It is difficult to assign a value for hydraulic or osmotic fluid permeability of the retinal pigment epithelium, since the precise hydrostatic pressure or osmotic pressure difference across the RPE is not known. Elevation of intraocular pressure also changes the suprachoroidal hydrostatic pressure, such that the pressure drop across the RPE may not change appreciably. Similarly, unstirred layer effects in the extravascular choroid following systemic mannitol injection would cause an underestimation of the true osmotic fluid permeability of the RPE.

It would appear that under normal circumstances, osmotic and hydrostatic forces play a definite but small role in fluid transport across the RPE. However, following damage to the RPE, such as with sodium iodate, the importance of these factors may be enhanced. One might also speculate that a similar situation could occur following retinal cryopexy where the hydraulic permeability of the RPE would be increased and the protein content of the extravascular choroid would be increased.

Key words: blood-retinal barrier, retinal pigment epithelium, permeability, carboxyfluorescein, mannitol, intraocular pressure, cynomolgus monkey

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References


Ocular Renin-Angiotensin: Immunohistochemical Evidence For the Presence of Prorenin in Eye Tissue

Stephen J. Sramek,* Ingolf H. L. Wallow,* Richard P. Day,† and Edward N. Ehrlich†

Angiotensin II (A2) is a vasoconstrictor generated by the renin-angiotensin system. A2 appears to act also as an angiogenic factor. Recent evidence suggests that renin is synthesized at many tissue sites and may generate A2 locally. Local A2 may have important functions in the normal and diseased eye. We examined eight human eyes by immunostaining with an antibody to prorenin, the biosynthetic precursor of renin. In all eyes, prorenin staining was extensive in the pars plicata of the ciliary body suggesting that the ciliary body synthesizes renin and this renin may be part of an ocular A2 generating system. Invest Ophthalmol Vis Sci 29:1749–1752, 1988

In the classic pathway angiotensin II (A2) is generated within the circulation by sequential cleavage of liver-derived angiotensinogen. Renin cleaves this substrate, forming angiotensin I (A1). Converting enzyme subsequently converts A1 into A2, a potent vasoconstrictor and stimulant of the synthesis of the mineralocorticoid aldosterone. A2 thereby affects blood pressure and electrolyte homeostasis. The classic pathway has generally been thought of as a renal feedback loop responding to afferent glomerular arteriolar pressure. However, recent observations sug-
Fig. 1. Photomicrograph of human renal cortex, stained with anti-prorenin using the avidin-biotin-peroxidase technique. Juxtaglomerular tissue right of center shows prominent staining (×150).

suggest that this system may have function in addition to the systemic or renal control of blood pressure and electrolytes. As recently reviewed, A2 appears to have angiogenic activity stimulating vessel growth in a rabbit cornea model of neovascularization. In addition, the synthesis of "local" extrarenal renin, which may generate A2, has been described in various organs. The precise function of local A2 is unknown.

Evidence has been presented that A2 may also be generated in the eye. Both renin- and angiotensin-converting enzyme activity have been found in the aqueous, ciliary body, retina and choroid of several species, including man. In addition, the retinal vasculature was shown to contain A2 receptors, and to constrict in response to externally administered A2. This report is the first description of immunostaining of normal ocular tissue with an antibody to a component of the renin-angiotensin system. We began with an antibody to prorenin, the biosynthetic precursor of renin.

Materials and Methods. Monoclonal antibody specific to human prorenin was raised by immunization with a peptide identical to the carboxyl terminal sequence of the human renin prosegment which has been used to raise polyclonal antisera specific for human prorenin. The antibody used in this study, 257B11A9, is an IgG1 which binds human amniotic fluid prorenin with an apparent dissociation constant of 0.35 nM and has no cross-reactivity with active human renin, or other aspartate proteases (Day RP, Gourley J, Duello T, unpublished observations).

Antibody was used at 10 μg/ml. Formalin-fixed, paraffin-embedded sections were stained using the avidin-biotin-peroxidase technique (Vector Laboratories, Burlingame, CA) as previously described. Sections were stained with and without pretreatment with 0.4% pepsin in 0.10 N HCl for 5 min at 37°C. Moderate improvement in contrast was observed when sections were pretreated with protease, although no change in staining pattern or grading occurred. Negative controls consisted of replacement of the primary antibody with nonimmune ascites fluid (MOPC21, 10 μg/ml, Bionetics Laboratory Products, Kensington, MD) or with antibody that had been preabsorbed with 100 μg/ml of the peptide antigen for 48 hr at 4°C (Day RP, Gourley J, Duello T, unpublished observations).

Tissue: Eight donor eyes from eight patients were selected from the University of Wisconsin Eye Bank according to the following criteria: less than 5 hr from time of death to time of fixation, donation from a Madison hospital to facilitate chart review, four patients under 40 years of age, and four patients over 40 years of age. Clinical information is summarized in Results. Human kidney obtained at autopsy was stained for a positive control in every experiment.

Results. The typical staining pattern obtained with
the monoclonal anti-prorenin antibody (aPR) in kidney is shown in Figure 1. Only areas consistent with juxtaglomerular cells were stained, and the staining was completely blocked by preabsorption of antibody with peptide antigen.

In ocular tissue the striking finding was specific staining of the ciliary body of all eyes examined. The pars plicata of eight of eight eyes (Fig. 2A) and the pars plana of six of eight eyes (Fig. 3A) were positive. The pattern was similar in all cases. Staining was limited to the basal portion of the nonpigmented ciliary epithelium, beginning in the pars plicata and extending onto the pars plana with a decreasing intensity, so that by approximately the posterior third staining was not evident (Fig. 3B). No staining of the ciliary body was detected in any of the negative control sections (Fig. 2B).

Two other areas were frequently positive. A thin layer of stain was evident on the inner surface of the internal limiting membrane of the retina posterior to the equator in five of eight eyes, and stain was present on the outer and inner surface of the lens capsule in eight of eight eyes. Although this stain was not seen with control or preabsorbed serum, the possibility of nonspecific “edge” staining cannot be completely ruled out.

The optic nerve and ciliary nerves stained in all eyes. However, this stain was present with preabsorbed serum, and we have observed similar nonspecific staining of these structures with other monoclonal antibodies (unpublished data).

Clinical and staining information is briefly summarized in Table 1. Staining of ocular tissue was observed in a wide range of age groups, both sexes, and in normotensive and hypertensive patients. No patient had been treated with an angiotensin converting enzyme inhibitor.

Discussion. In this paper we demonstrate immunoreactivity in normal ocular tissue using an antibody that reacts with prorenin (PR), an early component in the angiotensin II (A2) generating system. PR staining was located primarily in the ciliary body, and appeared concentrated in the basal portion of the nonpigmented epithelium. Its presence in the eye is a new finding and suggests that this proenzyme is synthesized and/or stored within the nonpigmented ciliary epithelium. PR may also be activated here since a previous demonstration has shown renin enzyme activity within the ciliary body. We cannot rule out the possibility that plasma components of the renin-angiotensin system are filtered by the ciliary body and that our PR staining would then reflect “accumulating” plasma PR. However, if plasma PR were filtered by the ciliary body, one would have to postulate selective concentration by the ciliary epithelium since plasma PR levels under most physiologic circumstances are low. Also, if our PR staining reflected filtration from the plasma, one would expect a gradient of staining decreasing from the vascular core of pars plicata and pars plana toward the vitreal surface. Such a gradient was clearly absent. Regardless of its source, whether locally produced and/or selectively concentrated, the presence of PR within the nonpigmented ciliary epithelium deserves increased attention.

The physiologic and pathophysiologic significance of the new finding remains to be specified by further work. Others have raised the concept that locally formed A2 may affect the retinal vasculature. Demonstration of renin in ciliary body and retina and of angiotensin converting enzyme in ciliary body, retina and aqueous is consistent with their proposal. Our finding would fit with this concept if PR or renin were secreted into the aqueous and transported to the retina by posterior aqueous flow. Presence in the eye of the enzymes necessary for A2 generation and presence of specific receptors in the retina for A2-me-
Table 1. Summary of clinical data and immunostaining

<table>
<thead>
<tr>
<th>ID</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Cause of death</th>
<th>Comment</th>
<th>Pars plicata</th>
<th>Pars plana</th>
<th>ILM*</th>
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<tr>
<td>HC-21</td>
<td>1½</td>
<td>M</td>
<td>Asphyxiation</td>
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<td>+</td>
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<td></td>
</tr>
<tr>
<td>HC-10</td>
<td>18</td>
<td>M</td>
<td>Massive head injury</td>
<td>Hypotension treated with dopamine</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HC-13</td>
<td>21</td>
<td>M</td>
<td>Closed head injury, elevated intracranial pressure</td>
<td>Hypotension treated with dopamine</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC-34</td>
<td>27</td>
<td>F</td>
<td>Closed head injury, elevated intracranial pressure</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC-51</td>
<td>45</td>
<td>M</td>
<td>Progressive renal failure and hepatobiliary</td>
<td>No history of hypertension, but B/P elevated during</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>obstruction secondary to metastatic adrenal cancer</td>
<td>week prior to death</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP-1</td>
<td>57</td>
<td>F</td>
<td>Septic shock, ischemic bowel</td>
<td>History of untreated hypertension</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HC-49</td>
<td>58</td>
<td>M</td>
<td>Small cell CA metastatic to brain</td>
<td>—</td>
<td></td>
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<tr>
<td>HC-50</td>
<td>64</td>
<td>M</td>
<td>Pneumonia</td>
<td>—</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>

* Internal limiting membrane.

diated vasoconstriction make it reasonable to suspect that the eye contains a local renin angiotensin system influencing retinal vasoconstriction and blood flow.

Key words: prorenin, renin, angiotensin, ciliary body, immunostaining

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