ideal since the viscosity is highest when the material is at rest under low shear stress. This means a pseudoplastic fluid is more viscous when it is nearly stationary and less viscous when it is flowing. In contrast chondroitin sulfate has the same viscosity at various shear rates and would not exhibit the same degree of anterior chamber support as a pseudoplastic fluid at rest. Further research is needed to identify the optimal viscosity of the agents used for corneal endothelial protection in specific clinical situations.

Key words: viscosity, endothelium, sodium hyaluronate, chondroitin sulfate, methylcellulose, intraocular lens

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References

Neuron-Specific Enolase-Containing Cells in the Rhesus Monkey Trabecular Meshwork

Richard A. Stone,* Yasuaki Kuwayama,* Alan M. Laties,* and Paul J. Marangoz†

Neuron-specific enolase (NSE) localizes immunohistochemically to a discontinuous band of cell clusters in the trabecular meshwork of the anterior segment of the rhesus monkey eye. To date, this enolase isomer has been found exclusively in neurons or in cells of the diffuse neuroendocrine system. On this basis, its presence provides presumptive evidence for neuroregulatory cells in the triticate trabecular meshwork. Invest Ophthalmol Vis Sci 25:1332–1334, 1984

Neuron-specific enolase (NSE), an isomer of the glycolytic enzyme enolase,1,2 has been localized histochemically only in central and peripheral neurons and in neuroendocrine cells.3–5 As a result, it is believed generally that NSE is a reliable marker for such tissues.3–6 We now report the immunohistochemical localization of NSE in cells at the primate trabecular meshwork, a finding that implies the presence of neuroregulatory cells in the aequous humor outflow pathway.

Methods. Antiserum: The primary antiserum used for immunohistochemical localization in this study was raised by injecting New Zealand white rabbits with highly purified rat brain NSE homogenized in Freund's complete adjuvant.7 It has been characterized previously1,3; and importantly, this antiserum does not cross-react with a 105 excess of non-neuronal enolase, indicating its specificity.

Tissue preparation: Eyes were obtained immediately after death from rhesus monkeys (Macaca mulatta) killed under deep pentobarbital anesthesia as part of the polio vaccine testing program of the Bureau of Biologics of the Food and Drug Administration. The eyes were immersion fixed for 4 hr at 4°C in 0.1 M phosphate buffer, pH 7.2, with 4% paraformaldehyde and transferred overnight to 0.1 M phosphate buffer with 30% sucrose at 4°C. Cryostat tissue sections, 16–20 μm thick, were thaw mounted on gelatin-coated slides, dried at room temperature, and stored at −20°C until stained by the following indirect immunofluorescence technique.

Immunohistochemical procedure: After washing in phosphate-buffered saline (PBS), pH 7.2, the tissue sections were incubated at 37°C for 1 hr with NSE antiserum diluted 1:500 or 1:1000 and containing 0.3% Triton X-100. After incubation, the tissue sections were washed twice in PBS and then reacted for...
one hour at 37°C with goat anti-rabbit IgG conjugated to fluorescein isothiocyanate (FITC) (Cappel Laboratories; Malvern, PA) at 1:300 dilution and containing 0.3% Triton X-100.

To assess immunologic specificity, the primary antiserum at 1:1000 dilution was absorbed with $10^{-6}$ M purified NSE by overnight incubation at 4°C. Then alternate consecutive sections were stained with nonabsorbed NSE antiserum diluted 1:1000 or with the preabsorbed NSE antiserum. As a second control, nonimmune rabbit antiserum was substituted for the NSE antiserum in the same immunohistochemical procedure. As a third control, FITC-conjugated antiserum alone was studied.

These studies conformed to the ARVO Resolution on the Use of Animals in Research.

**Results.** As evidence of its specificity, the NSE antiserum stained nerves in an expected distribution throughout the principal regions of the eye. Of present interest, it also stained discrete cells in the anterior portion of the trabecular meshwork. They formed a discontinuous circumferential band of cells appearing as either cell aggregates or isolated cells on tissue sections (Figs. 1A, B). A lesser intensity of the histochemical reaction product also differentiated these cells from nerves of the anterior segment.

Preabsorption specificity controls were done. When alternate consecutive sections showed a cluster of NSE immunoreactive meshwork cells, the intervening section treated with preabsorbed antiserum displayed no specific reactivity (Fig. 1C). Thus, these preabsorption conditions eliminated staining of both meshwork cells and ocular nerves. Furthermore, substituting nonimmune rabbit serum for the primary antiserum or using FITC-conjugated antiserum alone showed neither trabecular meshwork cells nor nerves.

**Discussion.** Once thought to occur only in neurons proper, NSE has been found more recently in one other special group of cells, variously called paraneurons, amine precursor uptake and decarboxylation (APUD) cells or, more recently, cells of the diffuse neuroendocrine system. Whatever term is chosen, a diverse group of neuroregulatory cells is encompassed, including isolated neurosecretory cells of gut and lung, pancreatic islet cells, medullary chromaffin cells of the adrenal gland, and Merkel's cell, a receptor in skin. All cells of the diffuse neuroendocrine system studied to date contain NSE. Many contain a biologically active peptide as well. As measured by biochemical assay or by the fainter intensity of histochemical stain, the concentration of NSE in these neuroendocrine cells is less than that in neurons, perhaps because of lower concentration or perhaps because of the presence of a hybrid form of the enzyme. From its characteristic distribution, NSE is now thought to be a reliable probe to identify cells of the diffuse neuroendocrine system. Thus, the histochemical localization of NSE to discrete cells in the anterior trabecular meshwork directly implies membership in the diffuse neuroendocrine system.

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Fig. 1. NSE immunoreactive cells in the trabecular meshwork of the rhesus monkey eye are visualized either as cell clusters (A) or as isolated cells (B) in tissue sections. Arrows indicate immunoreactive trabecular meshwork cells. Note also the prominent staining of iris nerves (A and B). Use of preabsorbed NSE antiserum, prepared as described in the text, fails to stain either meshwork cells or nerves (C). Figures 1A and 1C are adjacent sections. SC = Schlemm's canal; TM = trabecular meshwork (fluorescence micrographs, X128).
In fact, localization of NSE to discrete cells of the anterior meshwork hints at an unsuspected local neuroregulatory mechanism in the eye.

By light microscopic appearance, these immunoreactive cells correspond to the Schwalbe line's cells recently described in the same location by Raviola and likely to the clustered cells described in this region by Rohen et al. At the electron microscopic level, the cells seen by Raviola contained a well-developed Golgi apparatus and two different cytoplasmic inclusions: small homogeneous granules and larger lamellar bodies. Based on these ultrastructural characteristics, Raviola proposed that the Schwalbe line's cells could be secretory, perhaps elaborating a surfactant as do lung cells of similar ultrastructure. While the known cells of the diffuse neuroendocrine system also may contain homogeneous secretory granules, lamellar bodies are not recognized as one of their ultrastructural features. Thus, NSE-immunoreactive cells may be identical to Schwalbe line's cells, or they may represent yet a second specialized cell type at this location. Immunoelectron microscopy is required to decide among these alternatives.

To date, neuroendocrine cells in the anterior segment of the eye have not been described. By analogy with the distribution of NSE in non-ocular tissues, these immunoreactive meshwork cells are most likely neurosecretory, possibly elaborating a biologically active peptide directly into the aqueous humor outflow pathway. The alternative that they represent a specialized neuroreceptor to regulate intraocular pressure or to control the composition of aqueous humor cannot be ruled out with present knowledge. Certainly further work to define their function is indicated, especially immunostaining with antisera to known neuropeptide hormones.

**Key words:** neuron specific enolase, trabecular meshwork, eye, rhesus monkey, immunohistochemistry

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