Pancreatic Polypeptide-like Immunoreactive Nerves in the Guinea Pig Eye

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The indirect immunofluorescence technique with antisera either to avian pancreatic polypeptide or to bovine pancreatic polypeptide stains nerve fibers in the guinea pig eye. In all regions of the uvea, immunoreactive fibers are present around large blood vessels; an association of immunoreactive nerve fibers to melanocytes is seen. Immunoreactive nerves are found throughout the choroid, including the choriocapillaris. In the ciliary body, they are seen in individual ciliary processes. The iris dilator muscle and, to a lesser degree, its sphincter are innervated. The chamber angle of the anterior segment contains immunoreactive nerve fibers, but convincing innervation to the cornea is lacking. No retinal cells stain. With some exceptions, the distribution of peripheral nerve fibers parallels that of the adrenergic innervation. Appropriate controls are negative. Invest Ophthalmol Vis Sci 24:1620-1623, 1983

Biologically active peptides in ocular nerves recently have been demonstrated using immunohistochemical techniques. Both VIP-like and substance P-like immunoreactive nerves1,2 have been discovered in the eye. We have undertaken to study still another biologically active peptide, pancreatic polypeptide (PP).

First isolated from the chicken pancreas, PP has been localized not only in specific cells of the pancreas but also, like many other gut peptides, in neurons of the central and peripheral nervous systems.3 Of particular note, antiserum to avian PP stains a subgroup of the adrenergic neurons in the superior cervical ganglion.4 Since it is already known that adrenergic nerves arising from the superior cervical ganglion supply many parts of the eye, we investigated the guinea pig eye for PP-like immunoreactivity using antiser a directed against both avian PP (APP) and bovine PP (BPP).

The only previous reports mentioning the eye, an antiserum to APP has revealed a sparse innervation in the rat iris5 and an antiserum to BPP, has shown an innervation to chorialid vessels.6 We report here a prominent network of PP-like immunoreactive nerves to the eye in a distribution parallel to that of the adrenergic innervation.

Materials and Methods. Antisera: Antisera were prepared by injecting rabbits at multiple sites with highly purified APP or BPP homogenized in complete Freund's adjuvant. The initial injections were followed by injections of antigen homogenized in incomplete Freund's adjuvant. The high specificity of both antisera has been shown by radioimmunoassay.6,7

Tissue preparation: Pigmented guinea pigs were anesthetized with intraperitoneal sodium pentobarbital and were perfused through the left ventricle with a mixture of 4% paraformaldehyde and phosphate-buffered saline (PBS), pH 7.2. The eyes were removed immediately, post-fixed for 2-4 hours at 4°C in the same fixative, and then washed overnight at 4°C in 30% sucrose and PBS. Cryostat tissue sections, 16-20 μ thick, were thaw-mounted on gelatin-coated slides, dried at room temperature, and stored at −20°C until stained by the indirect immunofluorescence technique.

Immunohistochemical procedure: After a wash in PBS, pH 7.2, the tissue sections were incubated for one hour at 37°C with antisera either to APP or BPP in dilutions ranging from 1:50 to 1:1,000 and containing 0.3% Triton X-100. After incubation, the tissue sections were washed twice in PBS and then incubated at room temperature for 30 minutes with goat anti-rabbit IgG conjugated to fluorescein isothiocyanate (FITC) (Miles Biologicals, Elkhart, IN) at a dilution of 1:300 and containing 0.3% Triton X-100. The sections were washed twice, covered with a coverslip in a Tris-glycerin mixture, and examined for fluorescence with an epi-illumination system.

The immunologic specificity was assessed by incubating the appropriate primary antiserum overnight at 4°C with varying concentrations of either synthetic APP (Peninsula Laboratories, Inc., San Carlos, CA) or BPP (gift from Dr. R. E. Chance). The preabsorbed antiserum then was used instead of the primary antiserum in the immunohistochemical procedure. As another control, the primary antiserum was eliminated and the FITC-conjugated antiserum alone was studied.

Results. Prominent APP-like immunoreactive nerve fibers, having a form typical of a terminal autonomic network, are present in the guinea pig eye. Although visualized at lower dilutions, optimum fluorescence of nerve fibers occurred at an antiserum solution of 1:100.

The uveal innervation stands out. Its three major divisions—choroid, ciliary body, and iris—all receive substantial numbers of APP-like immunoreactive nerves. In the choroid, their density is moderate. Frequently, nerves are seen surrounding larger choroidal blood vessels (Fig. 1A). Fine nerve fibers also are seen to reach the choriocapillaris (Fig. 1B).

The ciliary body contains a well-defined APP-like immunoreactive nerve distribution. These nerves are plentiful toward deeper structures, but are more sparse near the sclera. Immunoreactive nerves are present around the large blood vessels. A rich network of fine nerve fibers pass down individual ciliary processes (Fig. 2); these nerves are seen in the stroma and around the blood vessels. We observed no nerve fibers crossing...
the basement membrane to enter the ciliary epithelium, at least within the limits of resolution of the fluorescence microscopy.

In the iris, the larger blood vessels, especially at the periphery, are surrounded by APP-like immunoreactive nerves (Fig. 3A). There is also a high density of nerve fibers along the anterior surface of the dilator muscle (Fig. 3B). Some, but not many, immunoreactive fibers are visible within the substance of the sphincter muscle (Fig. 3C). In general, they follow the long axis of the muscle cells of the iris sphincter.

Throughout the uvea, APP-like immunoreactive nerve fibers often are found in close association to melanocytes (Fig. 3D).

In the aqueous outflow structures that make up the chamber angle, APP-like immunoreactive nerves are found. Their processes are seen both within the pectinate ligament (Fig. 4) and among deeper structures of the ciliary cleft.

In the outer coat of the eye, APP-like immunoreactive nerves are seen around superficial blood vessels at the limbus. If the limbal blood vessels are discounted, nerves within the cornea itself are visualized so rarely as to be negligible.

No specific staining occurs in the retina.

Using the antisera to BPP, immunoreactive nerves also can be visualized. The fluorescence intensity of such nerves tends to be lower than that seen in tissue sections studied with the APP antiserum. When seen, however, BPP immunoreactive nerves are distributed in a pattern identical to that found for APP-like immunoreactivity.

Preabsorption of the APP antiserum (1:100) with 1 
μM APP or preabsorption of the BPP antiserum (1:100)
with 1 μM BPP eliminates all specific reaction. Similarly, tissue sections incubated with FITC alone show no nerves.

Fig. 1. A. Fluorescence micrograph of APP-like immunoreactive nerve fibers (arrows) surrounding large choroidal blood vessels (magnification, ×692). B. An oblique tissue section displays a BPP-like immunoreactive nerve (arrow) in the choriocapillaris. The retina is artifactually detached (magnification, ×451). Phr = photoreceptors. RPE = retinal pigment epithelium.

Fig. 2. APP-like immunoreactive nerves (arrows) are visible in ciliary processes (magnification, ×439).
Fig. 3. A. APP-like immunoreactive nerve fibers (arrows) occur around large vessels near the iris root (magnification, X633). B. A rich network of BPP-like immunoreactive nerves (arrows) is seen on the anterior surface of the dilator muscle (magnification, X474). C. A small number of APP-like immunoreactive nerves (arrows) are present within the iris sphincter. Because their course parallels the long axis of the sphincter muscle cells, these fibers are visualized best in oblique sections (magnification, X506). D. APP-like immunoreactive nerve fibers partially surround an iris melanocyte (magnification, X1,007). Dil = dilator; PE = iris pigment epithelium; Sph = sphincter.

**Discussion.** By currently accepted histochemical criteria, mainly loss of activity by specific preabsorption, the ocular nerves described in this report fairly can be designated PP-like immunoreactive. The identification of the neurohumor of these nerves must be considered tentative in view of the recent description of a series of structurally related, biologically active peptides. Such peptides contain 36 amino acids and have an amidated carboxy terminal tyrosine. APP and BPP are but two; others include Peptide YY and Neuropeptide Y. Peptide YY has been found in endocrine cells of the gut, but is absent from brain. Neuropeptide Y is present in large quantities in brain but absent from gut. Neither has been sought in the eye. Neuropeptide Y can cross react to antisera raised against APP and BPP. The neurohumor of the nerves seen in the present study could represent one or more of these closely related peptides or, in fact, an as yet undescribed similar peptide. Accumulating evidence suggests that the particular peptide of this class present in the mammalian nervous system is probably Neuropeptide Y, but complete identification of the PP-like immunoreactive material demonstrated in ocular nerves requires biochemical characterization.

The distribution of PP-like immunoreactive nerves described in this report parallels closely the known
distribution of ocular adrenergic nerves. Certainly such a pattern holds for the iris, the ciliary body, and the choroid. The cornea is perhaps an exception, but even this is not completely certain. A scant, adrenergic innervation to the cornea is demonstrable by the Falck-Hillarp technique for catecholamines, but this method commonly shows no nerves in individual specimens, wide variation among species, and important changes during life. Since the cornea has yielded conflicting results with antisera raised to other neuropeptides, technical considerations rather than true absence of nerves also may account for our failure to observe PP-immunoreactive nerves in the guinea pig cornea. For all these reasons, the statement that the cornea lacks PP-like immunoreactive nerves must be qualified. Additional studies, including selective denervation, are now in progress to uncover any relationship between PP-like immunoreactivity and the adrenergic innervation.

At present, assignment of a function for this PP-like immunoreactive material in the eye is speculative. Certainly interest is stimulated through the knowledge that a variety of gastrointestinal and vascular effects have been identified for PP and its biochemically related peptides. However, to our knowledge, physiologic studies have not yet been performed in the eye.

Note Added in Proof: In a just published letter (Allen JM, McGregor GP, Adrian TE, Bloom SR, Zhang SQ, Ennis KW, and Unger WG: Reduction of Neuropeptide Y (NPY) in the rabbit iris-ciliary body after chronic sympathectomy. Exp Eye Res 37:213, 1983), Neuropeptide Y-like immunoreactivity has been found by radioimmune assay in the anterior segment, providing additional confirmation of the presence of a peptide of this class in the eye.

Key words: pancreatic polypeptide, innervation, eye, immunohistochemistry, guinea pig

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References


