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# Commentary

## Prorenin and the (Pro)renin Receptor in Ocular Pathology

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In recent times, there has been considerable interest in the role of the (pro)renin receptor [(P)RR] in organ pathology. In this issue of *The American Journal of Pathology*, Satofuka and colleagues[1] report on the protective effects of a particular (P)RR inhibitor in an experimental model of age-related macular degeneration. The (P)RR receptor is part of the renin-angiotensin system (RAS), a hormonal cascade that influences blood pressure and cellular function including proliferation, angiogenesis, inflammation, and the stimulus of growth factor pathways. The RAS pathway is initiated by prorenin, the inactive precursor of renin, an enzyme that cleaves angiotensinogen to generate angiotensin I (Ang I). Subsequent conversion of Ang I by angiotensin-converting enzyme (ACE) results in angiotensin II (Ang II). Ang II can then bind to its receptors, of which the angiotensin type 1 (AT1-R) and angiotensin type 2 receptors are most well characterized. Blockade of the RAS with ACE or AT1-R inhibitors aims to reduce the deleterious effects of Ang II, and these interventions are commonly used for the treatment of cardiovascular and renal diseases.[2]

For many years, prorenin was viewed to have little function of its own and simply be the precursor of renin. However, observations that plasma prorenin exists in 10-fold higher amounts than renin,[3] and becomes elevated in diseases such as diabetic retinopathy,[4,5] led to speculation that prorenin might have its own receptor and elicit effects independently of Ang II. More than 20 years ago, prorenin and renin binding proteins were reported and a clearance receptor identified; however, neither were found to generate Ang II. The discovery of the (P)RR, which binds both renin and prorenin and induces signal transduction pathways that are independent of Ang II, led to the suggestion that the (P)RR might have a role in organ pathology. One research team has now generated a (P)RR blocker and reported significant protective effects in cardiorenal[6–8] and ocular disease.[9,10] This issue of the *AJP* contains a subsequent study by Satofuka and colleagues[1] that describes the protective effects of this (P)RR blocker in an experimental model of choroidal neovascularization (CNV) that is often used to study age-related macular degeneration. These findings are of considerable interest because they suggest an alternative approach for organ protection via RAS blockade. These results are discussed in the context that the mode of action of the (P)RR is yet to be fully elucidated, and recent evidence by other investigators suggests that this (P)RR blocker does not confer organ protection in cardiorenal disease.[11,12]

### *Prorenin and the (P)RR*

Renin is an aspartyl protease that consists of two homologous lobes. The cleft between the lobes contains the active site with two catalytic aspartic residues. Prorenin is the inactive form of renin, with an amino-terminal prosegment that folds over the cleft between the two lobes of renin to prevent access to the active site by angiotensinogen. Prorenin can become catalytically active when an irreversible process known as proteolytic cleavage removes the prosegment. *In vivo*, this mainly occurs in the juxtaglomerular cells of the kidney by enzymes such as proconvertase and cathepsin B. *In vitro*, low pH or cold can unfold the prosegment from the enzymatic cleft to expose the active site in a process known as nonproteolytical activation. Reversal of this process is possible by increasing pH and temperature. In 2002, Nguyen and colleagues[13] identified a (P)RR for renin and prorenin that is a 350-amino acid protein with a single transmembrane domain. *In vitro* studies indicate that binding to the (P)RR increases the catalytic efficiency of renin and causes nonproteolytic activation of prorenin, which must be because of a conformation change.[13,14]

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## The Ocular RAS

Local RAS exist in a variety of organs such as the kidney, adrenal, brain, and ovary. The eye also has a local RAS, with components expressed in the retina[4,15] and choroid.[16] Although prorenin and renin synthesis has been identified in retina,[15] the main source is likely to be the glomerular juxtaglomerular cells of the kidney, which release large amounts of prorenin and renin into the circulation. Prorenin and renin might then be sequestered into tissue sites. Throughout the past decade there has been increased interest in Ang II blockade as a treatment strategy. There is considerable evidence that ACE inhibition and AT1-R blockade (AT1-RB) prevent aspects of vascular and neuronal pathology in diabetic retinopathy[17] and retinopathy of prematurity.[18] The role of the RAS in age-related macular degeneration is not as extensively studied; however, there are reports that Ang II blockade is beneficial in experimental models.[16]

## Blockade of the (P)RR and Organ Disease

After the discovery of the (P)RR, Suzuki and colleagues[19] proposed that a site-specific binding protein interacts with a portion of the prosegment of prorenin, which was termed the handle region, to elicit a conformational change which renders prorenin enzymatically active. These investigators suggested that by inhibiting the handle region of the prosegment, that organ pathology attributable to the (P)RR would be suppressed. They constructed a synthetic handle region peptide (HRP) or decoy peptide corresponding with amino acids 10 to 19 of the prorenin prosegment that binds to the (P)RR.[19] This research group evaluated the effects of the HRP in a variety of organ pathologies. In rats with streptozotocin diabetes, administration of the HRP by miniosmotic pump for 24 weeks completely prevented the development of diabetic nephropathy including glomerulosclerosis and type IV collagen deposition.[6] In a subsequent study, Ichihara and colleagues[6] reported that the HRP attenuated cardiac fibrosis in stroke-prone spontaneously hypertensive rats fed a high-salt diet. In both situations, the HRP reduced both tissue Ang I and Ang II levels and the nonproteolytic activation of prorenin as assessed by immunohistochemistry.[6,19] In another study by the same group, the HRP was used in (P)RR transgenic rats.[8] The (P)RR rat generated is normotensive, has near normal Ang II levels, and develops nephropathy with aging. Remarkably, the HRP reduced glomerulosclerosis, proteinuria, and transforming growth factor-$\beta$ expression in kidney; however, ACE inhibition had no effect despite reducing kidney Ang II levels.[8]

Satofuka and colleagues[1,9,10] have studied the HRP [also termed a (P)RR blocker] in three models of ocular disease; endotoxin-induced uveitis, retinopathy of prematurity, and laser-induced CNV (current issue of the *AJP*). In all three pathologies, the HRP reduced vascular disease. In uveitis, the HRP administered 24 hours after the induction of lipopolysaccharide attenuated retinal leukocyte accumulation and protein leakage into the anterior chamber of the eye. Reductions in retinal gene and protein expression of inflammatory mediators were also detected after HRP administration.[10] In experimental retinopathy of prematurity, the HRP suppressed pathological angiogenesis, leukocyte accumulation, and intracellular adhesion molecule-1 and vascular endothelial growth factor expression.[9] In the murine model of CNV, the HRP clearly had similar effects as observed in retinopathy of prematurity, and also reduced macrophage infiltration. Experiments were also conducted in mice with CNV and genetic ablation of the AT1-R or angiotensinogen, or after treatment with the AT1-RB, losartan. In all situations, CNV was reduced indicating a role for the RAS in this pathology. The most interesting finding was that the HRP further suppressed CNV and macrophage infiltration, and was more beneficial than AT1-RB. Taken together these findings indicate a pathogenic role for prorenin and the (P)RR in a range of ocular diseases. Development of new (P)RR inhibitors might be warranted as a potential therapeutic strategy for diabetic retinopathy and age-related macular degeneration that could be used as either monotherapy or in combination with ACE inhibitors or AT1-RB.

Despite these positive effects of the HRP, other investigators have not yet reproduced these findings. Müller and colleagues[11] studied renovascular hypertension in two-kidney one-clip rats, which display high blood pressure, cardiac hypertrophy, and renal damage.[11] The HRP was infused for 2 weeks and had no effect on left ventricular hypertrophy, nephrosclerosis, or macrophage infiltration and interstitial collagen I accumulation in kidney. A further study by this group compared the renin inhibitor, aliskiren, with the HRP in double-transgenic rats overexpressing human renin and angiotensinogen genes. After 7 weeks, aliskiren, but not the HRP, reduced blood pressure and normalized albuminuria and renal tubular damage.[12] However, one study has reported that the HRP reduces left ventricular mass in spontaneously hypertensive rats, but the HRP did not influence myocardial collagen content, left ventricular function, and coronary and renal hemodynamics.[20]

## Outstanding Questions Relating to the Role of the (P)RR in Organ Pathology

It is unclear as to why different laboratories report varying effects of the HRP on organ pathology. It is possible that this might relate to the type of organ injury and the ratio of tissue and plasma renin to prorenin. The (P)RR binds both renin and prorenin, and preferentially prorenin.[14] In diseases such as diabetes, plasma prorenin is increased but renin is low,[5] which might indicate a preference for prorenin binding in target organs. This might be particularly relevant for ocular diseases such as diabetic retinopathy, in which plasma and vitreal prorenin levels are elevated by ~100-fold.[5] A similar situation might occur in retinopathy of prematurity, with elevated prorenin levels reported in retina.[18] Furthermore, in the model of CNV, Satofuka and colleagues[1] reported that prorenin and Ang II are elevated in retinal pigment epithelium-choroid compared to normal controls. On the other hand, in

the Goldblatt hypertension model and double-transgenic rats overexpressing human renin and angiotensinogen genes, both plasma renin and prorenin are elevated,[11,12] which might explain the lack of effect of the HRP. Yet to be fully understood is whether the HRP modulates not only prorenin binding but also renin binding, and the extent of renin's occupancy of the (P)RR.

Another consideration is whether the doses of HRP used in the various studies were sufficient to influence binding of prorenin to the (P)RR. As pointed out by Campbell,[21] the HRP is a small peptide, which might be quickly metabolized and cleared. Much higher doses of the HRP would be required to achieve the nanomolar HRP concentrations required to inhibit prorenin binding to the (P)RR. Even in the study by Feldt and colleagues,[12] who used 30-fold higher doses of the HRP than other investigators, there was no effect on kidney disease.

## *(P)RR Signal Transduction Pathways That Are Independent of Angiotensin II*

A number of the studies using the HRP have measured angiotensin levels, and found the HRP to reduce tissue angiotensin content without affecting blood pressure,[7,11,12] suggesting that organ pathology was attributable to prorenin-induced tissue angiotensin generation. On the other hand, there is evidence that prorenin induces cardiac and vascular damage independently of angiotensin, and that this occurs via the (P)RR. For instance, overexpression of the human (P)RR exhibits increased glomerulosclerosis, proteinuria, but not Ang II levels or hypertension.[8] In the initial study of the (P)RR, Nguyen and colleagues[13] reported that in renal mesangial cells, binding of renin to the (P)RR increases the catalytic efficiency of angiotensinogen conversion into Ang I. However, renin and prorenin binding also caused a rapid phosphorylation of the (P)RR on serine and tyrosine residues, which was associated with an induction of mitogen-activated protein kinases (MAPK) and phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2).[13] Other investigators have reported similar findings with prorenin binding to the (P)RR inducing transforming growth factor-$\beta$ and then plasminogen activator inhibitor-1, fibronectin, and collagen via ERK1/2.[22] Confirming these findings are reports that increases in ERK 1/2 and also heat shock protein 27 can be prevented by MAPK inhibition in cardiomyocytes and vascular smooth muscle cells.[12] Taken together, it is possible that inhibition of the (P)RR might provide organ protection that cannot be achieved with conventional Ang II blockade.

Based on this information and the findings by some investigators that the HRP protects against organ pathology, it would be reasonable to expect that the HRP inhibits MAPK activation and ERK1/2 phosphorylation. In transgenic (P)RR rats with kidney disease because of aging, the HRP reduced immunoreactivity and protein expression for phosphorylated-ERK1/2, -p38, -JNK, and transforming growth factor-$\beta$1; however, ACE inhibition had no effect.[8] In terms of ocular disease, to date this has only been studied for CNV. In the study by Satofuka and colleagues,[1] the HRP reduced phosphorylated ERK1/2 in mice with CNV, and also in AT1-R-deficient mice with CNV. However, the findings have not been reproduced in cultured vascular smooth muscle cells, where the HRP did not prevent renin- and prorenin-induced ERK1/2 phosphorylation, but a MAPK inhibitor was effective.[12]

## *Other Features of the (P)RR*

Perhaps relevant to ocular diseases, the highest levels of (P)RR expression are in the central nervous system, then the heart and placenta.[13] In contrast, kidney, liver, and pancreas show low (P)RR expression levels.[13] Importantly, an 8.9-kDa fragment of the (P)RR called M8–9 co-precipitates with a vacuolar proton-translocating ATPase (V-ATPase), also known as ATP6AP2. V-ATPase is involved with the acidification of intracellular vesicles and pH homeostasis, and there might be the potential for a link between the (P)RR and acid activation. V-ATPase is involved in neurotransmitter uptake and storage, endocytosis, and receptor recycling, which might be relevant to retinal biology. In addition, a role in cognitive function and brain development is suggested by the finding that a mutation in the (P)RR is present in patients with X-linked mental retardation and epilepsy.[23]

## *Summary and Perspectives*

Emerging evidence suggests that prorenin and the (P)RR influence certain organ pathologies. The eye might be particularly sensitive with increases in prorenin identified in diseases such as diabetic retinopathy,[17] retinopathy of prematurity,[18] and now experimental CNV.[1] We are yet to understand if other features of the (P)RR including its link with V-ATPase influences prorenin binding and activation, and whether this is important in the central nervous system and eye. The development of new (P)RR blockers will be an important next step in further defining the benefits of this strategy for ocular pathology.

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