Abstract

The discovery of specific renin/prorenin receptors, termed (pro)renin receptors, initiated several studies on the pathological role of prorenin. The association of high prorenin levels with the development of kidney injury in diabetes suggested that prorenin itself may induce diabetic nephropathy. Prorenin can be activated in two ways: a proteolytic and a nonproteolytic. When bound to a (pro)renin receptor, prorenin displays full enzymatic activity through the nonproteolytic way, but it not only facilitates angiotensin generation, but also causes activation of intra−cellular signaling independent of angiotensin. This review presents current data which characterize the role of the renin/prorenin-(pro)renin receptor system particularly in diabetic nephropathy and the results of studies on the inhibition of this system in experimental diabetic animals (Adv Clin Exp Med 2008, 17, 2, 123–128).

Key words: renin/prorenin, (pro)renin receptor, diabetic nephropathy, inhibition of (pro)renin receptor.

Streszczenie

Odkrycie receptorów reniny/proreniny, określanych jako receptor (pro)reniny, zapoczątkowało liczne badania nad oceną znaczenia proreniny w patologii. Związek dużych stężeń proreniny w osoczu z rozwojem uszkodzenia ne−rek u chorych na cukrzycę sugerował, że sama prorenina może wpływać na rozwój nefropatii cukrzycowej. Prore−nina może być aktywowana dwiema drogami: proteolityczną i nieproteolityczną. Po związaniu z receptorem (pro)reniny uzyskuje pełną aktywność enzymatyczną na drodze nieproteolitycznej, ale nie tylko zwiększa powstawanie angiotensyny, lecz również powoduje aktywację przekaźnictwa wewnątrzkomórkowego niezależnego od angiotensyny. W niniejszej pracy poglądowej przedstawiono aktualne dane charakteryzujące rolę układu reni−na/prorenina−receptor (pro)reniny w nefropatii cukrzycowej oraz wyniki badań nad hamowaniem tego układu w doświadczalnej cukrzycy u zwierząt (Adv Clin Exp Med 2008, 17, 2, 123–128).

Słowa kluczowe: renina/prorenina, receptor (pro)reniny, nefropatia cukrzycowa, hamowanie receptora (pro)reniny.
in the juxtaglomerular apparatus of the kidney [1]. Human renin is an aspartyl protease composed of 340 amino acids and with a molecular weight of 37,326 Da. The structure of mature renin consists of two regions, with the active catalytic center of the enzyme located between them.

Active renin has high substrate specificity to angiotensinogen and cleaves its N-terminus to angiotensin I (Ang I). Ang I is then transformed into angiotensin II (Ang II) by endothelial and soluble angiotensin-converting enzyme (ACE) and also by some other enzymes, such as chymase, particularly in the tissues, e.g. in the heart and the kidney [6]. Ang II can also be transformed into Ang 1–9 by angiotensin-converting enzyme II (ACE II). Then Ang 1–9 can be transformed to Ang 1–7 under the influence of ACE. The biological functions of Ang 1–7 are at least in part opposite to the well-known vasoconstrictive and pro-proliferative effects of Ang II. In contrast to the well-characterized circulating and local renin-angiotensin system (RAS), the role of the excess of prorenin in the circulation remained unknown for a long time, until the renin/prorenin receptors were discovered [1].

### Renin/Prorenin Receptors

Three renin/prorenin receptors have been described [1]. The mannose-6-phosphate receptor (M6P-R) or insulin-like growth factor II receptor was localized on human endothelial cells and on neonatal rat cardiocytes [7–9]. The M6P-R binds renin and the M6P-containing (pro)renin. After internalization of the M6P/(pro)renin-M6P-R complex, the prorenin is submitted to proteolysis and mature renin is liberated and degraded without the generation of angiotensin I. The M6P-R is considered a ubiquitous clearance receptor for prorenin/renin [1].

Peters et al. [9] observed in rats transgenic for the mouse ren-2d gene that unglycosylated ren-2d prorenin was able to bind to rat cardiocytes and was then internalized and activated, which resulted in intracellular Ang I and Ang II generation. The putative prorenin/renin receptor in cardiac cells was not well characterized [1, 10]. Nguyen et al. [10] identified a specific receptor for renin and prorenin on human mesangial cells in culture. Using immunofluorescence, the renin/prorenin receptors were also found in vascular smooth-muscle cells of renal cortical arteries and coronary arteries [11]. The specific receptor for renin/prorenin, named (pro)renin receptor, was characterized as a 350-amino-acid protein with a single trans-membrane domain. The gene encoding the renin/prorenin receptor, called (pro)renin receptor [(P)RR], is most probably located on chromosome X p11.4 and presents partial nucleotide sequence homology with the gene for proton ATPase accessory protein 2 (ATP6ap2) [12]. It was postulated that the (P)RR gene might originate from the fusion of two genes, one ancestral gene encoding the C-terminus transmembrane and cytoplasmic regions responsible for conserved cellular functions and one gene encoding the N-terminus ectodomain conserved in vertebrates and responsible for (pro)renin binding and signaling [12, 13]. The cloned renin receptor has a molecular weight of 45 kDa and is most probably subjected to dimerization induced by renin binding or complex formation with another, yet unidentified protein, which results in the molecular weight of 70–80 kDa observed for the receptor on primary mesangial cells [12, 14]. The (pro)renin receptor binds prorenin and renin with the same affinity [12].

As indicated by Suzuki et al. [15], (pro)renin binds to the receptor with a “handle” region of the molecule (I11P FLKR15P), which results in the dissociation of the “gate” region (T7P FKR10P) that covers the enzymatically active site of prorenin. When prorenin interacts with this specific receptor through the “handle” region of the N-terminal prosegment of the molecule, it undergoes a conformational change, achieving the full enzymatic activity of renin [16]. This transformation is called the nonproteolytic activation of prorenin [12]. The receptor also binds renin, and receptor-bound renin exhibited five-fold increased catalytic activity compared with mature renin in solution [1]. In addition to increased prorenin/renin activity, the binding of (pro)renin to the receptor triggers intracellular signaling and the phosphorylation of the mitogen-activated protein kinases p44 (ERK1)/p42 (ERK2) [1].

### The Effects of Renin/Prorenin Receptor Activation

Renin or prorenin bound to the specific receptor is neither internalized nor degraded [12, 14]. The binding has two consequences: it allows prorenin to reach full enzymatic activity with an increase in local renin effects and it leads to the direct activation of intracellular signal pathways [12]. Receptor-bound prorenin is subjected to a conformational change, most likely provoking unfolding of the prosegment and making the enzymatic active region accessible to angiotensinogen. Due to this nonproteolytic activation, receptor-
-bound prorenin can cleave angiotensinogen into angiotensin I (Ang I), which is subsequently transformed into angiotensin II (Ang II) by the angiotensin-converting enzyme (ACE) present at the cell surface. Locally produced Ang II can activate its AT1 receptor also expressed at the cell surface, which leads to the activation of mitogen-activated protein (MAP) kinases p44/p42 or extracellular regulated kinases 1/2 (ERK 1/2) [12]. It was demonstrated that a pentapeptide ("decoy peptide") reproducing the "handle" region of prorenin inhibited prorenin binding to the receptor and protected the tissues against damage attributed to renin-angiotensin system (RAS) stimulation [17, 18]. The prorenin or renin bound to the specific receptor also causes direct activation of MAP kinases p44/p42 or ERK 1/2 independently of Ang II [12, 19, 20].

The activation of the ERK 1/2 pathway is involved in the stimulation of cell hypertrophy and proliferation. It was shown that renin (or prorenin) binding to the receptor induces an increase in transforming growth factor β-1 (TGF-β1) expression in rat mesangial cells, which in turn upregulates the expressions of several profibrotic molecules, including plasminogen activator inhibitor 1 (PAI1), fibronectin, and collagen I [12, 14, 19]. In rat cardiomyocytes, the activation of renin/prorenin receptor by prorenin induces (independently of Ang II) MAP kinase p38 and heat shock protein-27 (Hsp-27) phosphorylation, which may regulate growth, motility, and survival of the cells [18, 20].

It was suggested that the (pro)renin receptor was downregulated in vivo by a high concentration of renin, although the mechanism of this negative feedback was not determined [12]. If this is indeed the case, then it seems possible that in low-renin states the (pro)renin receptor is upregulated and particularly sensitive to stimulation by prorenin. This hypothesis seems attractive in relation to several low-renin conditions, including diabetic kidney disease. The results of recent studies indicate that the renin/prorenin-(pro)renin receptor system may play a particularly important role in the kidney damage caused by diabetes.

**The Renin/Prorenin—(Pro)Renin Receptor System in Diabetes**

More than 20 years ago, Luetscher et al. [21] reported that increased inactive plasma renin in diabetes mellitus is a marker of microvascular complications. In 1996, Allen et al. [4] demonstrated that a significant increase in total serum renin (active renin + prorenin) was apparent up to five years before the onset of microalbuminuria in the patients with type 1 diabetes mellitus. It was also shown that the increase in serum total renin level is due to a high prorenin concentration, while the renin concentration is low or normal. A high prorenin level was identified as an early marker of nephropathy in type 1 diabetes [22]. The mechanisms responsible for the increased plasma levels of prorenin in diabetic patients at risk of developing kidney damage have not been elucidated. Under most steady-state conditions, renin and prorenin in plasma change in parallel, although the concentration of plasma prorenin exceeds that of plasma renin [23]. In the streptozotocin diabetic rat, prorenin-processing enzymes, such as cathepsin B, which are predominantly present in the macula densa of the juxtaglomerular apparatus, have been reported to be decreased [24]. It may be hypothesized that in diabetic subjects predisposed to kidney damage, the processing of prorenin to active renin in the juxtaglomerular cells is markedly inhibited, which results in elevated prorenin and reduced renin secretion, although other mechanisms, including hypervolemia due to sodium retention, may be involved in low levels of plasma renin. This hypothesis as well as the role of increased prorenin plasma levels in the development of diabetic kidney disease remain to be clarified. It was demonstrated, however, that transgenic rats expressing prorenin exclusively in the liver have severe renal histopathological changes mimicking diabetic nephropathy without hypertension [25].

In the streptozotocin diabetic rats, plasma prorenin level was significantly higher, while plasma renin activity (PRA) and plasma levels of Ang I and II were markedly lower than in healthy control rats [17]. In the kidneys of these rats, total renin content and the renin mRNA level were lower, but Ang I and Ang II content were significantly higher than in control rats, when renal histological changes developed [17]. In the juxtaglomerular area of the kidneys, the prorenin-positive cells were significantly greater in number compared with those of control rats. It was indirectly demonstrated that the kidneys of diabetic rats may have an increased level of nonproteolytically activated prorenin [17]. Taken together, during the development of diabetic nephropathy, intrarenal renin activity increases due to nonproteolytic activation of prorenin and the levels of Ang I and Ang II increase without any changes in renin, ACE, or angiotensin synthesis. The decreased prorenin processing in the juxtaglomerular cells, caused by the decrease in proteases including cathepsin B, can increase the ratio of prorenin to
renin in diabetic rat kidneys [17] and also in the circulation. Since both renin and prorenin bind competitively to the (pro)renin receptor, the relative increase in prorenin versus renin could cause more binding of prorenin to its receptor, leading the nonproteolytic activation of prorenin [17]. It is also possible that the affinity of prorenin to (pro)renin receptor might be increased in diabetic kidneys [17].

The increased binding of prorenin to its receptor also results in the activation of the ERK 1/2 pathways independently of the activation of Ang II, as proposed by Nguyen [12]. Most probably the dual mechanisms triggered by the increased prorenin binding to its receptor are involved in the development of diabetic glomerulosclerosis and proteinuria, but surprisingly without elevation of blood pressure [17].

The Effects of Prorenin--(Pro)Renin Receptor System Inhibition in Experimental Diabetic Nephropathy

A peptide with the structure of the "handle" region of the prorenin molecule (HRP – handle region peptide) was constructed. It was demonstrated that this HRP binds competitively to (pro)renin receptor as a decoy peptide and inhibits the nonproteolytic activation of prorenin [17]. Streptozotocin-induced diabetic rats treated with subcutaneous administration of HRP demonstrated normalization of the Ang I and Ang II renal content and complete inhibition of the development of diabetic nephropathy (glomerulosclerosis and proteinuria) without affecting hyperglycemia and blood pressure [17]. The administration of HRP did not affect plasma renin, Ang I, and Ang II levels and did not alter kidney cathepsin B mRNA level. The increased prorenin content in the kidneys was not affected by HRP treatment, but the level of activated prorenin was reduced toward the normal range, which indicates that HRP inhibited the nonproteolytic activation of prorenin [17]. It is also possible that HRP prevents the effects of direct activation of (pro)renin receptor by prorenin independently of the local RAS activation and can therefore completely prevent the development of diabetic kidney damage [17]. Prevention of proteinuria and the development of glomerulosclerosis in diabetic rats by HRP suggests that the inhibition of prorenin binding to the (pro)renin receptor and nonproteolytic activation of prorenin may have superior beneficial effects compared with ACE inhibitors or Ang II type 1 receptor blockers (ARB) [17].

Both ACE inhibitors and ARB increase renin secretion because they interrupt the normal feedback suppression by Ang II. The reactive augmentation of circulating active renin leads to a greater generation of Ang I, which increases the formation of Ang II via pathways dependent or independent of ACE [26]. As a consequence, an escape from the inhibitory effect of the RAS blockade phenomenon can occur.

An additional beneficial effect of (pro)renin receptor blockade in nephropathy that had already developed in diabetic rats was recently reported by Tekahashi et al. [16]. The authors treated heminephrectomized streptozotocin-induced diabetic rats presenting increased urinary protein excretion and significant glomerulosclerosis with (pro)renin receptor blocker (PRRB, formerly "handle region decoy peptide"), ACE inhibitor, or vehicle peptide by using subcutaneously implanted osmotic minipumps for 12 weeks. At the end of the observation, the expression of activated prorenin in the kidney was markedly suppressed in the rats with advanced diabetic glomerulosclerosis that were treated with PRRB and no increase in proteinuria and even significantly improved glomerulosclerosis were observed. The blood glucose level and blood pressure were unaffected by PRRB treatment. In the rats treated with ACE inhibitor the expression of activated prorenin was increased in the same range as in the vehicle-treated animals, and only attenuated increases in proteinuria and glomerulosclerosis were found when compared with the progressive changes observed in the vehicle-treated rats [16]. It was concluded that a long-term (pro)renin receptor blockade significantly reversed glomerulosclerosis and markedly inhibited the increase in proteinuria after significant nephropathy occurred in diabetic rats. The effects of PRRB treatment should be attributed to the lowering renal levels of activated prorenin and Ang II [16], but also, and probably predominantly, to the inhibition of RAS-independent pathways. It was suggested that the RAS-independent effects of prorenin receptor blockade might play a key role in the regression of nephropathy in diabetic animals. A recent study demonstrated that (pro)renin receptor stimulates matrix protein expression in rat mesangial cells through a RAS-independent mechanism [27]. It was also reported that (pro)renin receptor stimulation contributes to the development of nephropathy in diabetic Ang II type 1a receptor-deficient mice [28]. PRRB administration in such animals attenuated the development of proteinuria and glomerulosclerosis and also inhibi-
panied increased ERK 1/2 of p38 and c−Jun N−terminal kinase (JnK) activation in the diabetic kidney, clearly confirming independence of the effects mediated by RAS [28]. It was suggested that prorenin is the main ligand of (pro)renin receptor in vivo [29].

A study by Peters et al. [30] published this year left little doubt that prorenin has a physiological role in the generation of tissue RAS and perhaps a pathophysiological one as well [31]. Peters et al. [30] developed a cyp1a1ren−2 transgenic rat model in which prorenin levels and arterial pressure can be increased by oral administration of indole−3−carbinol (I3C). I3C treatment for 2 weeks in these transgenic rats resulted in an increase in plasma prorenin concentrations and arterial pressure in a dose−dependent manner. After 12 weeks of 0.125% I3C, the rats exhibited markedly elevated plasma prorenin concentration, increased blood pressure, and moderate hypertensive renal vasculopathy, but no histological sings of glomerulosclerosis. The authors concluded that high circulating prorenin levels per se do not cause glomerulosclerosis, but suggested that prorenin may be a permissive factor for the development of glomerulosclerotic lesions [30]. It was also suggested that any putative effects of prorenin on glomerulosclerosis may become discernable only if certain glomerulosclerosis−promoting factors, such as inflammation, are simultaneously present [30]. The coexistence of diabetes may probably act as such a promoting factor, but further studies are clearly needed. Many additional studies are also necessary to prove that agents which act as (pro)renin receptor antagonists and which are safe in humans are of potential therapeutic benefit in diabetic nephropathy. The results of experimental studies are promising.

References


