Prorenin and the (pro)renin receptor: do they have a pathogenic role in the retina?

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1. ABSTRACT

Prorenin, the inactive precursor of renin has been suggested to be an indicator of diabetic complications including retinopathy. This concept was originally based on findings that prorenin is elevated in the plasma and vitreous of patients with diabetic retinopathy. Experimental studies in animal models of diabetic retinopathy and retinopathy of prematurity, have confirmed these reports and localized prorenin to macroglial Müller cells and blood vessels. The identification of a (pro)renin receptor [(P)RR] which binds both prorenin and renin, and influences intracellular signaling pathways independently of angiotensin II, suggests that prorenin-(P)RR may be pathogenic under certain circumstances. Given recent evidence from clinical trials that angiotensin II blockade improves to some extent retinopathy in diabetic patients, the development of (P)RR antagonists could have promise as an adjunct treatment for retinal diseases where prorenin is up-regulated. This review will discuss the cellular location of the renin-angiotensin system in the retina, evidence that angiotensin II blockade is beneficial for both retinal vascular, neuronal and glial pathology and place this information in the context of the development of (P)RR inhibitors.

2. INTRODUCTION

Prorenin, the inactive precursor of renin, initiates the renin-angiotensin-aldosterone system (RAAS) (Figure 1). The eye has a long history in terms of its relationship with prorenin. Reports over two decades ago identified that prorenin is elevated in the plasma of patients with proliferative diabetic retinopathy (DR) (1, 2). This rise occurred before the onset of microalbuminuria, leading to the suggestion that prorenin may be a marker of diabetic complications (1). Subsequent studies confirmed these findings, and went on to identify that prorenin is present in ocular fluids and elevated there in patients with proliferative DR (3). This information contributed to speculation that prorenin may have its own receptor and elicit effects independently of angiotensin II (Ang II). Early studies identified prorenin and renin binding proteins and a clearance receptor; however, neither were found to generate Ang II. In recent times a (pro)renin receptor [(P)RR] has been discovered, which binds both renin and prorenin, and induces signal transduction pathways such as extracellular signal related kinase1/2 (ERK1/2) that are independent of Ang II (4).This important finding has led to new interest into the possible pathogenic effects of prorenin in organs
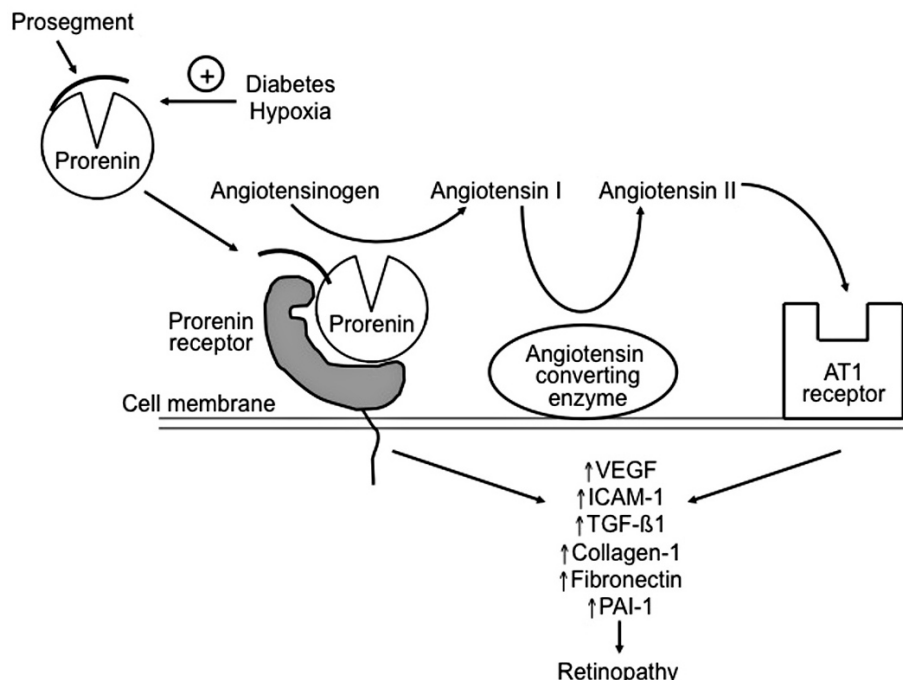
Figure 1. Potential consequences of prorenin binding to the (P)RR. The binding of prorenin to the (P)RR may directly activate second messenger systems that include phosphorylated-ERK1/2 (p-ERK1/2), mitogen activated protein kinase (MAPK), vascular endothelial growth factor (VEGF), transforming growth factor-beta 1 (TGF-beta1) and plasminogen activator inhibitor-1 (PAI-1), which may lead to organ pathology by mechanisms independent of angiotensin II. Binding of prorenin to the (P)RR may also cause its prosegment to unfold, thereby activating prorenin so that it is able to generate angiotensin peptides that stimulate the angiotensin II type 1 (AT1) receptor. (Adapted from Van den Heuvel *et al.* (91) and Wilkinson-Berka and Campbell (92)).

including the eye. In 2003, Suzuki and colleagues 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 (5). These investigators suggested that by inhibiting the handle region of the prosegment, that organ pathology due 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 (5).The HRP has been used by this group in a variety of experimental models of disease including the eye, and has shown remarkable protective effects (6-11).

DR, retinopathy of prematurity (ROP) and age-related macular degeneration (AMD), involve pathological changes to retinal vascular, neuronal and glial cells, which are known to express RAAS components. This review will provide an overview of the ocular RAAS and the outcomes of studies using Ang II blockade and the HRP to attenuate these diseases.

3. THE OCULAR RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

Local RAAS's exist in a variety of organs such as the kidney, adrenal, brain and ovary [reviewed (12)]. A local system also exists in the eye with expression localized to both retina (3, 13) and choroid (Figure 2) (14). Using immunohistochemical techniques, we reported renin expression in retinal Müller cells (13), a macroglial cell that expands almost the entire width of the retina, and has close anatomical connections with the inner retinal microvasculature. Other components of the RAAS including angiotensinogen, Ang II, angiotensin 1-7, the angiotensin type 1 receptor (AT1-R), the angiotensin type 2 receptor (AT2-R), angiotensin converting enzyme (ACE) and ACE2 have also been localized to either retinal Müller cells, neurons or the vasculature in a variety of species (Figure 2)(13, 15-19). Aldosterone may also have actions in the retina via the mineralocorticoid receptor (MR), with aldosterone synthase mRNA present and MR situated on vascular cells (20). The presence of 11β-hydroxysteroid 2 suggests that aldosterone rather than cortisol may influence MR's actions in retina (20).

In terms of prorenin and the (P)RR, prorenin has been localized to the vasculature and inner retinal neurons of the developing and adult rat eye (17)(Figure 3). The site of (P)RR expression has only recently been studied, with reports of immunolabelling in blood vessels of mouse retina (6), although unpublished studies by our group indicate (P)RR to also be located on neurons and glia, consistent with other components of the RAAS. The reasons for the differences in these expression patterns are not clear, but may relate to antibody and species diversity. Nevertheless, it is interesting to note that Contespas and
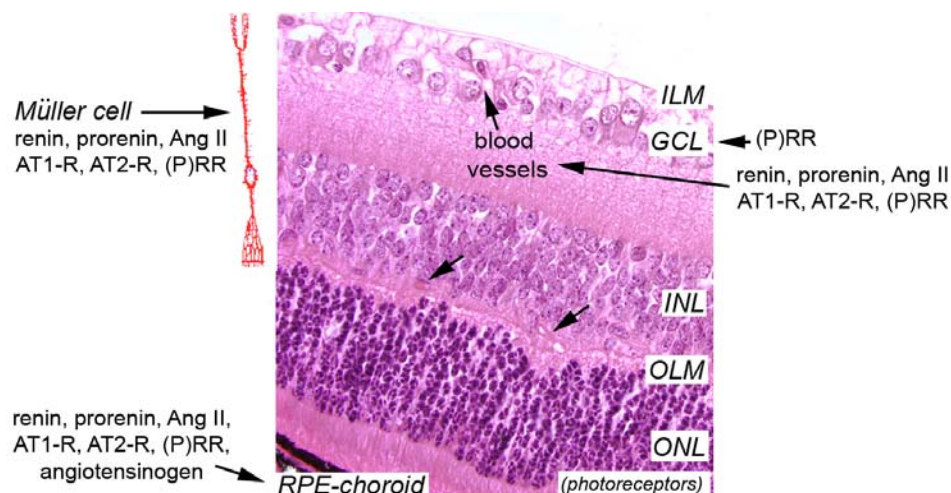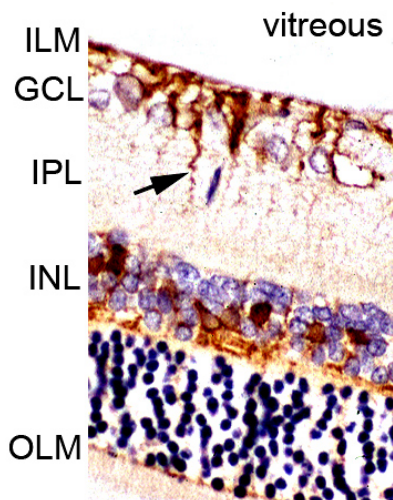
Figure 2. Three micron paraffin section of retina showing the cellular location of prorenin, the (P)RR and other RAAS components. ILM, inner limiting membrane, GCL, ganglion cell layer, INL, inner nuclear layer, OLM, outer limiting membrane, ONL, outer nuclear layer and RPE, retinal pigment epithelium. Counterstain, haematoxylin and eosin. AT1-R, angiotensin type 1 receptor, AT2-R, angiotensin type 2 receptor, (P)RR, (pro)renin receptor. Most components of the RAAS are localized to macroglial Müller cells whose nuclei reside in the INL and processes extend to the ILM and OLM (13, 17, 93), although other retinal glial cells such as astrocytes and amacrine cells do express angiotensin receptors (19). RAAS components including prorenin (17) and the (P)RR (6) are also found in retinal blood vessels (arrows) and the RPE-choroid complex (7, 14, 22, 23). Angiotensin converting enzyme is distributed on retinal blood vessels and angiotensin converting enzyme2 is localized to the INL and photorecepetors (18). In diabetes, vascular pathology and neuronal and glial cell deficits occur. In retinopathy of prematurity, a neurovascular lesion also develops, with neovascularization extending from the inner retina into the vitreous cavity. In age-related macular degeneration, pathology occurs primarily in the RPE-choroid complex and to photoreceptors.

colleagues reported high (P)RR protein expression in brain neurons (21), and particularly those areas of brain involved with Ang II mediated events (21). Additionally, these authors report (P)RR to be localized to the plasma membrane and synaptic vesicles of mouse neurons (21). Prorenin and the (P)RR has also been found in the choroid with expression in retinal pigment epithelial cells (22) and choroidal macrophages (7). This is consistent with previous studies in humans where renin, angiotensinogen and ACE mRNA were found in retinal pigment epithelium (23), perhaps suggesting a role for the (P)RR in choroidal pathologies such as AMD.

4. ANGIOTENSIN II'S ACTIONS IN THE RETINA

The idea that Ang II is up-regulated in organ pathology to become a major contributor to cellular dysfunction, is established in diseases such as diabetic nephropathy (24)(Figure 1). The eye is a relative newcomer to the field, however, over the past 10 to 15 years growing evidence indicates that prorenin, renin, ACE and/or Ang II are elevated either in the plasma or eyes of patients (3, 15, 25, 26), or animals with ROP or DR (27, 28). Experimental studies have the advantage of being able to evaluate the direct effects of exogenous Ang II on cellular function to determine both its pathological actions and mechanisms of action. To date, retinal vascular cells rather than neurons or glia have been most extensively studied. In cultured retinal pericytes, the administration of Ang II under normal conditions stimulates migration via transforming growth factor-beta and platelet derived growth factor (29), reactive

oxygen species formation, nuclear factor-kappa beta (NF-kappa beta) expression, and exacerbates apoptosis when cells are exposed to advanced glycation end-products (30, 31). In cultured retinal endothelial cells, Ang II has mitogenic effects enhancing vascular endothelial growth factor (VEGF)-stimulated endothelial cell proliferation, which involves angiopoeitin2 and Tie2 and protein kinase C (32-34). The effects of *in vivo* administration of Ang II has been not as extensively studied; however there is evidence that Ang II increases retinal NADPH oxidase expression in diabetic animals (35), and aldosterone exacerbates retinal angiogenesis and inflammation in rats with ROP (20). Overall, this information combined with findings evaluating the effects of pharmacological blockade of Ang II (discussed below), suggest that Ang II is a potent inducer of retinal growth factors, inflammatory factors and reactive oxygen species which may then stimulate pathological inflammation, angiogenesis and perhaps neuronal and glial dysfunction in the retina. There still remains much to learn about Ang II and aldosterone's role in retina, and particularly that of prorenin and the (P)RR, which is an emerging area of investigation. For instance, what are the stimuli for increased production of RAAS components in retinal disease, how do RAAS components influence the function of vascular cells, glia and neurons, and which RAAS intervention combination provides the best outcome for the different retinal diseases in which the RAAS has a causative role? The next section will discuss current information about the role of the RAAS in ROP, DR and AMD

Figure 3. Three micron paraffin section of retina labeled with prorenin. Counterstain, haematoxylin. ILM, inner limiting membrane, GCL, ganglion cell layer, IPL, inner plexiform layer, INL, inner nuclear layer, OLM, outer nuclear layer. Brown labeling denotes prorenin labeling in Müller cell processes (arrow) which extend from the ILM to the OLM.

5. ANGIOTENSIN II AND RETINOPATHY OF PREMATURITY

5.1. Vascular Effects

ROP is the leading cause of lifelong visual impairment in premature babies in developed countries (36). ROP is characterized by changes to the immature vasculature of the developing eye, and can be mild with no visual defects to severe with retinal neovascularization and subsequent retinal detachment and blindness. A major factor in the development of ROP is exposure to changes in the concentration of inspired oxygen. Briefly, when premature babies are exposed to high levels of inspired oxygen to assist breathing, normal retinal blood vessel growth is blunted. This is most likely due to a suppression of angiogenic growth factors such as VEGF. Once babies are returned to room air, the relative hypoxia in the retina leads to an increase in inflammatory and angiogenic factors, which stimulate pathological angiogenesis and vascular leakage. This situation can be reproduced in experimental models of ROP, in which animals are exposed to varying levels of inspired oxygen during postnatal retinal development. Emerging evidence indicates that ROP is also associated with deficits in retinal function, with losses in rods and cones as well as glial dysfunction leading to a decline in visual acuity (37). Current treatments for ROP involve procedures such as laser photocoagulation, which can be destructive, and hence preventative measures are sought.

The idea that the RAAS is causative in the development of ROP was suggested by findings that transgenic Ren-2 rats, which exhibit elevated extra-renal prorenin and renin, have more severe pathological retinal angiogenesis than Sprague Dawley rats with ROP (27). In addition, prorenin and renin levels are higher in retina from rats with ROP compared to controls. This information may translate to patients, as infants with ROP have elevated levels of plasma and vitreal prorenin (25). Furthermore, AT1-R may be a genetic risk factor for ROP, with a single nucleotide polymorphism in the AT1-R associated with the development of ROP (38).

More definite evidence for a causative role for the RAAS in ROP comes from studies showing that blockade of ACE and AT1-R attenuates vascular disease in ROP. In 2000, our group made the first report that ACE inhibition and AT1-R blockade (AT1-RB) reduced pathological angiogenesis in a rat model of ROP (27). Subsequent studies confirmed the anti-angiogenic effects of AT1-RB in rodent ROP (39), and also found reductions in inflammation and vascular leakage (40, 41). AT1-RB's protective effects in ROP is associated with a reduction in the expression of pro-angiogenic and pro-inflammatory factors such as VEGF, monocyte chemoattractant protein and intercellular adhesion molecule-1 (ICAM-1) (20, 27, 42). Blockade of the MR has also been reported to reduce both angiogenesis and inflammation in ROP (20).

5.2. Neuronal and Glial Effects

It is established that glial cells contribute to the development of the retinal vasculature, by providing a major source of VEGF, and a template for the growth of blood vessels from the optic disk (43). In ROP, retinal astrocytes degenerate in the first few days following transfer from hyperoxia to room air (44). The general view is that in ROP, ganglion cells located close to the retinal surface compensate for the lack of astrocyte-derived VEGF, to themselves increase VEGF production (44). This occurs to such an extent that retinal blood vessels breach the retinal surface to enter the vitreous cavity, resulting in pathological angiogenesis. In a recent study of ROP, we determined that both astrocytes and ganglion cells express the AT1-R (19). We reported that in rats with ROP, AT1-RB had dual benefits, which may account for the restoration of retinal function. Firstly, AT1-RB restored astrocyte survival resulting in revascularization of the peripheral retina. Secondly, AT1-RB reduced VEGF expression in the ganglion cell layer (27) and reduced pre-retinal neovascularization at the retinal surface (45). Overall, it can be surmised from these findings that Ang II has complex actions in ROP, stimulating inflammation, glial cell apoptosis and growth factor production to result in pathological angiogenesis.

6. ANGIOTENSIN II AND DIABETIC RETINOPATHY

6.1. Experimental Studies

The beneficial effects of ACE inhibition and AT1-RB on the retinal vasculature in ROP have also extended to experimental models of DR. These interventions attenuate retinal vascular leakage and the formation of acellular capillaries (28, 46-48). Whether ACE inhibition and AT1-RB reduce retinal angiogenesis in DR is not clear, as rodent models do not progress to the proliferative form of the disease. However, in transgenic

Ren-2 rats, which exhibit proliferating endothelial cells in the retina and iris after long-term diabetes, Ang II blockade reduces this pathology including the increase in ocular VEGF expression (28). As in ROP, inflammation is viewed to be important in the development of vascular disease. Diabetic rats exhibit increased leukostasis and ICAM-1 expression (49), which can be attenuated with either ACE inhibition or AT1-RB (50-52). The resident immune cells of the retina, microglia, may contribute to the inflammatory status of the retina in diabetes (53), by releasing pro-inflammatory factors. It has been suggested that in this situation, activated microglia may then contribute to the death of retinal neurons (54). In terms of RAAS blockade, AT1-RB has been reported to attenuate NF-kappa beta expression and microglia accumulation in animals with both diabetes and hypertension (52). Diabetes may also induce neuronal and glial deficits in DR, with apoptosis of retinal neurons (55, 56), gliosis (57), decreases in the number and length of photoreceptors (58) and functional deficits in the early stages of experimental diabetes (59, 60).

6.2. Hypertension

Hypertension is likely to contribute to DR (61, 62); however, the mechanisms by which this occurs are not fully defined. There is evidence from experimental studies that mechanical stretch, the *in vitro* counterpart of hypertension, increases the expression of the RAAS and VEGF systems in cardiac myocytes (63), and in the eye upregulates VEGF in retinal cells (64). This data is supported by studies in spontaneously hypertensive rats (SHR) with streptozotocin diabetes, which exhibit increased leukostasis, ICAM-1, NF-kappa beta and VEGF expression, reactive oxygen species and apoptosis of neuronal and glial cells compared to normotensive diabetic rats (28, 52, 65). However, it is possible that if the RAAS is sufficiently elevated in the setting of combined diabetes and hypertension that further retinal pathology occurs. This has been shown in streptozotocin diabetic transgenic Ren-2 rats (with an enhanced RAAS), which develop an increase in proliferating endothelial cells and VEGF expression in retina and iris, compared to age-matched diabetic SHR, despite comparable hypertension between the two rat strains (28). This effect may extend to retinal neurons and glia with unpublished studies from our group indicating that hypertension alone promotes deficits in retinal function (electroretinogram) in the diabetic SHR; which is exacerbated in the diabetic Ren-2 rat. Our report that blood pressure reduction alone with atenolol is insufficient to restore retinal function, and that AT1-RB provides the greatest benefit, further supports the idea that overactivity of the RAAS is a significant stimulator of vascular, neuronal and glial dysfunction in DR (48, 66). A study comparing atenolol and ACE inhibition in diabetic SHR reported a similar finding, with only ACE inhibition attenuating increased basement membrane thickening and VEGF expression (67). However, overall, these results do not completely exclude a role for hypertension in DR. A randomized clinical trial comparing atenolol and the ACE inhibitor captopril in 1148 patients with type 2 diabetes (T2D), reported that tight blood pressure control with both treatments reduced the progression of retinopathy as assessed on retinal photographs and by visual acuity (61, 62). When interpreting these findings it important to consider that the experimental and clinical studies differ not only in the species studied, but the type of diabetes, the time course of treatment and the end points measured.

6.3. Clinical studies of Diabetic Retinopathy

Almost all patients with type I diabetes (T1D) will develop retinopathy over a 15 to 20 year period, and approximately 20% to 30% will advance to the blinding form of DR (68). In terms of T2D, greater than 60% will have retinopathy. The effects of Ang II blockade have been studied in clinical trials of both T1D and T2D. The United Kingdom Prospective Diabetes Study (UKPDS) trial comprised 1148 hypertensive patients with T2D allocated to tight control of blood pressure (<150/85 mmHg) and 390 patients to less tight blood pressure control (<180/105 mmHg) (62). After nine years of follow-up, the ACE inhibitor, captopril, was associated with a 34% reduction in the rate of progression of DR. In contrast, the Appropriate Blood Pressure Control in Diabetes (ABCD) trial reported no difference in the progression of DR in hypertensive T2D patients who had intensive (132/78 mmHg) or moderate (138/86) blood pressure control (69). More recently, the Action in Diabetes and Vascular Disease Controlled Evaluation (ADVANCE) trial reported the findings of a study of 11,140 T2D patients performed by 215 collaborating centres in 20 countries (70). Patients were randomised to receive either a fixed combination of the ACE inhibitor, perindopril plus the diuretic indapamide, or placebo. After a mean follow up period of 4.3 years, those assigned fixed therapy had a mean reduction in systolic blood pressure of 5.6 mmHg and diastoiic blood pressure of 2.2 mmHg. Fixed combination therapy was associated with a reduction in the risk of major vascular events including death. However, there was no effect of perindopril plus indapamide on the incidence of new or worsening microvascular eye disease.

In terms of T1D, the EURODIAB Controlled trial of Lisinopril in Insulin-dependent Diabetes (EUCLID) trial evaluated the effect of the ACE inhibitor lisinopril on the progression of DR in normotensive normoalbuminuric patients (71). After 2 years, lisinopril reduced the progression of DR by 50% and progression to proliferative DR by 80%. The studies limits include a short follow-up period of 2 years and differences in baseline glycaemic levels between groups. In 2008, a large randomized clinical trial in T1D and T2D patients with either hypertension or normotension was completed. The DIabetic REtinopathy Candesartan Trial (DIRECT) involved over 5000 patients recruited from 309 centres worldwide (72, 73). DIRECT reported that candesartan reduced the incidence of DR in T1D patients by 33%, and improved regression in T2D patients by 34%; however there was no significant effect on progression. A greater benefit has recently been reported in a study of 258 normotensive and normoalbuminuric T1D patients, which after a 5 year follow-up showed a reduction in progression with either ACE inhibition (65%, enalapril) or AT1-RB (70%, losartan) (74). These recent studies provide promise for the use of Ang II blockade in the treatment of DR.

7. ANGIOTENSIN II AND AGE-RELATED MACULAR DEGENERATION

AMD is the most common cause of blindness in the elderly. AMD can be classified into early and late stages (75, 76). The early stage is associated with minimal visual impairment and comprises drusen and pigmentary abnormalities in the macula. Drusen are accumulations of acellular, amorphous debris subjacent to the basement membrane of the retinal pigment epithelium (RPE). There are two forms of late AMD: the atrophic or "dry" form and the neovascular and exudative or "wet" form. Dry AMD involves the choriocapillaris, RPE and photoreceptors, and does not involve vascular leakage. The "wet" form includes detachment of the RPE and choroidal neovascularization, which lead to leakage and fibrovascular scarring. Hypertension is a contributor to AMD (77, 78), although the mechanisms by which it influences pathology remain unclear. The role of the RAAS in AMD has not been extensively explored. However, there is evidence that components of the RAAS are expressed in the choroid-RPE (15, 23). A study by Nagai and colleagues reported that in patients with AMD, AT1-R, AT2-R and Ang II are present (14), and in mice the use of laser photocoagulation to induce choroidal neovascularization, leads to increased expression of angiotensinogen, AT1-R, AT2-R and Ang II (14). There is evidence that ACE inhibition and AT1-RB are beneficial in these experimental models of choroidal neovascularization and inflammation (14, 79, 80); however, studies in models of dry and wet AMD have not yet been performed.

8. (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 due to 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 2002, Nguyen and colleagues identified a (P)RR for renin and prorenin that is a 350-amino acid protein with a single transmembrane domain (4). *In vitro* studies indicate that binding to the (P)RR increases the catalytic efficiency of renin and causes non-proteolytic activation of prorenin which must be due to a conformation change (4, 81).

8.1. (P)RR and Vacuolar-ATPase

Relevant to the eye are that the highest levels of (P)RR expression are in the central nervous system, then the heart and placenta (4). Furthermore, a role in cognitive function and brain development is suggested with the finding that a mutation in the (P)RR is present in patients with X-linked mental retardation and epilepsy (68). Of importance with regard to the specific actions of the (P)RR is that a 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-ATPases are involved with the acidification of intracellular vesicles and pH homeostasis and there may be the potential for a link between the (P)RR and acid activation. Also of possible relevance for the retina is that the v-ATPases are present on the plasma membranes of cells where they facilitate pH homeostasis in macrophages and neutrophils and angiogenesis by endothelial cells (68).

8.2. (P)RR Signal Transduction Pathways that are Independent of Angiotensin II

One of the most interesting features of the (P)RR is that this receptor appears to promote organ pathology independently of Ang II. Experiments with cells expressing the (P)RR in the presence of angiotensin receptor antagonists, showed that both renin and prorenin induce signaling in an angiotensin-independent manner, resulting in mitogen-activated protein kinase (MAPK) activation (MAPK p42/44 and p38) and subsequent heat shock protein (HSP) 27 phosphorylation (4, 82, 83). This may have relevance for a variety of organ pathologies given evidence that binding of renin to the (P)RR in mesangial cells induced an increase in transforming growth factor-beta$_1$ and the subsequent synthesis of plasminogen-activator inhibitor-1, fibronectin and collagen-1 (82, 84).

9. FUNCTIONS OF (P)RR IN THE OCULAR VASCULATURE

Whether prorenin and the (P)RR significantly contribute to ROP, DR or AMD is a relatively new area of scientific research. The cellular location of prorenin and the (P)RR may give clues to its mode of action(s). Their localization to the retinal vasculature, may indicate that the (P)RR is involved in modulating ocular angiogenesis (6, 17). Although studies showing a direct evaluation of prorenin in the eye are lacking, the effects of a putative (P)RR blocker has been evaluated in a number of experimental models of ocular disease (6-9). These studies utilize the HRP developed by Suzuki *et al* (5), which has been reported to reduce organ pathology and the expression of pro-fibrotic factors in diabetic nephropathy (85), cardiac fibrosis in stroke-prone spontaneously hypertensive rats fed a high-salt diet (10), and (P)RR transgenic rats (86). For ocular disease, the studies by Satofuka and colleagues report similar protective effects, particularly in the vasculature, which has been most extensively studied. In rats with endotoxin-induced uveitis, HRP administered subcutaneously at doses of 1 and 0.1.mg each day reduced leukocyte adhesion in the retinal vascular and expression of ICAM-1, interleukin-6 and MCP-1 (9). In mice with ROP and laser-induced choroidal neovascularization, HRP had similar anti-inflammatory effects and also reduced pathological angiogenesis (7). In 2009, this group extended their studies to a model of DR. Here, diabetes was associated with an increase in retinal prorenin, but not the (P)RR. HRP administered for 4 weeks, reduced inflammation, and VEGF and ICAM-1 gene expression (8). Of interest is that in diabetes, HRP reduced leukostasis to a greater extent than the AT1-RB, losartan (8).

Despite these findings, there is some controversy as to how HRP influences prorenin-(P)RR mediated events in the eye and other organs. For instance, other groups have not reported organ protective effects with HRP, or that HRP blocks renin and prorenin related induction of p-ERK1/2 (87, 88). Müller and colleagues studied renovascular hypertension in 2-kidney 1-clip rats, which display high blood pressure, cardiac hypertrophy and renal damage (88). 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 (88). A separate study by this group compared the renin inhibitor, aliskiren, with HRP in double transgenic rats overexpressing human renin and angiotensinogen genes. Following 7 weeks, aliskiren, but not the HRP reduced blood pressure, normalized albuminuria and renal tubular damage (87).

The reasons for the differences in findings between these laboratories are not fully understood. In order to further elucidate the role of (P)RR in the retina we studied the location of (P)RR and made a comprehensive study of HRP in three experimental models of retina. As previously reported, (P)RR was located to retinal blood vessels (6); however, the most intense and extensive immunolabelling was in retinal neurons and glia (unpublished findings) which is consistent with recent studies in brain neurons (21). In both the developing retina and ROP, HRP was anti-angiogenic and in ROP and diabetes, HRP reduced inflammation with a concomitant reduction in retinal VEGF and ICAM-1 mRNA (unpublished findings). In short, HRP demonstrated similar anti-angiogenic and anti-inflammatory effects in retina as reported previously.

10. FUNCTIONS OF (P)RR IN RETINAL NEURONS AND GLIA

The original report of (P)RR distribution in tissues indicated high levels of mRNA in the brain. Subsequent studies indicated (P)RR expression on primary brain neurons (89), and brain neurons associated with RAAS mediated events (21). The role of the (P)RR in neurons has not yet been fully explored; however, studies in zebrafish point towards a role for (P)RR in embryogenesis and eye development (90). In zebra fish, (P)RR, is expressed at a very early stage of development, and a mutation in the (P)RR gene, which has high homology with an accessory protein of vacuolar-ATPase (ATP6AP2), resulted in the death of zebrafish before the end of embryogenesis (90). Of particular interest is that the mutant was characterized by severe malformations of the central nervous system and of the eye (90). In support of an important role for the (P)RR in neuronal function, is that the only known mutation in (P)RR in humans resulted in the presence of a mRNA with an in-frame deletion of exon 4 [4-(P)RR], along with normal (P)RR mRNA. Patients suffered from X-linked mental retardation and epilepsy without detectable cardiovascular or renal abnormalities (68). When this information is put into the context of retinal disease, it should be noted that the retina is largely comprised of neuronal and glial cells. Our recent and unpublished findings indicate that although (P)RR is expressed on blood vessels it has an extensive expression pattern in retinal neurons and glia. This information together with data that HRP modulates (P)RR expression in retina, and HRP causes deficit in the electroretinogram, a measure of neuronal and glial activity, is consistent with the idea that the (P)RR has important functions in the central nervous system.

11. FUNCTIONS OF (P)RR IN THE RETINAL PIGMENTED EPITHELIUM

As mentioned previously, RAAS components including renin are expressed in the RPE, suggesting a role for the RAAS in choroidal pathology such as AMD (14, 15, 23). A recent study adds to this body of information by reporting that (P)RR is expressed in human RPE cells, and at this site is functional, as shown by prorenin-induced increases in ERK1/2 phosphorylation (22). Of interest is that in RPE from eyes with AMD and hypertension, (P)RR and type I collagen expression are increased (22), suggesting a role for (P)RR in dry AMD.

12. CONCLUSION

The role of prorenin and the (P)RR in the retina is a new area of biomedical research. The findings of prorenin and (P)RR expression in retina, and the up-regulation of prorenin in retinal diseases such as ROP and diabetes, is suggestive of a potential role for prorenin in retinal pathologies. It is as of yet undetermined how prorenin influences retinal disease, and whether this involves the (P)RR. Evidence to date would indicate that HRP is bioactive in retina; however, these findings may need to be placed in the context that the (P)RR has identical homology with an accessory protein of v-ATPase, and thus, some of the actions of HRP may be linked to the activity of this enzyme. Certainly, further research in prorenin-(P)RR biology is warranted given the need to improve the outcomes of trials such as DIRECT which highlight the benefits of angiotensin II blockade for DR (72, 73).

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