metabolic degradation remains the same. In the cornea (Table I and II) vidarabine monophosphate appears to be more rapidly metabolized after topical application compared to iontophoretic topical application. Therefore the catabolism in the aqueous humor must be slower, diffusion from the cornea higher, or some combination of the two in order to maintain the same amount of vidarabine monophosphate and vidarabine for 20 and 60 min.

Erlanger used iontophoresis as a procedure for obtaining high levels of drugs in eye tissues. Although iontophoresis is an uncomplicated, safe, well-documented method for assuring penetration of charged drugs, the technique has not been widely used. Harris has reviewed the uses of iontophoresis in medicine and dentistry and cited the advantages of iontophoresis in drug delivery to surface tissues, giving numerous examples. Iontophoresis offers the advantages of assuring penetration of the drug to the desired site, i.e., corneal stroma and aqueous humor, and is an alternative to topical application, systemic administration, or direct injection into the eye tissue.

The radioactive vidarabine monophosphate was supplied through the courtesy of Drs. T. J. Petrick and A. J. Glazko, Warner Lambert–Parke, Davis & Co., Ann Arbor, Mich. Scanning electron microscopy was performed by Mid-America Micronalysis, Milwaukee, Wisc.

From the Departments of Cell and Molecular Biology, Pharmacology, Oral Biology, Ophthalmology, and Physiology, Medical College of Georgia, Augusta. Supported in part by grants from Parke, Davis & Co. (J. M. H. and N. H. P.), National Eye Institute EY-01413 (K. G.), and NIDR-DE-04917 (J. M. H.). Present address: Harvard Medical School, Eye Research Institute of Retina Foundation, Boston, Mass. Submitted for publication Sept. 13, 1977. Reprint requests: James M. Hill, Ph.D., Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, Georgia 30901.

Key words: iontophoresis, vidarabine, vidarabine monophosphate, hypoxanthine arabinoside, corneal epithelium, cornea, aqueous humor

REFERENCES

Histochemical demonstration of cyclic guanosine 3′,5′-monophosphate phosphodiesterase activity in retinal photoreceptor outer segments. RICHARD M. ROBB.

A technique for the histochemical demonstration of cyclic guanosine monophosphate phosphodiesterase in retina is described. Enzyme activity was identified on photoreceptor outer segment lamellae, a finding in agreement with previous biochemical data on isolated outer segment preparations. The distribution of phosphodiesterase activity for cyclic guanosine monophosphate was similar to that found previously in rod outer segments for cyclic adenosine monophosphate, suggesting that the same enzyme may hydrolyze both nucleotides.

The cyclic nucleotide phosphodiesterase which hydrolyzes cyclic adenosine 3′,5′-monophosphate (cyclic AMP) to 5′-adenosine monophosphate has previously been demonstrated on photoreceptor outer segment lamellae by histochemical means. Although a similar enzyme hydrolyzing cyclic

0146-0404/78/0517-0476$00.50/0 © 1978 Assoc. for Res. in Vis. and Ophthal., Inc.
guanosine 3',5'-monophosphate (cyclic GMP) has been identified in photoreceptor outer segment preparations by biochemical techniques, its histochemical demonstration has been inconsistent and uncertain. Since levels of cyclic GMP are high in the retina and since they appear to be light-sensitive, the ultrastructural localization of cyclic GMP phosphodiesterase has been a matter of continuing interest. This report presents a successful method for the histochemical localization of cyclic GMP phosphodiesterase activity in retinal photoreceptor cells.

**Method.** Normal adult mice of the C57BL/6J inbred strain were used in the study, in anticipation of the desirability of subsequently applying the technique to animals of the same strain with recessively inherited retinal degeneration (rd). The animals were killed with an intraperitoneal injection of pentobarbital and were immediately decapitated. Eyes were removed in room illumination and were immersed in a solution containing 60 mM Tris-maleate buffer (pH 7.4), 2 mM MgCl₂, and 0.25M sucrose (TMS buffer). The eyes were opened under an operating microscope,
Fig. 2. Photoreceptor outer segments of retina incubated with cyclic GMP plus xanthine inhibitor of phosphodiesterase activity. Reaction product is diminished compared to Fig. 1. (×25,000.)

and the retina was separated gently from the pigmented epithelium. Isolated portions of the retina were then fixed for exactly 3 min with 2% glutaraldehyde in 0.05M cacodylate-nitrate buffer (pH 7.4) containing 0.25M dextrose. The retina was then returned to TMS buffer to be dissected into pieces approximately 0.5 by 1.0 mm.

The pieces of retina were first incubated for 30 min at room temperature in TMS buffer to which was added 5'-nucleotidase in the form of snake venom of Ancistrodon piscivorus piscivorus, 1 mg. of dry venom per milliliter. The tissue was then incubated for 30 min at 37°C with slow agitation in TMS buffer containing 2 mM lead nitrate, 5'-nucleotidase (1 mg. of dry venom per milliliter), and 3 mM cyclic GMP. Control experiments were run without the cyclic nucleotide substrate in the second incubation, or with cyclic nucleotide plus 2 mM 1-methyl, 3-isobutyl xanthine, a known inhibitor of phosphodiesterase.

After incubation the retinal tissue was briefly washed in distilled water, dehydrated in graded alcohols, treated with propylene oxide, and embedded in Epon 812. Thin sections were cut, stained with uranyl acetate, and examined with the electron microscope.

Results. A lead phosphate precipitate at or near the site of phosphodiesterase activity is the expected result of the above histochemical technique. Such a precipitate was seen along photoreceptor outer segment lamellae (Fig. 1) when tissue fixation times were kept at or below 3 min.
Fig. 3. Photoreceptor outer segments incubated without added cyclic GMP substrate. No reaction product has formed as the result of phosphodiesterase activity. (×24,000.)

Fig. 4. Inner retina incubated with cyclic GMP, showing reaction product (arrows) beneath basement membrane (asterisks) of Müller cells and on plasma membranes (double arrows) of inner retinal cells. (×30,000.)

With longer fixation times the reaction product became scanty and unpredictable. With fixation times shorter than 3 min the precipitate was heavier, but it tended to obscure the underlying tissue, the morphology of which was already compromised by such a brief period of fixation. The reaction product was much diminished by the addition of a phosphodiesterase inhibitor to the second incubation (Fig. 2). No reaction product at all was seen in experiments in which the cyclic GMP substrate was omitted (Fig. 3). The only other place in the retina at which reaction product was
consistently seen was along the inner retinal surface and along cell membranes of the inner retina (Fig. 4). This localization of enzyme activity had also been noticed in earlier experiments with cyclic AMP as substrate.  

Discussion. The previous difficulties in demonstrating cyclic GMP phosphodiesterase activity histochemically in retina appear to have been due to excessive fixation of the tissue. Glutaraldehyde fixation for more than brief periods evidently denatures or alters the enzyme in such a way that it will not react with cyclic GMP. The hydrolysis of cyclic AMP is not as sensitive to fixation, but it too is reduced by prolonged exposure to glutaraldehyde. 1 I have recently used the 3 min fixation time for both cyclic AMP and cyclic GMP and find slightly more reaction product with the former when the conditions of incubation are otherwise similar. The distribution of reaction product on the outer segments is identical with the two substrates, a finding which suggests that one and the same enzyme may be capable of hydrolyzing both cyclic nucleotides.

No precipitate was seen consistently on photoreceptor inner segments, nuclei, or synaptic areas. On the vitreal side of the retina the restriction of precipitate to areas near the retinal surface may reflect limited diffusion of reactants, as discussed previously. 2 The fact that the reaction product was found beneath the basement membrane of the Müller cells rather than on its surface suggests that the precipitate reflects true enzyme activity and not an artifactual deposit.

From the Departments of Ophthalmology, The Children's Hospital Medical Center and Harvard Medical School, Boston, Mass. This work was supported by U. S. Public Health Service grant 5R01 EY01451 from the National Eye Institute, Bethesda, Md. Submitted for publication Feb. 3, 1978. Reprint requests: Dr. Richard M. Robb, Department of Ophthalmology, The Children's Hospital Medical Center, 300 Longwood Ave., Boston, Mass. 02115.

Key words: phosphodiesterase, cyclic GMP, histochemistry, retina, photoreceptor cells

REFERENCES


The role of hemolysin in corneal infections with Pseudomonas aeruginosa. Mary K. Johnson and James H. Allen.

Cultures of Pseudomonas aeruginosa considered to be of proven virulence were found to have higher titers of extracellular hemolysin than cultures of lesser virulence. Intracorneal injection of purified hemolysin produced extensive corneal opacification with extensive leukocytic infiltration of the tissue. It is suggested that the hemolysin plays a role in the pathogenesis of P. aeruginosa infections by effecting lysis of host cells and/or subcellular organelles, leading to the release of enzymes destructive to corneal tissue.

The role of various factors elaborated by Pseudomonas aeruginosa in the destruction of ocular tissue has been studied by several investigators. Fisher and Allen 1, 2 produced ulceration of the cornea with cell-free extracts and with partially purified protease preparations from this organism. More recently, the role of proteolytic enzymes in Pseudomonas ulcers has been studied by other investigators. 3, 4 The production of damage to corneal ground substance by enzymes from Pseudomonas was reported by Brown et al. 5 and by Gray and Kreger. 6 The possible role of host enzymes in tissue degradation was discussed by the latter authors and further explored by Kessler et al., 7 who concluded that corneal destruction by P. aeruginosa depends not only on the protease, which rapidly destroys the cornea, but also on host-derived enzymes which are capable of degrading both collagen and proteoglycans.

In a preliminary survey in which we compared various characteristics of strains of P. aeruginosa of proven virulence with those of strains of undetermined virulence, we noted that the former appeared to be more hemolytic on human blood...