Distribution of Transthyretin in the Rat Eye

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We reported previously synthesis of transthyretin (TTR), or prealbumin, a transport protein for thyroxine and retinol, in the eyes of rats and cows and showed that in the rat eye, TTR mRNA is localized exclusively in the retinal pigment epithelium (RPE). We now demonstrate by immunohistochemistry that TTR has a more widespread distribution in the rat eye than does its mRNA. Intense immunoreactivity for TTR was found in the RPE, ciliary epithelium, iris epithelium, corneal endothelium, optic nerve fiber layer of the retina, and lens capsule. Depending on the method of processing, immunoreactivity of varying intensity was found also in other ocular structures. In particular, the retinal ganglion cells were strongly immunoreactive on frozen sections but not on paraffin sections. Although vitreous humor was not included in the sections of adult rat eye, sections of a 25-mm rat embryo showed intense immunoreactivity in the vitreous humor. Since plasma TTR does not cross Bruch’s membrane into the retina, our findings suggest that ocular TTR is synthesized, at least in part, in the RPE and is transported to specific locations within the eye. Although the physiologic role of ocular TTR is unknown, it is possible that it participates in retinol cycling within the eye. The widespread ocular distribution of TTR may account for the occurrence of various forms of ocular amyloidosis in the familial amyloidotic polyneuropathies, a group of dominantly inherited disorders caused by point mutations in the TTR gene.

Plasma transthyretin (TTR) is a 55-kD tetrameric protein1 which is synthesized in the liver.2,3 TTR is an important plasma transport protein for thyroxine, and holoretinol-binding protein (the complex of retinol and retinol-binding protein, the chief plasma transport protein for retinol [vitamin A]), exists in the serum predominantly in complex with TTR.4,5 TTR is synthesized also by the choroid plexus epithelium,6-11 but the precise role of TTR within the central nervous system has not yet been determined.

A mutant form of TTR12-14 forms the major component of the amyloid deposits in the familial amyloidotic polyneuropathies (FAPs), a group of dominantly inherited disorders in which ocular amyloidosis features prominently.15-17 However, plasma TTR does not cross Bruch’s membrane into the retina.18 For this reason, and because of the importance of retinol in the visual process, we suspected that an independent source of TTR might exist within the eye. We established that TTR is synthesized in abundance within the eye, and that the retinal pigment epithelium (RPE) is the unique site of ocular TTR synthesis.20

The current report describes the ocular distribution of TTR, which is considerably more extensive than that of its mRNA.

Materials and Methods

Materials

Rabbit polyclonal antiserum to rat TTR has been characterized previously.19 Biotinylated secondary antibody, avidin, and biotinylated peroxidase were obtained from Vector Laboratories (Burlingame, CA). M1 embedding compound was obtained from Lipshaw (Detroit, MI). All other reagents were from Sigma (St. Louis, MO). Rat embryos were obtained from Rockland (Gilbertsville, PA).

Immunohistochemistry

Animal experimentation was performed in accordance with the ARVO Resolution on the Use of Animals in Research.
Adult male Sprague-Dawley albino rats (Camm Breeding Laboratories, Wayne, NJ) were sacrificed by decapitation or by cardiac perfusion under pentothal anesthesia with 10% paraformaldehyde in phosphate buffered saline (PBS; 10 mM sodium phosphate, pH 7.4, with 0.9% NaCl). For frozen sections, all of which were obtained from perfused rats, eyes and brains were removed and immersed for at least 1 day in the perfusion fixative or in Bouin’s fixative, after which they were immersed in 15% or 30% sucrose in PBS, embedded in M1 compound, sectioned at 6–16 μm on a cryostat, and placed on slides coated with gelatin or gelatin and poly-l-lysine. For paraffin sections, eyes and brains were removed, fixed in 10% unbuffered formalin, embedded in paraffin, sectioned at 8 μm, and placed on gelatin-coated slides. Rat embryos were received from the supplier (Rockland, Gilbertsville, PA) in 10% formalin and were embedded in paraffin.

Immunohistochemistry was performed by a previously described modification of the avidin–biotin peroxidase technique. Primary antiserum was used usually at dilutions ranging from 1:4000 to 1:32,000. Each experiment included negative controls, in which primary antiserum was omitted from the first incubation; in each case, this omission eliminated staining entirely. Brain sections containing choroid plexus, the unique site of brain TTR synthesis, served as positive controls. The brain sections were fixed and processed identically to the sections of eye. At all concentrations of antiserum tested, staining of brain sections was limited to the choroid plexus epithelium, as reported previously. Adequacy of perfusion was assessed by the absence of erythrocytes from eye sections after incubation for 10 min in PBS (pH 7.2) containing 0.05% dianinobenzidine and 0.01% hydrogen peroxide. This treatment imparts a dark brown color to any erythrocytes present. The paraformaldehyde-fixed eyes contained no erythrocytes, and the eyes fixed in Bouin’s solution contained erythrocytes only in the extraocular tissues.

Specificity of the primary antiserum was demonstrated by western blotting, as reported previously. This report establishes a widespread ocular distribution for immunoreactive TTR protein. The distribution includes the RPE, retinal ganglion cells, optic nerve fiber layer, epithelia of the iris and ciliary body, corneal endothelium, and lens capsule. In addition, we detected TTR immunoreactivity in ocular adnexae such as the lacrimal gland (on frozen sections), consistent with the reported presence of TTR in human tears. Although the vitreous was lost from all of our preparations of adult rat eye, we detected intense TTR immunoreactivity in the vitreous body of the adult human eye (unpublished data) and in the vitreous body of the developing eye in embryonic rat, suggesting that the vitreous of the adult rat as well is probably rich in TTR.

Whereas this study localizes TTR immunoreactivity to certain layers of the retina, in some cases we have been unable to assign the immunoreactivity to individual cell types, for example, in the nerve fiber layer and at the junction of the inner nuclear and inner plexiform layers. Because of the intense immunoreactivity seen in the somata of the ganglion cells,
Fig. 1. TTR immunoreactivity of retinal structures in the albino rat. Frozen sections. Primary antiserum diluted 1:8000. (A) Bouin's fixative, ×485. (B) Paraformaldehyde fixation, ×615. In the section in (B), the RPE, which stains intensely, was artefactually detached from the photoreceptor layer and lies outside the field of the photograph. Layers were identified by phase contrast microscopy and by comparison to similar sections stained with hematoxylin and eosin. NFL, nerve fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; RC, layer of rods and cones; RPE, retinal pigment epithelium; CC, choriocapillaris.

we suspect that axons from these cells account for the immunoreactivity observed in the nerve fiber layer (Figs. 1A, B). Identification of cell types stained in the photoreceptor layer and other retinal layers will require electron microscopic or double-labeling studies.

In the rat eye, TTR mRNA is synthesized only within the RPE, raising the possibility that TTR is secreted by the RPE and transported to specific ocular locations. Since TTR is exported from both liver and choroid plexus, and since the TTR precursor protein contains a presequence which probably serves as an export signal, it seems very likely that TTR, likewise, is exported from the RPE. Pino has shown that for 30 min after intraperitoneal administration, radiolabeled TTR is present in the choriocapillaris but does not cross Bruch’s membrane into the retina. Therefore, it appears that retinal TTR is derived from the RPE. However, Pino did not report the fate of labeled TTR at times beyond 30 min after administration, nor did he describe anterior ocular structures. Therefore, the possibility of slow accumulation of plasma TTR within the retina is not ex-
cluded, and it remains to be determined whether TTR in the more anterior portions of the eye is derived from plasma or from RPE.

The functions of ocular TTR are unknown. The retinal photoreceptors require a constant supply of retinol for use in the visual cycle, and we have suggested that ocular TTR (possibly in concert with locally synthesized retinol binding protein) may play a role in the intraocular processing of retinol. Since other ocular structures are not known to have exceptional needs for vitamin A, the reason for so widespread a distribution of ocular TTR is not immediately apparent. It is possible that TTR in other locations may function in its thyroxine-transporting capacity or in some capacity as yet undetermined.

Several differences in TTR immunoreactivity were observed among the various preparations used. Most strikingly, the intense immunoreactivity of the retinal...
Fig. 3. TTR immunoreactivity in anterior ocular structures of the albino rat. Formaldehyde fixation, paraffin section, primary antiserum diluted 1:8000; X96. c, cornea; i, iris; cb, ciliary body; l, lens; r, retina. Arrow, retinal pigment epithelium.

Fig. 4. TTR immunoreactivity of cornea, lens, and lacrimal gland of the albino rat. (A) Cornea. Formaldehyde fixation, paraffin section, primary antiserum diluted 1:8000; X292. Immunoreactivity is strong in corneal endothelium (en), but minimal in epithelium (ep). (B) Lens. Same section as in (A). Intense immunoreactivity is present in lens capsule (arrow). X182. (C) Lacrimal gland. Paraformaldehyde fixation, frozen section. Primary antiserum diluted 1:8000; X146. Glandular epithelium and basement membrane are immunoreactive.
ganglion cells seen in frozen sections was entirely absent in paraffin sections. Since immunoreactivity was generally weaker in paraffin sections than in frozen sections, it appears that TTR was partially removed during paraffin processing, or that denaturation by heat and organic solvents reduced its immunoreactivity. However, this does not explain the complete removal of intense immunoreactivity from the retinal ganglion cells, sclera, and lacrimal gland, while reactivity in other regions, and in control sections of choroid plexus, was relatively preserved.

Differential loss of staining may imply that TTR in the retinal ganglion cells is in a different physical state than that in certain other ocular structures, such as the cornea and RPE. For example, Pettersson et al have demonstrated microheterogeneity of plasma TTR, dependent in part on the nature of a solitary cysteiny1 residue and on the ability of TTR to dissociate into monomers.26 They have suggested that these differences may have functional implications. Association of TTR with different macromolecules in various cell types may result in the masking of antigenic
determinants or in an alteration of susceptibility to removal or denaturation during processing.

The synthesis and distribution of ocular TTR appear to explain the prominence of ocular amyloidosis in the FAPs. This group of autosomal dominant disorders is characterized by the deposition of amyloid fibrils in the peripheral nervous system and in a variety of systemic organs.\textsuperscript{15-17} The various FAP phenotypes are caused by single amino acid substitutions in the TTR monomer.\textsuperscript{12-14} Affected individuals are heterozygous, with one normal and one mutant allele. It is known that the amyloid in these disorders is composed primarily of the mutant TTR,\textsuperscript{12,13} but the factors determining amyloid deposition are not yet understood.

Various patterns of ocular amyloidosis have been described in the FAPs. Vitreous opacities are the most common abnormality and are found in FAP type I,\textsuperscript{15-17} FAP type II,\textsuperscript{15-17} the Ashkenazi Jewish variant,\textsuperscript{27} and in familial oculeoleptomeningeval amyloidosis (FOA).\textsuperscript{28} Pupillary scalloping due to ciliary body involvement occurs in about 25% of patients with FAP type I and has been considered pathognomonic.\textsuperscript{16} Corneal lattice dystrophy due to amyloid fibril deposition is a characteristic feature of FAP type IV.\textsuperscript{29} Cataracts and glaucoma have been described as features of FAP types III\textsuperscript{30} and IV,\textsuperscript{29} respectively. These pathologies are consistent with our demonstration of TTR in the ciliary body, cornea, and lens of the adult rat and in the vitreous body of the rat embryo. Presumably, deposition of abnormal TTR in these locations leads to the formation of amyloid, but the reason for differential involvement in the various phenotypes is still unknown. If, as suggested above, TTR exists in different physical states in various structures, this might explain the varying susceptibility to different mutations. Notably, although the most intense immunoreactivity in the rat was found in the retinal ganglion cells and RPE, TTR-derived amyloid in the retina has been described only recently, in a single case of FOA.\textsuperscript{31}

**Key words:** transthyretin, retinal pigment epithelium, familial amyloidotic polyneuropathy, retinol, retinal ganglion cells

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**References**


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