AH, Biswas KB, Nakagawa T, Ichihara A, Inagami T, Suzuki F. Prorenin has high affinity multiple binding sites for (pro)renin receptor. Biochim Biophys Acta. 2009b; 1794:1838–1847. [PubMed: 19733264]

Nabi AH, Suzuki F. Biochemical properties of renin and prorenin binding to the (pro)renin receptor. Hypertens Res. 2009; 33:91–97. [PubMed: 19942927]

Nguyen G, Delarue F, Burckle C, Bouzhir L, Giller T, Sraer JD. Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. J Clin Invest. 2002; 109:1417–1427. [PubMed: 12045255]

Nurun NA, Uddin NM, Nakagawa T, Iwata H, Ichihara A, Inagami T, Suzuki F. Role of “handle” region of prorenin prosegment in the non-proteolytic activation of prorenin by binding to membrane anchored (pro)renin receptor. Front Biosci. 2007; 12:4810–4817. [PubMed: 17569611]

O’Brien E, Barton J, Nussberger J, Mulcahy D, Jensen C, Dicker P, Stanton A. Aliskiren reduces blood pressure and suppresses plasma renin activity in combination with a thiazide diuretic, an angiotensin-converting enzyme inhibitor, or an angiotensin receptor blocker. Hypertension. 2007; 49:276–284. [PubMed: 17159081]

Pitt B. “Escape” of aldosterone production in patients with left ventricular dysfunction treated with an angiotensin converting enzyme inhibitor: implications for therapy. Cardiovasc Drugs Ther. 1995; 9:145–149. [PubMed: 7786835]

Quadri S, Siragy HM. (Pro)renin receptor contributes to regulation of renal epithelial sodium channel. J Hypertens. 2016; 34:486–494. [PubMed: 26771338]

Rabelo LA, Alenina N, Bader M. ACE2-angiotensin-(1–7)-Mas axis and oxidative stress in cardiovascular disease. Hypertens Res. 2011; 34:154–160. [PubMed: 21124322]

Re RN. Mechanisms of disease: local renin-angiotensin-aldosterone systems and the pathogenesis and treatment of cardiovascular disease. Nat Clin Pract Cardiovasc Med. 2004; 1:42–47. [PubMed: 16265259]

Riccioni G. The role of direct renin inhibitors in the treatment of the hypertensive diabetic patient. Ther Adv Endocrinol Metab. 2013; 4:139–145. [PubMed: 24143271]

Rong R, Ito O, Mori N, Muroya Y, Tamura Y, Mori T, Ito S, Takahashi K, Totsune K, Kohzuki M. Expression of (pro)renin receptor and its upregulation by high salt intake in the rat nephron. Peptides. 2015; 63:156–162. [PubMed: 25555681]

Ryan US, Ryan JW, Whitaker C, Chiu A. Localization of angiotensin converting enzyme (kininase II). II. Immunocytochemistry and immunofluorescence. Tissue Cell. 1976; 8:125–145. [PubMed: 178068]

Sakai K, Agassandian K, Morimoto S, Sinnayah P, Cassell MD, Davisson RL, Sigmund CD. Local production of angiotensin II in the subfornical organ causes elevated drinking. J Clin Invest. 2007; 117:1088–1095. [PubMed: 17404622]

Schefe JH, Unger T, Funke-Kaiser H. PLZF and the (pro)renin receptor. J Mol Med (Berl). 2008; 86:623–627. [PubMed: 18335187]

Schelling P, Ganten U, Sponer G, Unger T, Ganten D. Components of the renin-angiotensin system in the cerebrospinal fluid of rats and dogs with special consideration of the origin and the fate of angiotensin II. Neuroendocrinology. 1980; 31:297–308. [PubMed: 7003424]

Schmid J, Oelbe M, Saftig P, Schwake M, Schweda F. Parallel regulation of renin and lysosomal integral membrane protein 2 in renin-producing cells: further evidence for a lysosomal nature of renin secretory vesicles. Pflugers Arch. 2013; 465:895–905. [PubMed: 23229015]

Sernia C, Mowchanuk MD. Brain angiotensinogen: in vitro synthesis and chromatographic characterization. Brain Res. 1983; 259:275–283. [PubMed: 6297674]

Shan Z, Cuadra AE, Sumners C, Raizada MK. Characterization of a functional (pro)renin receptor in rat brain neurons. Exp Physiol. 2008; 93:701–708. [PubMed: 18326551]

Shan Z, Shi P, Cuadra AE, Dong Y, Lamont GJ, Li Q, Seth DM, Navar LG, Katovich MJ, Sumners C, Raizada MK. Involvement of the brain (pro)renin receptor in cardiovascular homeostasis. Circ Res. 2010; 107:934–938. [PubMed: 20689062]

Shi P, Grobe JL, Desland FA, Zhou G, Shen XZ, Shan Z, Liu M, Raizada MK, Sumners C. Direct pro-inflammatory effects of prorenin on microglia. PLoS One. 2014; 9:e92937. [PubMed: 25302502]

Sihn G, Rousselle A, Vilianovitch L, Burckle C, Bader M. Physiology of the (pro)renin receptor: Wnt of change? Kidney Int. 2010; 78:246–256. [PubMed: 20505655]

Siragy HM. Rationale for combining a direct renin inhibitor with other renin- angiotensin system blockers. Focus on aliskiren and combinations. Cardiovasc Drugs Ther. 2011; 25:87–97. [PubMed: 21213120]

Suzuki F, Hayakawa M, Nakagawa T, Nasir UM, Ebihara A, Iwasawa A, Ishida Y, Nakamura Y, Murakami K. Human Prorenin Has “Gate and Handle” Regions for Its Non-proteolytic Activation. Journal of Biological Chemistry. 2003; 278:22217–22222. [PubMed: 12684512]

Suzuki-Nakagawa C, Nishimura M, Tsukamoto T, Aoyama S, Ebihara A, Suzuki F, Nakagawa T. Participation of the extracellular domain in (pro)renin receptor dimerization. Biochem Biophys Res Commun. 2014; 444:461–466. [PubMed: 24472541]

Takahashi H, Ichihara A, Kaneshiro Y, Inomata K, Sakoda M, Takemitsu T, Nishiyama A, Itoh H. Regression of nephropathy developed in diabetes by (Pro)renin receptor blockade. J Am Soc Nephrol. 2007; 18:2054–2061. [PubMed: 17538189]

te Riet L, van den Heuvel M, Peutz-Kootstra CJ, van Esch JH, van Veghel R, Garrelds IM, Musterd-Bhaggoe U, Bouhuizen AM, Leijten FP, Danser AH, Batenburg WW. Deterioration of kidney function by the (pro)renin receptor blocker handle region peptide in aliskiren-treated diabetic transgenic (mRen2)27 rats. Am J Physiol Renal Physiol. 2014; 306:F1179–1189. [PubMed: 24694588]

Te Riet L, van Esch JH, Roks AJ, van den Meiracker AH, Danser AH. Hypertension: renin-angiotensin-aldosterone system alterations. Circ Res. 2015; 116:960–975. [PubMed: 25767283]

Tigerstedt R, PGB. Niere und Kreislauf. Arch Physiol. 1898:8.

Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. J Biol Chem. 2000; 275:33238–33243. [PubMed: 10924499]

van den Meiracker AH, Jan Danser AH. Aliskiren: the first direct renin inhibitor for hypertension. Curr Cardiol Rep. 2007; 9:470–476. [PubMed: 17999872]

Vijayaraghavan K, Deedwania P. Renin-angiotensin-aldosterone blockade for cardiovascular disease prevention. Cardiol Clin. 2011; 29:137–156. [PubMed: 21257105]

Vinturache AE, Smith FG. Angiotensin type 1 and type 2 receptors during ontogeny: cardiovascular and renal effects. Vascul Pharmacol. 2014; 63:145–154. [PubMed: 25451565]

von Lutterotti N, Catanzaro DF, Sealey JE, Laragh JH. Renin is not synthesized by cardiac and extrarenal vascular tissues. A review of experimental evidence. Circulation. 1994; 89:458–470. [PubMed: 8281681]

Wadia JS, Dowdy SF. Transmembrane delivery of protein and peptide drugs by TAT-mediated transduction in the treatment of cancer. Adv Drug Deliv Rev. 2005; 57:579–596. [PubMed: 15722165]

Wang F, Lu X, Liu M, Feng Y, Zhou SF, Yang T. Renal medullary (pro)renin receptor contributes to angiotensin II-induced hypertension in rats via activation of the local renin-angiotensin system. BMC Med. 2015; 13:278. [PubMed: 26554902]

Wang F, Lu X, Peng K, Zhou L, Li C, Wang W, Yu X, Kohan DE, Zhu SF, Yang T. COX-2 mediates angiotensin II-induced (pro)renin receptor expression in the rat renal medulla. Am J Physiol Renal Physiol. 2014; 307:F25–32. [PubMed: 24740788]

Wilkinson-Berka JL, Heine R, Tan G, Cooper ME, Hatzopoulos KM, Fletcher EL, Binger KJ, Campbell DJ, Miller AG. RILLKKMPSV influences the vasculature, neurons and glia, and (pro)renin receptor expression in the retina. Hypertension. 2010; 55:1454–1460. [PubMed: 20368504]

Wilkinson-Berka JL, Miller AG, Binger KJ. Prorenin and the (pro)renin receptor: recent advances and implications for retinal development and disease. Curr Opin Nephrol Hypertens. 2010; 20:69–76. [PubMed: 21099684]

Xa LK, Lacombe MJ, Mercure C, Lazure C, Reudelhuber TL. General lysosomal hydrolysis can process prorenin accurately. Am J Physiol Regul Integr Comp Physiol. 2014; 307:R505–513. [PubMed: 24965790]

Xia H, de Queiroz TM, Sriramula S, Feng Y, Johnson T, Mungrue IN, Lazartigues E. Brain ACE2 overexpression reduces DOCA-salt hypertension independently of endoplasmic reticulum stress. Am J Physiol Regul Integr Comp Physiol. 2015; 308:R370–378. [PubMed: 25519733]

Yamazato M, Yamazato Y, Sun C, Diez-Freire C, Raizada MK. Overexpression of angiotensin-converting enzyme 2 in the rostral ventrolateral medulla causes long-term decrease in blood pressure in the spontaneously hypertensive rats. Hypertension. 2007; 49:926–931. [PubMed: 17325232]

Yang G, Gray TS, Sigmund CD, Cassell MD. The angiotensinogen gene is expressed in both astrocytes and neurons in murine central nervous system. Brain Res. 1999; 817:123–131. [PubMed: 9889347]

Yang T. Crosstalk between (Pro)renin receptor and COX-2 in the renal medulla during angiotensin II-induced hypertension. Curr Opin Pharmacol. 2015; 21:89–94. [PubMed: 25681793]

Zhang Y, Gao X, Michael Garavito R. Structural analysis of the intracellular domain of (pro)renin receptor fused to maltose-binding protein. Biochem Biophys Res Commun. 2011; 407:674–679. [PubMed: 21420935]

Zhuo J, Moeller I, Jenkins T, Chai SY, Allen AM, Ohishi M, Mendelsohn FA. Mapping tissue angiotensin-converting enzyme and angiotensin AT1, AT2 and AT4 receptors. J Hypertens. 1998; 16:2027–2037. [PubMed: 9886893]

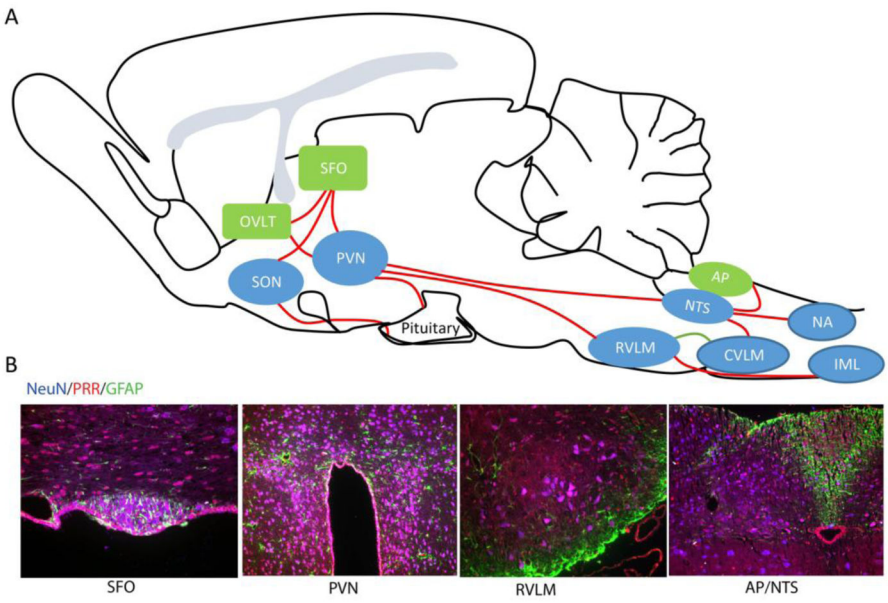

Figure 1. Expression of the PRR in brain regions involved in BP regulation

(A) A schematic showing the key cardiovascular regulatory brain nuclei. (B) Brain tissues from C57Bl/6J mice were stained with antibodies against the mouse PRR (red), the neuronal marker NeuN (blue), and the astrocyte marker GFAP (green). PRR immunoreactivity is predominantly co-localized with NeuN in SFO, PVN, NTS, RVLM, and AP. Abbreviations: SFO, subfornical organ; PVN, paraventricular nucleus of the hypothalamus; NTS, nucleus of the tractus solitaries; RVLM, rostral ventrolateral medulla; AP, area posterma; SON, supraoptic nucleus; OVLT, organum vasculosum of the lamina terminalis; CVLM, caudal ventrolateral medulla; NA, nucleus accumbens; IML, intermediolateral nucleus; PRR, (pro)renin receptor; GFAP, glial fibrillary acidic protein; NeuN, neuronal nuclei.

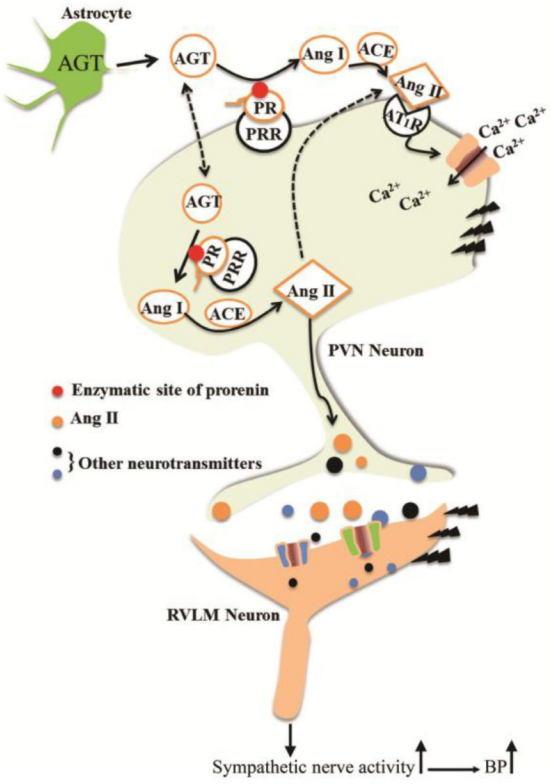

Figure 2. Proposed pathways for extra- and intracellular formation of Ang II in neurons

In presympathetic neurons, prorenin (PR) binds intracellular PRRs, stimulating the intracellular formation of Ang II, which is subsequently secreted into the extracellular space. Alternatively, extracellular prorenin binds to PRRs on the neuronal membrane and metabolizes extracellular AGT secreted by astrocytes or neurons to generate Ang I. ACE, located on the external surface of cell membranes or in the interstitial uid, converts Ang I to Ang II. Intracellular Ang II can be transported to axon terminals to act as a neurotransmitter. Extracellular Ang II binds to AT_1R to modulate neuronal activity and neurotransmitter release at the synapse. Abbreviations: PR, prorenin; PRR, (pro)renin receptor; AGT, angiotensinogen; Ang II, angiotensin II; ACE, angiotensin converting enzyme; AT_1R, angiotensin II type 1 receptor; PVN, paraventricular nucleus of the hypothalamus; RVLM, rostral ventrolateral medulla nucleus. (Modified from Li W et al., Hypertension, 2013, 65:352–361.)

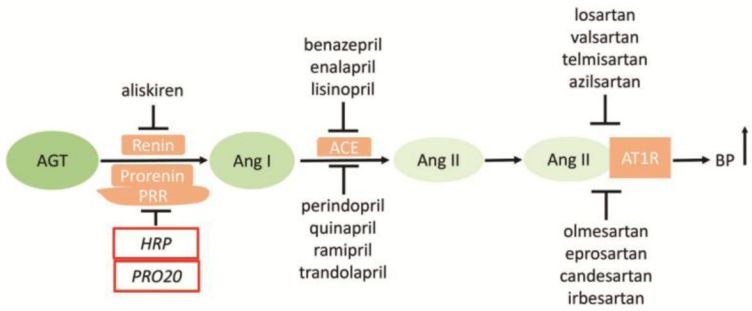

Figure 3. Antihypertensive drugs targeting the RAS

Schematic illustration of clinically approved antihypertensive agents that inhibit the RAS. Potential new RAS antagonists targeting the PRR (red square): HRP and PRO20 have been tested *in vitro* or in animals. Abbreviations: AGT, angiotensinogen; Ang I, angiotensin I; Ang II, angiotensin II; ACE, angiotensin converting enzyme; AT_1R, angiotensin II type 1 receptor; BP, blood pressure; HRP, handle region peptide.

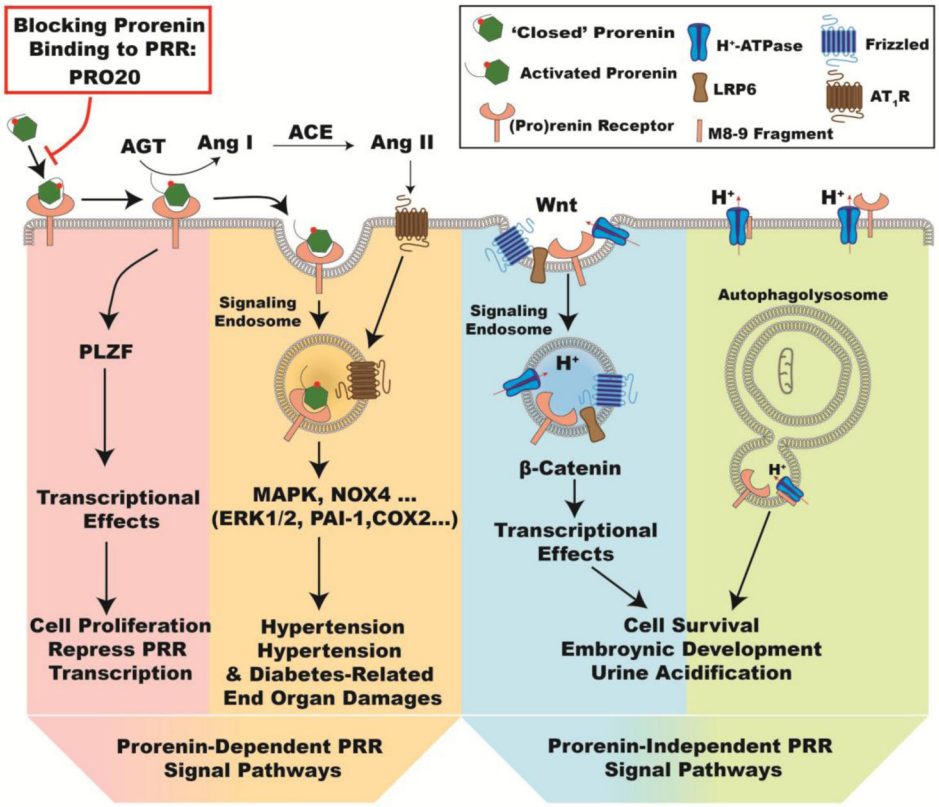

Figure 4. Proposed mechanism for PRO20 blockade of prorenin/PRR activation as a therapeutic strategy

The PRR mediates prorenin (ligand)-dependent signaling by promoting Ang II formation or Ang II-independent downstream signals. Activation of these prorenin-dependent signaling pathways is responsible for cell proliferation, hypertension, and diabetic end-organ damage. The PRR also plays important roles in Wnt signaling pathways and autophagy that do not require prorenin. The latter signaling pathways are important for cell survival, embryonic development, and urine acidification. According, we propose that blocking the binding of prorenin to the PRR will prevent prorenin/PRR activation and Ang II formation, thereby preventing activation of downstream signaling. In addition, blocking activation of prorenin/PRR will not affect the prorenin-independent roles of the PRR in cell survival, embryonic development, or urine acidification. Abbreviations: AGT, angiotensinogen; Ang I, angiotensin I; Ang II, angiotensin II; ACE, angiotensin-converting enzyme; AT_1R, angiotensin II type 1 receptor; PLZF, promyelocytic zinc finger; MAPKs, mitogen-activated protein kinases; ERK1/2, extracellular signal-regulated kinases 1 and 2; PAI-1, plasminogen activator inhibitor-1; COX2, cyclooxygenase 2; NOX4, NADPH oxidase 4; LPR6, low-density lipoprotein receptor-related protein 6.

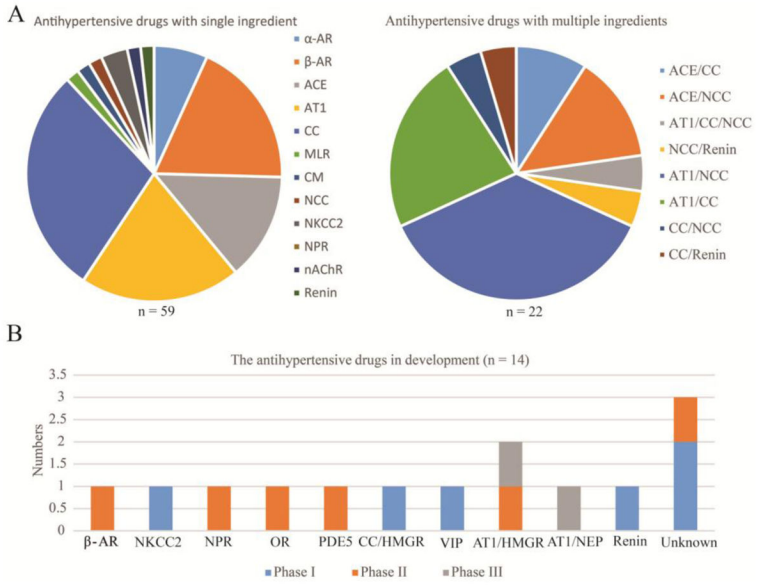

Figure 5. A global view of antihypertensive drug development

(A) Approved anti-hypertensive drugs with a single ingredient or multiple ingredients. (B) Antihypertensive drugs in development. Abbreviations: AR, adrenergic receptor; CC, calcium channel; HMGR, HMG CoA reductase; CM, calcium metabolism; MLR, mineralcorticoid receptor; NCC, Na/Cl transporter; NKCC2, Na-K-Cl cotransporter 2; NPR, natriuretic peptide receptor; nAChR, nicotinic acetylcholine receptor; OR, opioid receptor; PDE5, phosphodiesterase 5; VIPR, vasoactive intestinal peptide receptor.