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

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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.

Contents

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, 1898), reflecting its importance in the pathogenesis of hypertension and other cardiovascular diseases (Re, 2004; Hsueh & Wyne, 2011; Vijayaraghavan & Deedwania, 2011; Ellis et al., 2012; Te Riet et al., 2015). 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

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.

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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 et al., 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 (Danser et al., 1989; Hsueh & Baxter, 1991; von Lutterotti et al., 1994; Bader et al., 2001). 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 et al., 2013; Xa et al., 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 (Hirose et al., 1981; Hermann et al., 1984). 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 (Zhang et al., 2011; Suzuki-Nakagawa et al., 2014). 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 & Suzuki, 2009; Nabi et al., 2009a). 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 (Nabi & Suzuki, 2009; Nabi et al., 2009a; Li et al., 2015). 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 (Nabi et al., 2009b; Morales et al., 2012). 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; Rong et al., 2015; Quadri & Siragy, 2016). 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 (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; 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 et al., 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 (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 et al., 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, 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 et al., 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 (pro) renin receptor is a master regulator of blood pressure in the brain renin–angiotensin system

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 (Bunnemann et al., 1992; Johren et al., 1995; Lenkei et al., 1996; Zhuo et al., 1998; Allen et al., 2000). 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 (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, 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 et al., 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) (Yamazato et al., 2007; Feng et al., 2008; Xia et al., 2015). AGT in the brain is synthesized and secreted from astroglial cells (Sernia & Mowchanuk, 1983; Campbell et al., 1984; Deschepper et al., 1986) and neurons (Yang et al., 1999), and its levels are high in cerebrospinal fluid (Schelling et al., 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 et al., 2010). Although enzymes such as tonin and cathepsins are abundant in the CNS (Klickstein et al., 1982; Lomez et al., 2002), 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 (Li et al., 2012b, 2014). 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 Fig. 1. However, in the lateral cortex of the brain, some PRR immunostaining is detectable in astrocytes (Shan et al., 2008; Li et al., 2012a, 2012b). 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 (Li et al., 2012a). 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 (Li et al., 2012a). These changes were associated with a concomitant decrease in AT_1R expression and vasopressin levels in the SFO and PVN, respectively (Li et al., 2012a). 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 (Li 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 (Li 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 (Li 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 Fig. 2.

3. The development of (pro)renin receptor 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 et al., 2015). A major category of antihypertensive drugs encompasses those that target the RAS, including ACE-inhibitors, AT_1R blockers, and renin inhibitors (Fig. 3). These drugs function at different steps in the RAS cascade, providing important treatment options for patients (Feig et al., 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).

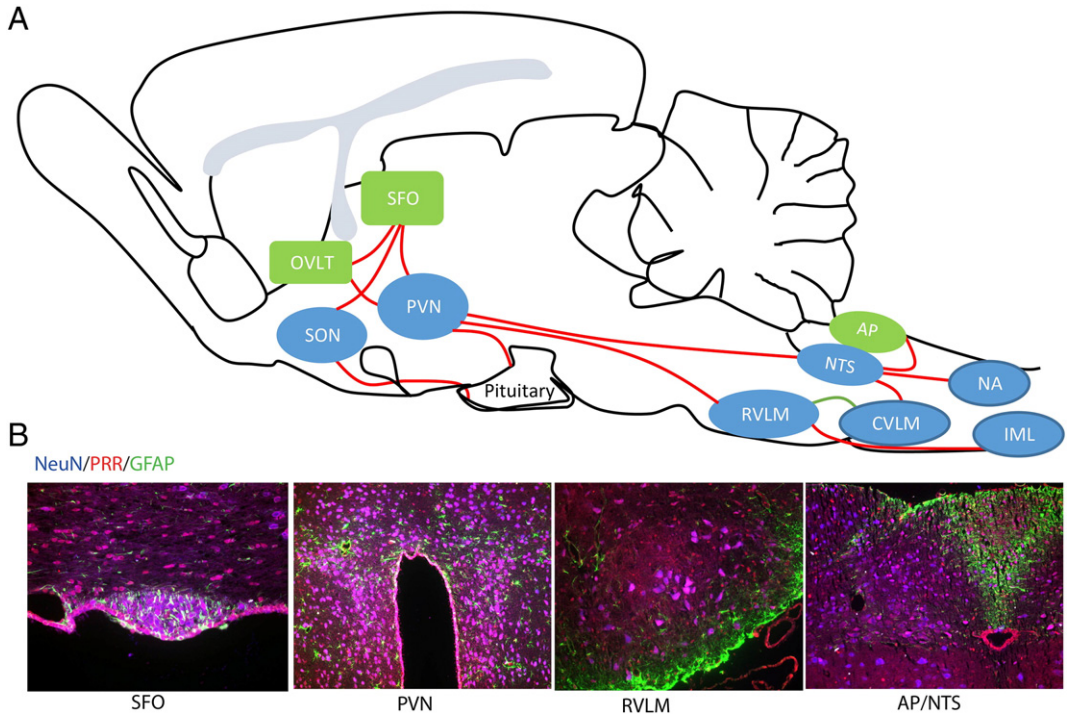

Fig. 1. Expression of the PRR in brain regions involved in BP regulation. (A) A schematic showing the key cardiovascular regulatory brain nuclei. (B) Brain tissues from C57Bl/6J mice were stained with antibodies against the mouse PRR (red), the neuronal marker NeuN (blue), and the astrocyte marker GFAP (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.

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 et al., 2008a) 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-amino-acid 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 (Suzuki et al., 2003; Ichihara et al., 2004), 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 et al., 2008b). 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 et al., 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 et al., 2010a). 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 (Li et al., 2015), 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 (Fig. 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 (Li et al., 2015). 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)-

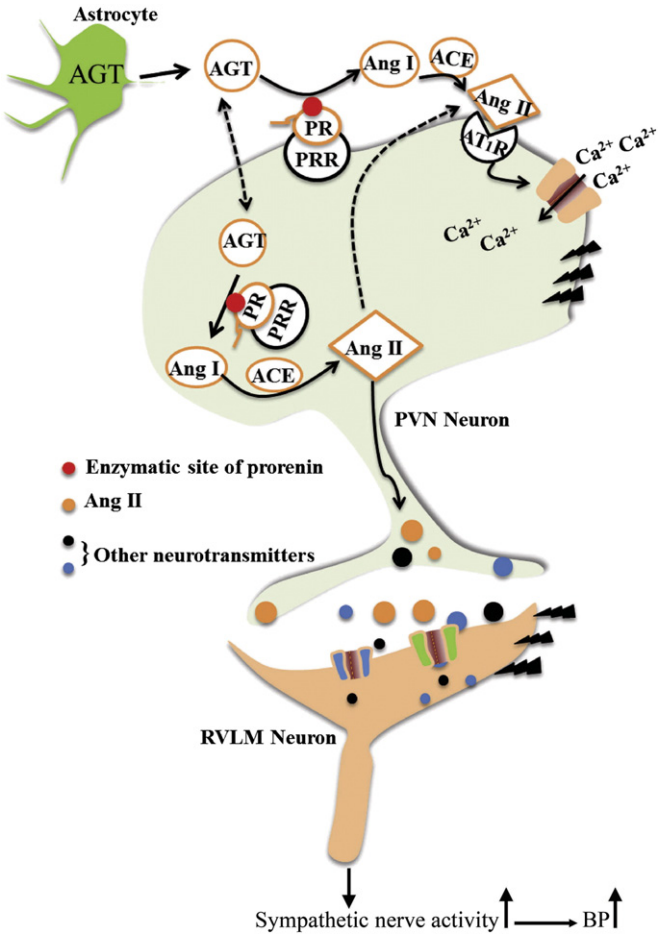

Fig. 2. Proposed pathways for extra- and intracellular formation of Ang II in neurons. In presympathetic neurons, prorenin (PR) binds intracellular PRRs, stimulating the intracellular formation of Ang II, which is subsequently secreted into the extracellular space. Alternatively, extracellular prorenin 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 fluid, 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, 2015, 65:352–361.)

induced calcium influx in neuronal cells with a half-maximal inhibitory concentration (IC_{50}) of 81 nmol/L (Li et al., 2015). 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 (Li et al., 2015). 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/(pro)renin receptor 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; Ichihara, 2012; Daryadel et al., 2016). 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 (Li et al., 2015), and the establishment of its importance in tissue fibrosis, inflammation and end-organ damage (Balakumar & Jagadeesh, 2010; Wilkinson-Berka et al., 2010b), 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. Fig. 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 et al., 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 (Li et al., 2012b; Danser, 2015; Feng, 2015). PLZF is a repressive auto-regulatory signaling molecule that is also

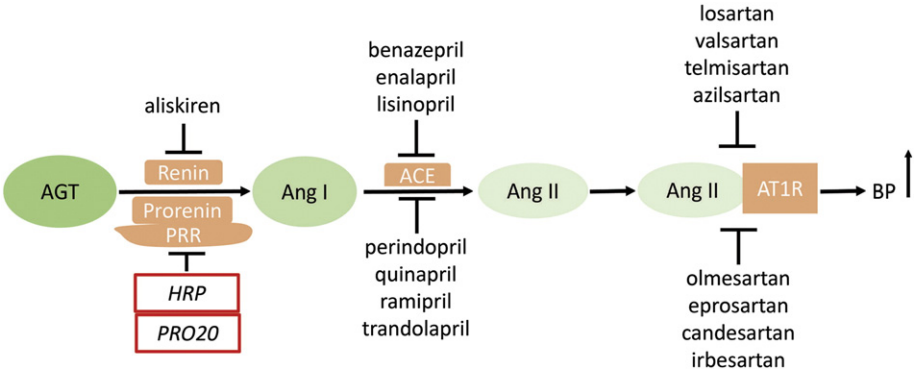

Fig. 3. Antihypertensive drugs targeting the RAS. Schematic illustration of clinically approved antihypertensive agents that inhibit the RAS. Potential new RAS antagonists targeting the PRR (red square): HRP and PRO20 have been tested in vitro or in animals. Abbreviations: AGT, angiotensinogen; Ang I, angiotensin I; Ang II, angiotensin II; ACE, angiotensin converting enzyme; AT_1R, angiotensin II type 1 receptor; BP, blood pressure; HRP, handle region peptide.

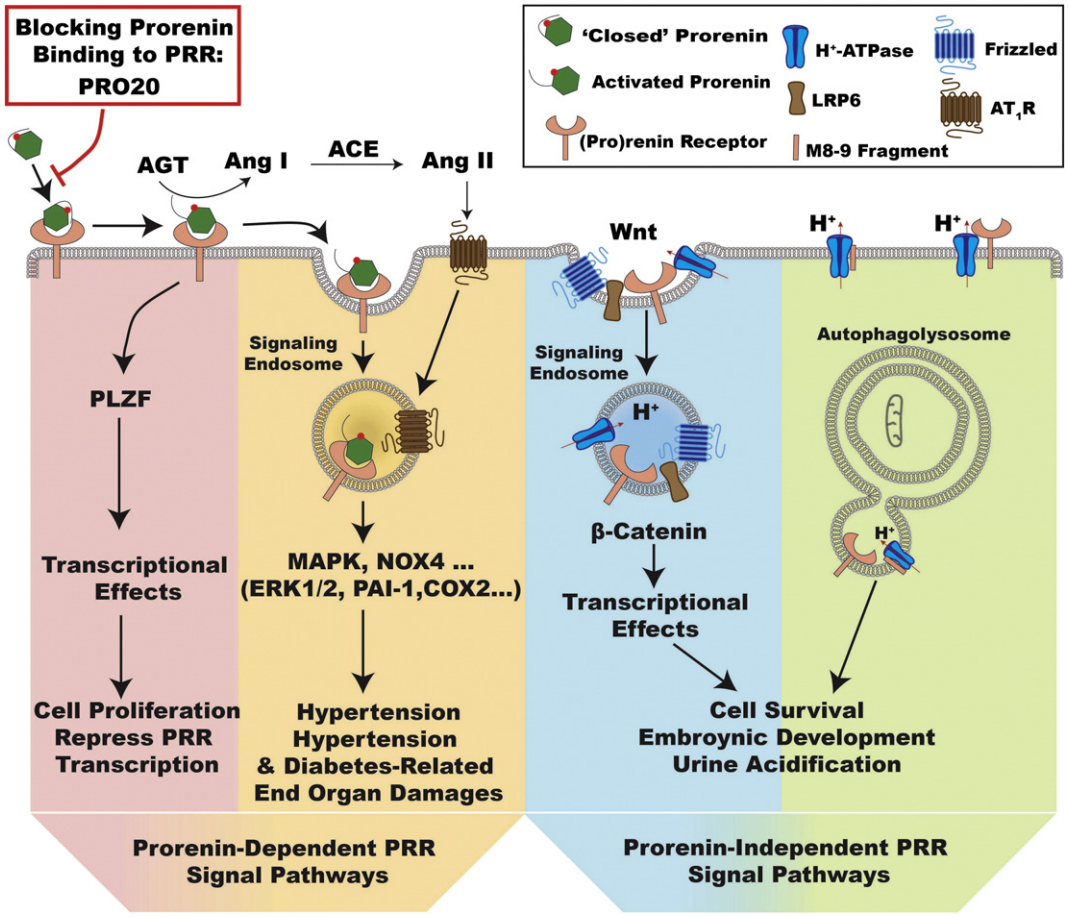

Fig. 4. Proposed mechanism for PRO20 blockade of prorenin/PRR activation as a therapeutic strategy. The PRR mediates prorenin (ligand)-dependent signaling by promoting Ang II formation or Ang II-independent downstream signals. Activation of these prorenin-dependent signaling pathways is responsible for 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.

induced by the binding of prorenin to the PRR (Schefe et al., 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 (Fig. 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; Muller et al., 2008; Li et al., 2015; 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 (Fig. 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 (Fig. 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 suggests 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

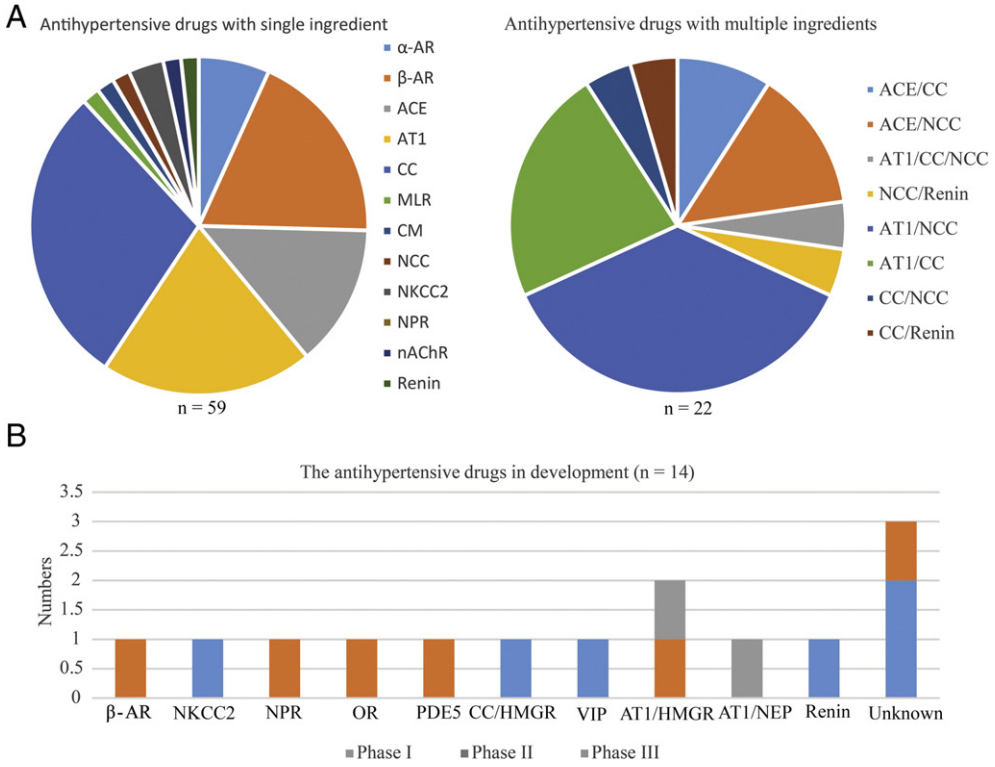

Fig. 5. A global view of antihypertensive drug development. (A) Approved anti-hypertensive drugs with a single ingredient or multiple ingredients. (B) Antihypertensive drugs in development. Abbreviations: AR, adrenergic receptor; CC, calcium channel; HMGR, HMG CoA reductase; CM, calcium metabolism; MLR, 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.

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.

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Disclosures

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

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