Renin/prorenin receptors

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The existence of a tissue renin–angiotensin (RAS) system independent of the circulating RAS has prompted the search for cellular binding sites for angiotensinogen and for renin in order to explain their tissue uptake. Two receptors that bind with similar affinity mature renin and prorenin were identified, the mannose-6-phosphate receptor (M6P-R) and a specific receptor. The M6P-R is a clearance receptor that binds exclusively the glycosylated forms of renin and prorenin. Binding of renin and prorenin to the M6P-R is followed by internalization and degradation, and the intracellular proteolysis of prorenin in mature renin did not provoke any generation of intracellular angiotensins. In contrast to the M6P-R, (pro)renin bound to the specific receptor was not degraded. Instead, receptor-bound renin showed increased catalytic activity, and receptor-bound prorenin exhibited full catalytic activity. This 'gain of activity' was explained by a conformational change of the (pro)renin molecule upon binding. Furthermore, (pro)renin binding provoked a rapid activation of the mitogen-activated protein kinases p44/p42, indicating that the receptor has mediated specific, angiotensin II-independent effects of (pro)renin. This receptor represents an elegant concept to explain the existence of active prorenin *in vivo*, and it provides a pathological role for prorenin in situations with paradoxical low renin and high prorenin concentrations such as in diabetes. Experimental models of rats overexpressing the receptor either in vascular smooth muscle cells and developing high blood pressure or with ubiquitous expression associated with glomerulosclerosis and proteinuria confirm a role for the receptor in cardiovascular and renal diseases.

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The renin–angiotensin system (RAS) is classically described as a circulating system with a single end point, the generation of angiotensin II (Ang II) that plays major role in the control of blood pressure and fluid, and salt balance through binding to its receptors. But the activation of the RAS is also involved in physiological processes such as development, learning and memory, in tissue growth, and in pathological diseases such as inflammation, in macro- and micro-vascular hypertrophy (atherosclerosis and diabetic retinopathy) and vascular remodeling (including in tumors), fibrosis and obesity, all processes in relation with a tissue RAS. The existence of a local generation of angiotensins independent of circulating angiotensins is now admitted, and the prerequisite is the presence of renin in tissue, but except in eye, adrenal, testis, ovaries, and brain where prorenin synthesis was shown, renin and prorenin in tissues accumulate in the interstitial fluid by diffusion or are taken up by binding sites and/or receptors.[1,2] Renin is an aspartyl-protease that exists in two forms, the proenzyme prorenin and mature renin. Prorenin is transformed into mature renin by cleavage of the 43 amino acids of the pro-segment. Mature renin in the circulation originates from the myo-epithelioid cells of the juxta-glomerular apparatus that are responsible for the synthesis and the processing of prorenin into mature renin and the release of renin in the circulation. Prorenin, although synthesized by a restricted number of tissues, represents up to 90% of total plasma renin in normal subjects. This excess of prorenin that cannot be activated in the circulation has intrigued for a long time, and the hypothesis of a functional role of prorenin was suggested. The discovery of receptors for renin and prorenin has revived the interest for a physiological role of prorenin and for (pro)renin specific functions independent of Ang II generation.

Several renin binding proteins (RnBPs) were described, on cell membranes prepared from rat tissues with no further characterization,[3] and in the intracellular compartment. The protein identified in the intracellular compartment was called renin binding protein was shown to be identical to the enzyme *N*-acyl-D-glucosamine 2 epimerase. Although renin binding protein could inhibit renin activity *in vitro*, mice RnBP−/− have normal plasma renin activity and normal blood pressure, making unlikely a role for renin binding protein in the control of renin activity *in vivo*.[4]

To date, two receptors have been identified and characterized, the mannose-6-phosphate receptor (M6P-R) and the

specific receptor, and a third receptor on rat cardiomyocytes was described.

THE RENIN AND PRORENIN RECEPTORS

A receptor for unglycosylated prorenin on rat adult cardiomyocytes

Using rats transgenic for the mouse *ren-2*^{d} renin gene (coding for unglycosylated prorenin) and with an inducible expression restricted to the liver, Peters *et al.*[5] have shown an increased synthesis of *ren-2*^{d} renin associated with high levels of *ren-2*^{d} prorenin in plasma and, surprisingly, also within cardiac cells. In addition, they showed that the unglycosylated *ren-2*^{d} prorenin was able to bind *in vitro* to rat cardiomyocytes and that bound-prorenin was internalized, whereas mature *ren-2*^{d} renin was weakly bound and internalized. Prorenin internalization was associated with an increased intrinsic catalytic activity and, remarkably, with intracellular Ang I and Ang II generation. Unfortunately, this receptor was not further characterized.

The M6P-R

The M6P-R, also called insulin-like growth factor II receptor, is an ubiquitous receptor that was shown to bind renin and prorenin on neonatal rat cardiomyocytes[6] and on human endothelial cells[7] with an affinity of about 1 nM. In contrast to the receptor for unglycosylated (pro)renin described by Peters *et al.*, the M6P-R binds equally well renin and prorenin but exclusively the M6P containing (pro)renin. The M6P-R/(pro)renin complex is then internalized, prorenin activated in mature renin by proteolysis and renin was subsequently degraded. Intracellular prorenin activation did not result in intra or extracellular angiotensin generation. Therefore, this receptor appears to be most likely a clearance receptor.

The specific receptor

A receptor of mature renin was first identified on human mesangial cells in culture. Renin binding to the receptor did not involve the active site and binding induced an increase of [^{3}H] thymidine incorporation and of plasminogen activator inhibitor-1 synthesis.[8] This receptor is a 350-amino-acid protein with a single trans-membrane domain cloned by screening a human adult kidney expression library with labeled renin. This receptor also binds prorenin, the inactive precursor of renin. Receptor-bound renin exhibited increased catalytic activity ($\times 5$) and receptor-bound prorenin exhibited full enzymatic activity comparable to that of mature renin in solution. This 'gain of activity' of renin and prorenin was explained by a conformational change induced by the binding. In addition, binding of (pro)renin to the receptor triggered an intracellular signaling and the phosphorylation of the mitogen-activated protein kinases p44(ERK1)/p42(ERK2).

Immunofluorescence studies performed on frozen tissues have initially localized the receptor in glomerular mesangial cells and in vascular smooth muscle cells of renal cortical arteries and coronary arteries.[9]

POTENTIAL SIGNIFICANCE OF THE SPECIFIC (PRO)RENIN RECEPTOR

The specific receptor provides a functional role for prorenin

In plasma, prorenin represents 70–90% of total circulating renin in normal subjects, and up to 95% of in diabetic patients.[10,11] Prorenin is catalytically inactive when its pro-segment covers the cleft containing the active site. Prorenin can acquire a catalytic activity after transforming in mature renin by cleavage of the prosegment (an irreversible process known as 'proteolytic activation') or by physical means such as low pH or cold that unfold the propeptide from the enzymatic cleft ('non-proteolytic' activation that can be reversed by increasing the pH or the temperature). The physiological proteolytic activation or processing of renin is probably due to a proconvertase in the renin-producing cells of the juxta-glomerular apparatus. *In vitro*, limited proteolysis by kallikrein, plasmin, and trypsin can also remove the propeptide to yield the mature renin and nonproteolytic activation of prorenin can also be observed when prorenin is bound to an antibody against a called 'handle' region of the pro-segment[12] triggering a conformational change of the molecule provoking the opening of the access to the catalytic site of prorenin. Since *in vivo*, no antibody to the handle region exists, the authors hypothesized that renin binding sites, in particular the renin receptor could play this role especially in situations with high prorenin concentrations like in diabetes. Subsequently, Ichihara *et al.*[13] showed that the infusion of a decoy peptide corresponding to the handle region of prorenin in streptozotocin-induced diabetic rats could completely prevent diabetic nephropathy assessed on morphological and functional data and could also normalize the kidney content of Ang II. Furthermore, the decoy peptide did not modify the plasma content of Ang I and Ang II in diabetic rats nor did it affect tissue Ang I and Ang II in control rats. This study provides the first evidence that prorenin can contribute to Ang I and Ang II formation in the kidney of diabetic rat, and it supports experimental data showing that *in vivo* prorenin was catalytically active.[14]

If the pathogenic role of the receptor-activated prorenin in diabetic nephropathy can be confirmed, for example, by using renin receptor-deficient animals, then these results would have major therapeutic consequences, implying that an inhibitor of prorenin and renin active site or a compound able to inhibit both (pro)renin binding and activity would be beneficial in diabetic nephropathy.

Signaling of the renin receptor via ERK1/2 activation is important for cell function in the central nervous system

In the central nervous system, the role of the RAS is involved in a wide variety of functions, including the control of cell growth and death, the neuro-endocrine regulation, cognitive properties, the modulation of cardiovascular functions such as autonomic activity, salt intake, and drinking. We have recently found a specific role for the renin receptor in cognitive functions and brain development related to the mutation in the renin receptor gene (*ATP6AP2*). The patients

suffered X-linked mental retardation and epilepsy, and no apparent cardiovascular or renal dysfunction. This mutation resulted in the deletion of exon 4 in 50% of renin receptor mRNA and functional studies showed that, although the mutated receptor could bind renin and increase renin catalytic activity as described for wild-type receptor, renin binding did not activate the mitogen-activated protein kinases ERK1/2.[15] The reason why the central nervous system is more sensitive to the lack of renin receptor is not known yet, but our findings suggest that activation of the ERK1/2 system via the renin receptor system is important in brain development and to maintain normal neurological functions. Its impairment can apparently not be compensated by other extracellular stimuli as for cardiac, vascular, and renal tissues.

The renin receptor may be important for cell metabolism

A 8.9 kDa fragment of the renin receptor called M8.9 and corresponding to the cytoplasmic, the trans-membrane and part of the extracellular domain was described to co-precipitate with the vacuolar proton-ATPase in chromaffin granules.[16] Since there is only one gene for the renin receptor and the M8.9 protein, it is likely that both proteins derive from the same transcript, but the mechanisms leading to the generation of the 8.9 kDa fragment are not known yet. *In silico* research showed that the renin receptor protein is highly homologous in human, mouse, and rat, as well as in chicken, fish, *Xenopus*, and *C. elegans* especially the segment associated with the vacuolar proton-ATPase,[17] suggesting an important conserved function for the renin receptor. Indeed, the ablation of the renin receptor gene in embryonic stem cells is not compatible with their participation in embryonic development after injection into blastocysts (C Burckle and M Bader, personal communication) and renin receptor gene appears to be essential for early zebrafish development since its inactivation is lethal before the end of the embryogenesis.[18] Therefore, it is likely that the renin receptor should possess two types of function, one function as a plasma membrane protein acting as a receptor for prorenin and one function independent of the RAS, and related to the M8-9 fragment associated with the vacuolar proton-ATPase.

Rats overexpressing the renin receptor have cardiovascular and renal dysfunctions

To study the role of the renin receptor *in vivo*, rats overexpressing the receptor were generated in which the human receptor gene was expressed in smooth muscle tissue under the control of the mouse smooth muscle myosin heavy chain gene or under the control of the cytomegalovirus promoter. Overexpression of the human receptor under the control of smooth muscle myosin heavy chain gene induced a strong expression in vascular smooth-muscle cells, and the rats developed at the age of 6 months, a cardiovascular phenotype with an increase of systolic blood pressure and of heart rate as compared to their littermates, and these alterations progressively worsened with aging. Although kidney function and plasma renin were normal in transgenic rats, the plasma aldosterone and in aldosterone/renin ratio were higher in transgenic animals. The mechanism of the increased aldosteronemia is not fully understood, but it could underlie the cardiovascular phenotype of these rats.[19] On the other hand, overexpression of human receptor under the control of cytomegalovirus promoter induced an ubiquitous but very modest increase of receptor expression. These rats have no increased blood pressure or kidney Ang II content; however, they had increased urinary protein excretion compared to control rats and significant glomerulosclerosis at the age of 28 weeks (Kaneshiro Y *et al.*, Transgenic Rats Overexpressing the Human Prorenin Receptor Gene Develop Glomerular Sclerosis without Hypertension or Diabetes. *J Am Soc Nephrol* 2005; **16**: 74A–75A). Whether or not glomerulosclerosis could be linked to ERK1/2 activation by the renin receptor remains to be determined. However, the role of (pro)renin receptor activation in glomerular fibrosis is supported by the excellent work of Huang *et al.*, who showed that activation of the (pro)renin receptor increased TGFβ synthesis by human and rat mesangial cells. This upregulation of TGFβ was responsible in part of the increased synthesis of plasminogen activator inhibitor-1 and fibrotic matrix components such as fibronectin and collagen I. Using the siRNA technique, these authors unequivocally demonstrated that the increase of TGFβ was due to activation of the renin receptor.[20] Finally, the data from transgenic rats confirm that the specific renin receptor plays a role in the control of cardiovascular and renal physiopathology, but the challenge now is to decipher the mechanisms underlying the receptor functions.

SUMMARY AND PERSPECTIVES

Two major facts have emerged from the studies on the (pro)renin receptors. First, the receptors binds not only mature renin but also prorenin resulting in prorenin activation. Whether prorenin is proteolytically activated within the cell or nonproteolytically activated at the cell surface, its activation is associated with angiotensins generation providing the first demonstration of a physiological role of prorenin. Second, experimental data on transgenic rats confirm a link between the overexpression of the receptor and cardiovascular and renal dysfunctions possibly involving direct activation of the receptor by (pro)renin. It would be of interest to know now if the renin inhibitors clinically available soon will be able to interfere with receptor-bound renin and prorenin activity, and with receptor activation or expression, and to compare them with angiotensin converting enzyme inhibitors and angiotensin receptor blockers for their tissue-protective effects in view of therapeutic use.

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