A New Look at the Eye
Aldosterone and Mineralocorticoid Receptors As Novel Targets in Retinal Vasculopathy

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Very so often, a study emerges that is refreshing, enlightening, and provocative and that has major clinical significance. Such is the study by Wilkinson-Berka et al1 in this issue of Circulation Research, which challenges the widely held belief that adrenal-derived aldosterone is a hormone that simply regulates volume and sodium balance. Here, we learn that similar to what has been suggested in the heart, brain, and vasculature,2 the eye has a dynamic aldosterone–mineralocorticoid receptor (MR) system that plays an important pathological role in the development of retinal vasculopathy.

Angiogenic ocular conditions, including retinopathy of prematurity, diabetic retinopathy, and age-related macular degeneration, are the leading causes of irreversible vision loss in developed countries.3,4 Pathological processes contributing to visual loss in these conditions include microaneurysms, hemorrhages, and exudates from new and poorly developed vessels, retinal detachment caused by fibrosis, and neovascular glaucoma, with a resultant increase in intraocular pressure. Exact mechanisms for the development of retinopathy remain elusive, but ischemic changes in vascular permeability, inflammation, and growth factor–induced angiogenesis are fundamental pathological features.

Among the many factors that have been implicated in vascular permeability and angiogenesis is vascular endothelial growth factor (VEGF), which signals through protein kinase C, mitogen-activated protein kinases, and reactive oxygen species (ROS) through VEGF receptor 1 (fms-like tyrosine kinase-1) and VEGF receptor 2 (fetal liver kinase-1).5,6 VEGF expression is increased by glucose, advanced glycation end products, transforming growth factor-β, and insulin-like growth factor-1, all of which are modulated by the renin–angiotensin–aldosterone system.5,6 Blocking effects of VEGF with ranibizumab, and bevacizumab modify vasoproliferation and hence prevent retinal neovascularization.7 However, although this treatment provides some benefits, it does not always prevent progression of the disease.7 Moreover, VEGF is pleiotropic affecting a broad spectrum of endothelial, neuronal, and glial behavior, thereby confounding the validity of anti-VEGF strategies, especially in chronic diseases. This raises the possibility that targeting upstream regulatory systems, such as the renin–angiotensin–aldosterone system, may be more effective in treating retinal vasculopathy, especially because angiotensin (Ang) II and aldosterone are proinflammatory, fibrogenic, and angiogenic and because the eye possesses a functionally active local intracrine renin–angiotensin system (RAS).

All components of the RAS have been identified in the eye. Renin, angiotensin-converting enzyme, angiotensinogen, Ang II, and Ang II receptors have been shown at the mRNA and protein levels in humans, rats, and rabbits in the vasculature, neurons, and glia.8,9 In animal models of diabetes and in patients with diabetes, elements of the RAS are elevated, and these correlate with the severity of retinopathy.10,11 Experimental studies and clinical observations demonstrated that interruption of the RAS, with angiotensin-converting enzyme inhibitors or Ang II type 1 (AT₁) receptor (AT₁R) blockers ameliorating many of the vascular abnormalities that develop in diabetic retinopathy and retinopathy of prematurity. Prorenin has also been implicated in retinal vascular injury, because handle region peptide, which binds to (pro)renin receptor as a decoy peptide and inhibits the nonproteolytic activation of prorenin, inhibited retinal neovascularization in mice with oxygen-induced retinopathy.12 Moreover recent findings from the Diabetic Retinopathy Candesartan Trials (DIRECT), which examined 1421 patients with type 1 diabetes and 1905 patients with type 2 diabetes (DIRECT-Prevent 1 and DIRECT-Prevent-2 respectively), showed that AT₁R blockade with candesartan significantly reduced retinopathy in type 1 diabetes13 and had beneficial effects on retinopathy progression in type 2 diabetes,14 supporting a role for the RAS in the pathophysiology of retinopathy.

In addition to a functional intracrural RAS,8,9 there is now evidence that the eye possesses functionally active MRs through which aldosterone stimulates angiogenesis and inflammation, particularly in the context of high salt conditions.1 The physiological significance of the aldosterone–MR in the eye is uncertain. Under physiological conditions, it may be important in maintaining ocular pressure,15 and in pathological conditions, aldosterone may contribute to vascular injury.1 Aldosterone, classically implicated in volume homeostasis and Na balance,2 is now recognized to be a potent profibrotic and proinflammatory mediator in the heart, vasculature, and kidneys, with effects mediated through activation of NADPH oxidase and increased generation of ROS (oxidative stress).2 Wilkinson-Berka et al1 extend this concept to the eye. Using a rat model of oxygen-induced retinopathy, which has features of retinopathy of prematurity in humans, it

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is shown that MR antagonism with spironolactone improves retinal angiogenesis by attenuating leukostasis and decreasing proinflammatory responses. This protective effect was exaggerated in rats with oxygen-induced retinopathy that were challenged with aldosterone and salt. Molecular mechanisms underlying aldosterone–MR effects were probed in cultured bovine retinal endothelial cells, where it was shown that aldosterone increases proliferation and tubulogenesis to a similar magnitude as VEGF and that this is associated with activation of the MR, as evidenced by cytosol:nuclear translocation.1

Processes by which aldosterone induces its angiogenic and proinflammatory effects in oxygen-induced retinopathy were attributed, at least in part, to oxidative stress.1 ROS are involved in inflammation, endothelial dysfunction, cell proliferation, migration, extracellular matrix deposition, fibrosis, angiogenesis, and vascular remodeling. These effects are mediated through redox-sensitive regulation of multiple signaling molecules including mitogen-activated protein kinases, protein tyrosine phosphatases, tyrosine kinases, ion channels, and proinflammatory genes, such as monocYTE chemoattractant protein-1, interleukin-6, and nuclear factor κB.10 Among the various mechanisms through which aldosterone influences redox state are downregulation of glucose-6-phosphate dehydrogenase (G6PD),17 which is a major source of reduced NADPH, and upregulation of NADPH oxidase, a key enzyme involved in vascular ROS generation.18 Wilkinson-Berka et al1 demonstrated that aldosterone decreases G6PD mRNA expression in retinal endothelial cells and that retinal nicotinamide adenine dinucleotide phosphate reduced oxidase 4 (NADPH oxidase [Nox]4), a Nox2 homolog, is upregulated in oxygen-induced retinopathy (Figure).1 Growing evidence indicates an important role for NADPH oxidase in retinal vascular inflammation. However, it is still unclear as to which Nox homolog is primarily responsible for retinal ROS production. In oxygen-induced retinopathy, Nox4 expression was increased, especially in the presence of aldosterone and salt.1 Nox4 is expressed in nonphagocytic cells, including vascular endothelium, is localized to the endoplasmic reticulum, and, unlike Nox2, it only requires p22phox, and not p47phox, p40phox or p67phox for its activation.19 Of the Nox proteins, Nox4 has been shown to be strongly proangiogenic, possibly through upregulation of VEGF signaling.19,20 Nox2, p22phox, and p47phox, classic subunits of NADPH oxidase, have also been implicated in NADPH oxidase–derived ROS in vascular retinopathy.21

There are some limitations in the study by Wilkinson-Berka et al1 that warrant consideration. Firstly, although oxidative stress is implicated as a major cause of vascular injury in oxygen-induced retinopathy, there is no evidence in the study that bioavailability of ROS, such as O2−, H2O2, ONOO−, and NO, is indeed increased. Moreover the fact that retinal gene expression of G6PD is reduced and that of Nox4 is increased does not necessarily mean that protein levels are altered. It is also possible that other sources of ROS, such as mitochondria, contribute to oxidative stress in retinal vascular injury. Secondly, spironolactone was administered systemically and not locally into the eye. As such it is possible that retinal effects described in rats with oxygen-induced retinopathy may be attributable to systemic actions of spironolactone through hemodynamic changes and not necessarily through local actions in the eye. Finally, it remains to be determined whether the eye itself is a source of aldosterone. Wilkinson-Berka et al1 suggest that this may be so because genes for the enzymes responsible for aldosterone synthesis, namely 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), 11β-HSD2, and aldosterone synthase are expressed in retina. However, until such time as these enzymes are demonstrated at the protein level and that retinal-derived aldosterone is detectable, it is still questionable as to whether the eye produces and secretes aldosterone. Moreover, if this were indeed so, physiologically significant levels would need to be demonstrated as discussed in the provocative study by Gomez-Sanchez et al who challenge the concept that aldosterone is produced in extraadrenal tissue such as the heart.22

**Figure.** Schematic demonstrating possible mechanisms by which aldosterone mediates effects through MRs in retinal endothelial cells to induce angiogenesis and inflammation, which underlie vascular retinopathy. Aldosterone binds to its MR in the cytoplasm, which translocates to the nucleus. Activated MR inhibits G6PD and increases Nox4-based NADPH oxidase, localized in the plasma membrane and endoplasmic reticulum (ER) to generate the ROS superoxide (O2−) and H2O2. Increased bioavailability of ROS (oxidative stress) stimulates redox-sensitive pathways, possibly involving VEGF, to promote angiogenesis, inflammation, and fibrosis, which underlie retinal vasculopathy.
Nevertheless, despite these shortcomings, the study under discussion provides novel data in well-conducted in vitro and in vivo experiments, demonstrating that the retina possesses a rich and functionally important aldosterone–MR system, which, under pathological conditions, plays an important role in retinal vasculopathy. The clinical significance of this derives from the findings that mineralocorticoid antagonism by spironolactone improves pathological angiogenesis possibly by suppressing redox-sensitive inflammatory processes. These data suggest that MR antagonism may be vasculoprotective in the retina and, as such, may be an interesting therapeutic modality in the treatment of vascular retinopathies. Considering the encouraging outcomes of the DIRECT trials, together with the positive results discussed here, combination AT1R blockade and MR antagonism may provide added therapeutic advantage. Moreover, if such treatments could be administered topically and directly into the eye, benefits may be greatly enhanced in the management of retinal vascular disease. Although premature at this stage to advocate such maneuvers, these aspects are certainly worth pursuing in future studies because they may suggest attractive new strategies in the improved treatment of angiogenic ocular conditions, thereby preventing the devastating complications of retinopathy that lead to irreversible vision loss.

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References

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