The (Pro)Renin Receptor and the Kidney

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Summary: Prorenin binding to the (pro)renin receptor not only causes a nonproteolytic activation of prorenin leading to the activation of the renin-angiotensin system (RAS), but also stimulates the receptor's own intracellular signaling pathways independent of the RAS. Within the kidneys, the (pro)renin receptor is present in the glomerular mesangium and podocytes, which play an important role in the maintenance of the glomerular filtration barrier. Therefore, prorenin-receptor blockers, which competitively bind to the receptor as a decoy peptide, have superior benefits with regard to proteinuria and glomerulosclerosis in experimental animal models with elevated plasma prorenin levels such as diabetes and hypertension compared with conventional RAS inhibitors, possibly by inhibiting both the nonproteolytic activation of prorenin and RAS-independent intracellular signals.
Semin Nephrol 27:524-528 © 2007 Elsevier Inc. All rights reserved.
Keywords: *Angiotensin, mesangium, nonproteolytic activation, podocytes, prorenin*

In the kidneys, stimulation of the (pro)renin receptor exerts 2 major pathways: the activation of the renin-angiotensin system (RAS) by the conversion of prorenin to the active form of prorenin by a conformational change instead of the proteolytic cleavage of the pro-segment of prorenin,[1,2] and the stimulation of the RAS-independent intracellular pathways via the (pro)renin receptor.[2–5] As shown in Figure 1, when the (pro)renin receptor binds to the handle region of inactive prorenin, the receptor-bound prorenin gains its enzyme activity (ability to generate angiotensin I) without proteolytic cleavage of the prosegment of prorenin in COS-7 cells,[6,7] presumably as a result of a conformational change. On the other hand, the receptor triggers its own intracellular signaling pathways independent of the RAS. Studies have shown that stimulation of the (pro)renin receptor by renin/prorenin activates tyrosine phosphorylation, leading to activation of extracellular-signal–related protein kinases[2] and up-regulates transforming growth factor-β1 and matrix proteins without involving angiotensin II generation in human and rat mesangial cells.[4] However, the physiologic roles of the (pro)renin receptor in the kidneys remain undetermined.

RENAL LOCALIZATION

Studies have shown that the (pro)renin-receptor protein and messenger RNA (mRNA) are expressed in the mesangium cells of human kidneys.[2,4] However, we recently found that (pro)renin-receptor mRNA also is present in human cultured podocytes (unpublished data), and a recent preliminary study reported that (pro)renin-receptor mRNA is present in rat glomerular epithelial cells.[8] Double immunohistochemical analyses using a polyclonal antirat (pro)renin-receptor antibody showed that the (pro)renin receptor was colocalized with podocalyxin (a podocyte marker), but not with Thy1.1 (a mesangium marker), nephrin, (a podocyte foot process marker), or reca-1 (an endothelium marker) in rat kidneys (Fig. 2A). In addition, electron microscopic analyses appeared to indicate the predominant presence of rat (pro)renin receptor in podocytes, excluding the foot processes, and its absence in mesangial cells (Fig. 2B). Thus, rat (pro)renin receptor was

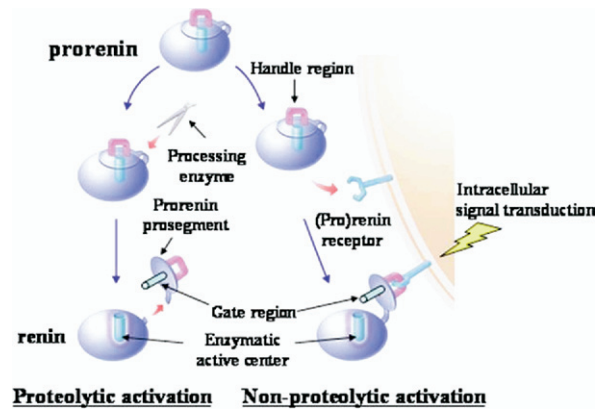**Figure 1.** Prorenin binding to the (pro)renin receptor causes the nonproteolytic activation of prorenin and receptor-mediated intracellular signal transduction.

present predominantly in the major processes and cell bodies of podocytes in rat kidneys. Within the glomerulus, podocytes play an important role in the maintenance of the glomerular filtration barrier, and podocyte injury leads to proteinuria and initiates glomerulosclerosis, resulting in the progressive loss of renal function.[9] Therefore, the podocyte (pro)renin receptor may contribute to proteinuria and renal injury through a RAS-dependent pathway, a RAS-independent pathway, or both pathways in chronic kidney diseases.

PATHOPHYSIOLOGIC ROLES IN THE KIDNEYS OF DIABETIC AND HYPERTENSIVE MODELS

The (pro)renin receptor binds to the handle region of inactive prorenin, thereby activating the receptor-bound prorenin.[6,7] Therefore, the handle region peptide competitively binds to the receptor as a decoy peptide and inhibits both the nonproteolytic activation of prorenin and RAS-independent intracellular signals. As shown in Figure 3, when the decoy peptide was administered as a prorenin-receptor blocker (PRRB) to streptozotocin-induced diabetic rats, PRRB significantly inhibited the increase in renal angiotensin II levels and the development of proteinuria and glomerulosclerosis,[1] suggesting that the nonproteolytic activation of prorenin by binding to the (pro)renin receptor plays an important role in the development of nephropathy. In addition, as

shown in Figure 4, although an angiotensin-converting enzyme inhibitor failed to inhibit the glomerulosclerosis with increased mitogen-activated protein kinase (MAPK) activation and proteinuria that developed in streptozotocin-induced diabetic angiotensin II type 1a receptor–deficient mice, despite a significant decrease in renal angiotensin II levels, PRRB markedly abolished these conditions, despite the absence of any change in the increase in renal angiotensin II levels.[5] Therefore, angiotensin II–independent glomerular mitogen-activated protein kinase activation by the (pro)renin receptor also may contribute to the development of diabetic nephropathy.
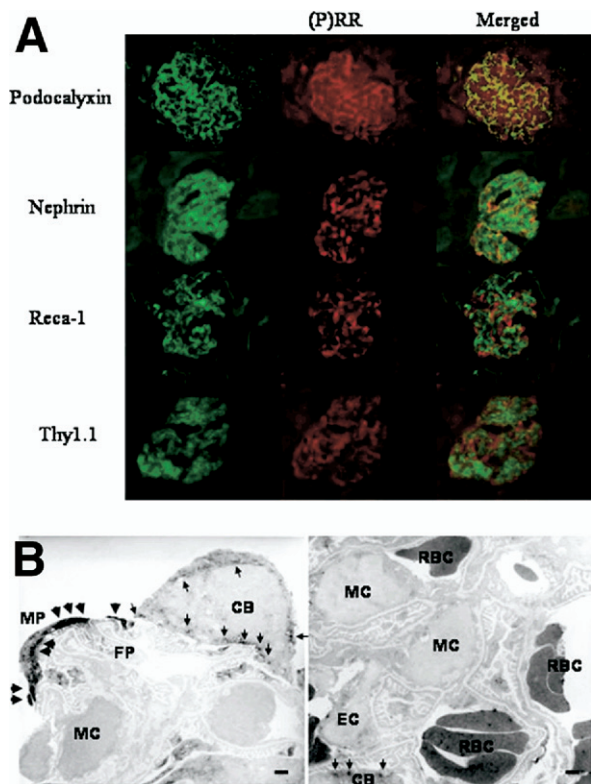
Figure 2. (A) Double fluorescent immunohistochemistry images showing rat (pro)renin receptor ([P]RR, red) and podocalyxin, nephrin, reca-1, or Thy1.1 (green). Merged images were obtained for (P)RR and podocalyxin. (B) Electron microscopy of the podocytes in the rat kidneys. The majority of (P)RR was present in the major processes (MP) of podocytes, as shown by the large arrows, and a few were observed in the cell body (CB) of the podocyte, as shown by the small arrows, but not in the foot processes (FP) of the podocyte, in the mesangial cells (MC), or in the endothelial cells (EC). RBC, red blood cells in capillary lumen. Scale bars: 1 μm.
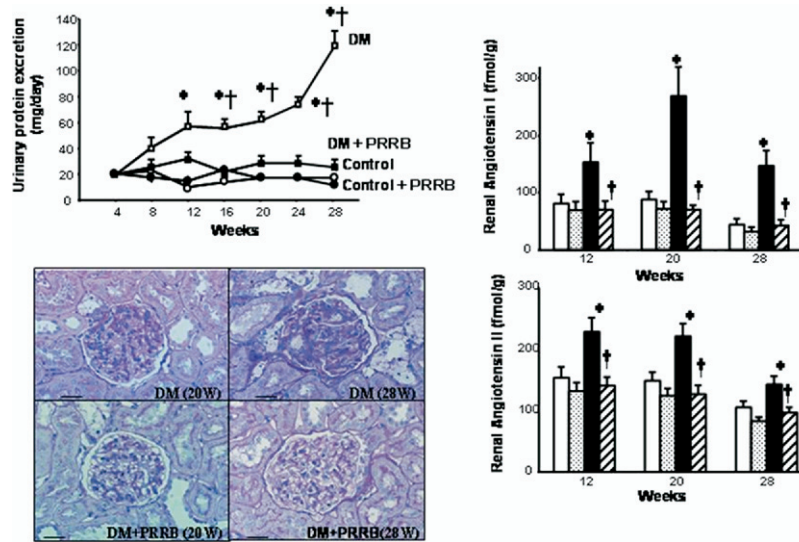
Figure 3. PRRB significantly inhibited the development of proteinuria and glomerulosclerosis and the increases in renal angiotensin I and II levels in diabetic rats (DM). □, Control; ▨ , control + PRRB; ■, DM; ▨ , DM + PRRB. * $P < .05$ for DM versus control. † $P < .05$ for PRRB versus vehicle. Scale bars: 50μm. Reprinted with permission from Ichihara et al.[1]

Dilated afferent arterioles and enlarged glomeruli are observed in the early stage of diabetic nephropathy, thus glomerular hyperfiltration is well known to play a role in the development of diabetic nephropathy. The increased expression of macula densa cyclooxygenase-2 (COX-2) is observed in the kidneys of diabetic animals[10,11] and is thought to account for the hyperfiltration state of the glomeruli as a result of afferent arteriolar dilation. Figure 5 shows that the macula densa COX-2 mRNA and protein levels were significantly higher in human (pro)renin receptor gene transgenic rats than in wild-type rats,[12] suggesting the involve-

ment of the (pro)renin receptor in the mechanism of increased macula densa COX-2 expression. Thus, the (pro)renin receptor also may contribute to the glomerular hyperfiltration state resulting from afferent arteriolar dilation through an increase in macula densa COX-2 expression in diabetic animals.

The plasma prorenin levels of stroke-prone spontaneously hypertensive rats (SHRsp) have been reported to be high,[13,14] and increased plasma prorenin levels cause end-organ damage in the kidneys independently of blood pressure.[15] Studies have shown that (pro)renin re-
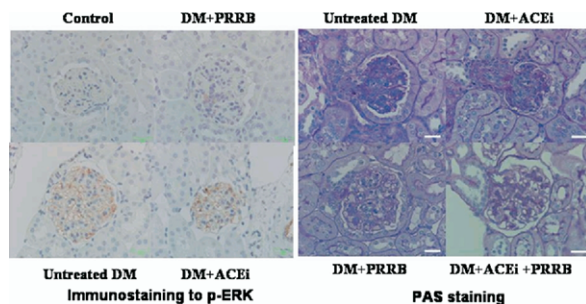
Figure 4. Renal expression of phosphorylated extracellular-signal–related protein kinases (p-ERK) and renal morphology in control rats, untreated diabetic rats (DM), and DM treated with PRRB, an angiotensin-converting enzyme inhibitor (ACEi), or both. Scale bars: 25μm. Reprinted with permission from Ichihara et al.[5]
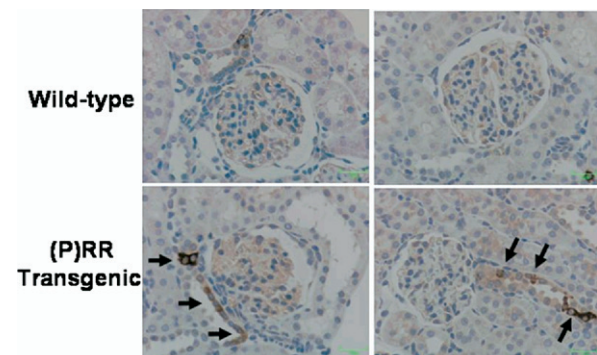
Figure 5. Enhanced COX-2 expression of tubular cells adjacent to the macula densa, shown by arrows, in (pro)renin receptor ([P]RR) transgenic rats compared with wild-type rats. Scale bars: 25μm. Reprinted with permission from Kaneshiro et al.[12]
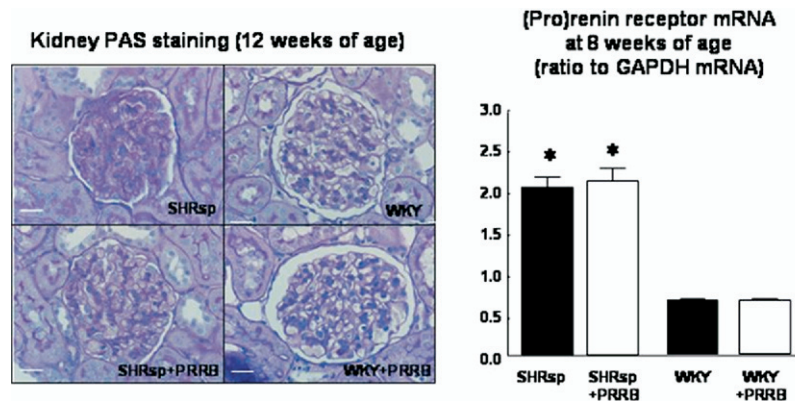
Figure 6. Renal morphology and (pro)renin receptor mRNA expression in stroke-prone spontaneously hypertensive rat (SHRsp), Wister-Kyoto rats (WKY), SHRsp treated with PRRB, and WKY treated with PRRB. *$P < .05$ for SHRsp versus WKY. Abbreviations: PAS, periodic acid schiff; GAPDH, glyceraldehyde-3-phosphate dehydrogenase. Reprinted with permission from Ichihara et al.[16]

ceptors, which bind to prorenin and stimulate its enzyme activity, are localized in prorenin-sensitive organs such as the kidneys.[2] These findings suggest that the (pro)renin receptor sequesters prorenin and activates it on the surface of the cells of critical organs susceptible to end-organ damage, and that the activated prorenin in turn generates angiotensin I and II locally, thereby exerting local actions that lead to tissue damage. In SHRsp, which have increased renal levels of (pro)renin-receptor mRNA, the (pro)renin receptor activated prorenin in the glomeruli of the kidneys, where tissue angiotensin I and II concentrations were increased markedly; consequently, end-organ damage occurred (Fig. 6).[16] Because prorenin activation was blocked almost completely by PRRB, resulting in a decrease of angiotensin I and II levels and attenuation of damaged morphology in the kidneys of the hypertensive animals, the (pro)renin receptor also may play a significant role in the development of hypertensive renal damage.

CONCLUSIONS

In the kidneys, the (pro)renin receptor is present predominantly in the mesangium and podocytes, and its blockade had beneficial effects on the kidneys of experimental diabetic and hypertensive animal models with elevated plasma prorenin levels through inhibition of both the nonproteolytic activation of prorenin and RAS-independent intracellular signals.

Thus, the glomerular (pro)renin receptor appears to contribute to the maintenance of the glomerular filtration barrier.

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