(Pro)renin Receptor: A Treatment Target for Diabetic Retinopathy?

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Many lines of evidence implicate the renin-angiotensin system in the pathogenesis of diabetic retinopathy. A recent multicenter trial showed that angiotensin II type 1 (AT1) receptor blockade (ARB) reduced the incidence of retinopathy in type 1 diabetic patients and improved the regression of retinal disease in type 2 diabetic patients (1,2). However, the failure of ARB to prevent progression of diabetic retinopathy indicates a role for mechanisms additional to angiotensin II in its pathogenesis. In this issue of *Diabetes*, Satofuka et al. (3) provide evidence that prorenin and the (pro)renin receptor, acting in part through mechanisms unrelated to angiotensin II, may contribute to the pathogenesis of diabetic retinopathy. This evidence holds promise for new therapies for diabetic retinopathy and other complications of diabetes.

Prorenin is a high–molecular weight biosynthetic precursor of renin. It has a low intrinsic activity of <2% of the activity of renin (4) because it has an amino-terminal 43–amino acid prosegment that masks the active site, thereby preventing access by the renin substrate—angiotensinogen. Renal juxtaglomerular cells are the only known site of renin production, whereas the kidney and a number of extrarenal tissues including adrenal, ovary, testis, placenta, and retina produce prorenin (5–7). Plasma prorenin concentrations are ~10- to 20-fold higher than those of renin (4). Prorenin concentrations in plasma and vitreous fluid are increased in diabetic subjects (6,8), and plasma prorenin is a powerful marker for both prevalent and incident microvascular complications of diabetes, including nephropathy, retinopathy, and neuropathy (9,10).

Before the discovery of the (pro)renin receptor, there was uncertainty regarding whether prorenin was biologically active (4). The (pro)renin receptor binds both renin and prorenin and is reported to increase the catalytic efficiency of renin and activate prorenin (11). Thus, binding of renin and prorenin not only stimulates the (pro)renin receptor but also increases angiotensin II formation, leading to AT1 receptor stimulation (Fig. 1). Suzuki et al. (12) proposed that a region of the prorenin prosegment, called the handle region, participates in the binding of prorenin to its receptor. They further suggested that synthetic handle region peptides (HRPs), corresponding to amino acids 10–19 of the prorenin prosegment, interfere with prorenin binding. In support of this hypothesis, they showed that HRP blocked the binding of prorenin to recombinant prorenin receptors expressed by COS-7 cells, with a K_i of 6.6 nmol/l (13). Ichihara et al. (14) tested this hypothesis in vivo by administering HRP to various experimental models of disease; rat HRP completely prevented the development of nephropathy in diabetic rats and caused regression of established diabetic nephropathy (15). HRP is referred to as (pro)renin receptor blocker (PRRB) in the current study by Satofuka et al. (3).

In an elegant series of experiments, Satofuka et al. (3) showed that PRRB reduced leukostasis in retinal vasculature of diabetic rats and mice; PRRB reduced leukostasis to a greater extent than the ARB losartan in wild-type mice and also reduced leukostasis in AT1A receptor gene knockout mice. In addition, PRRB reduced the diabetes-induced elevation in retinal expression of vascular endothelial growth factor (VEGF) and intracellular adhesion molecule-1 (ICAM-1) in wild-type mice and VEGF but not ICAM-1 expression in AT1A receptor gene knockout mice. Moreover, PRRB reduced the diabetes-induced elevation in retinal expression of phosphorylated extracellular signal–related kinase (ERK)1/2 in AT1A receptor gene knockout mice. The finding that PRRB reduced phosphorylated ERK1/2 expression in cultured brain capillary endothelial cells stimulated with prorenin, but not in cells stimulated with angiotensin II, was consistent with a specific effect of PRRB on the actions of prorenin.

Satofuka et al. (3) did not evaluate retinal neovascularization because diabetic rodents do not develop proliferative diabetic retinopathy. However, it is important to note that PRRB attenuated neovascularization in other models of ocular disease (16). Also relevant to diabetic retinopathy are previous studies showing that prorenin, renin, and angiotensin II are expressed in ganglion cells and Müller cells in adult and neonatal rat retina (17). Given that neuronal and glial dysfunction contribute to diabetic retinopathy, which can be prevented by ARB (18), it is important to determine whether PRRB has similar protective effects.

A critical question in the interpretation of these data is whether the actions of PRRB demonstrate a role for prorenin and the (pro)renin receptor in diabetic retinopathy. Satofuka et al. showed that PRRB reduced the immunostaining of retinal vessels by an antibody directed to the prorenin prosegment (3). In the absence of evidence for specificity of immunostaining, additional information would have been helpful, such as immunostaining with an antibody that recognized both renin and prorenin. Moreover, measurement of retinal angiotensin peptides might have clarified the effects of PRRB on angiotensin formation in the retina.

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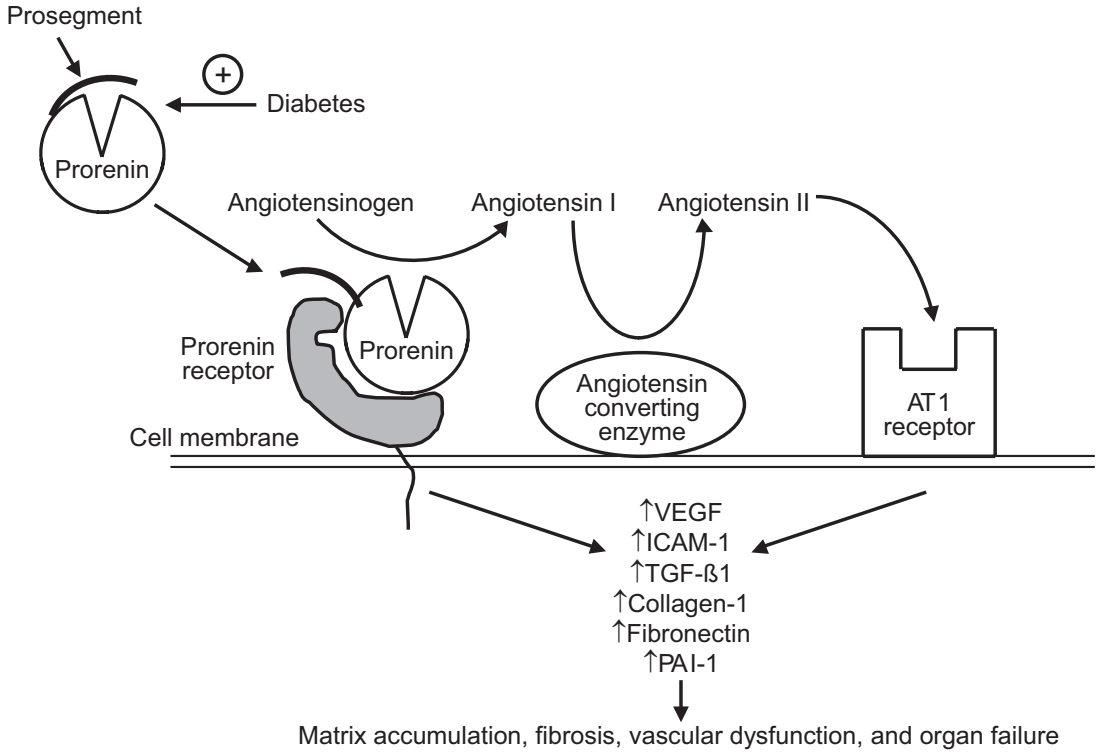

FIG. 1. Schematic outline of the potential consequences of prorenin binding to the (pro)renin receptor. There are two main mechanisms by which prorenin may contribute to diabetes complications (4,20). First, prorenin binding to its receptor may directly activate second messenger systems that include phosphorylated ERK1/2, mitogen-activated protein kinase, VEGF, transforming growth factor-β 1 (TGF-β1), and plasminogen activator inhibitor 1 (PAI-1), which may lead to organ pathology by mechanisms independent of angiotensin II. Second, the binding of prorenin to the receptor may cause its prosegment to unfold, thereby activating prorenin so that it is able to generate angiotensin peptides that stimulate the AT1 receptor. The failure of PRRB to reduce diabetes-induced retinal expression of ICAM-1 in AT1A receptor gene knockout mice in the studies of Satofuka et al. suggests that its reduction of ICAM-1 expression in wild-type mice was mediated by reduced angiotensin II levels. Adapted from van den Heuvel M, Batenburg WW, Danser AH. Mol Cell Endocrinol 2009;302:213–218.

It is of interest that one of the "negative" controls for PRRB was PRRB heated at 100°C for 10 min. The investigators assumed that heating would denature PRRB; however, this treatment does not denature an octapeptide, although it may promote oxidation of its methionine residue. There is, therefore, uncertainty regarding why heated and unheated PRRB had different effects.

Another concern is whether the concentrations of PRRB in vivo were sufficient to prevent prorenin binding to the (pro)renin receptor (4). Satofuka et al. (3) found that a 5,000-fold molar excess of PRRB (10 μmol/l) produced only 50% inhibition of prorenin (2 nmol/l)-stimulated ERK1/2 phosphorylation in brain-derived capillary endothelial cells. By contrast, the effects of PRRB in mice were seen with peak plasma concentrations of ~100 nmol/l, produced by daily intraperitoneal injections of 1 mg/kg. The effects of 0.1 mg/kg were similar to the effects of 1 mg/kg, and 0.01 mg/kg suppressed ocular inflammation in a previous study (19). Moreover, the investigators report that 1.8–3.6 μg/kg per day using an osmotic minipump prevented and reversed diabetic nephropathy in rats (14,15). These data suggest that PRRB has effects in vivo at concentrations below those required to prevent prorenin binding to its receptor (4).

Despite these concerns about the mechanisms by which the effects of PRRB were mediated, its actions in diabetic retinopathy and nephropathy hold promise for the development of new and more effective therapies to treat and prevent these conditions.

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