tributions of norepinephrine and prostaglandins in the mediation of the increase in facility, however, must await further study.

In conclusion, the reduction by indomethacin of the increased outflow facility occurring after sympathetic denervation may point to a role of prostaglandins in the mediation of this ganglionection effect. Because aspirin was not as effective in this regard, however, a special action of indomethacin in preventing the facility increase must also be considered.

From the Department of Medical Pharmacology, University of Uppsala, Uppsala, Sweden. This work was supported by grants 2 R01 EY00231, EY01217, and 1 KO7 EY00075 from the U. S. Public Health Service, National Eye Institute. Submitted for publication Nov. 21, 1977. Reprint requests: Dr. Brenda K. Colasanti, Department of Pharmacology, West Virginia University Medical Center, Morgantown, W. Va. 26506.

Key words: outflow facility, cervical ganglionection, indomethacin, aspirin, prostaglandins, rabbit

REFERENCES

Abnormal retinogeniculate projections in a congenitally microphthalmic cat. T. L. Hickey and N. R. Cox.

The retinogeniculate projections from the normal eye of a unilaterally microphthalmic cat are abnormal in that optic tract fibers cross laminar borders and end, inappropriately, in geniculate layers that would normally receive input from the microphthalmic eye. This congenitally induced abnormal retinogeniculate projection is quite similar to that seen in cats with one eye surgically removed shortly after birth. Although most cells are shrunken in the laminae normally innervated by the microphthalmic eye, cells in the region of the abnormal projection appear normal. The normal pattern of geniculate lamination is also disrupted in that cell-free interlaminar regions are considerably more difficult to define in the microphthalmic cat.

Central visual system anomalies, including abnormal retinogeniculate projections, occur in a variety of microphthalmic animals such as rats, and cats. Although, in some of these studies, teratologic agents were used to produce the microphthalmia, others have observed central nervous system disorders in congenitally microphthalmic animals. Recently, a congenitally microphthalmic cat became available to us through a breeding colony used to study feline GM2 gangliosidosis, a lysosome storage disease resulting from a β-hexosaminidase deficiency.

Methods. The kitten reported on here was one of three offspring resulting from the mating of a 2-year-old heterozygote male and a 2-year-old heterozygote female. Of the three kittens, one was sacrificed immediately due to a large, subcutaneous, fluid-filled, fibrous sac over the dorsal thorax, a second died at 4 days of age, and the third, the microphthalmic kitten, was allowed to survive for 83 days. No other examples of microphthalmia or other visual system abnormalities have appeared in this colony in more than 100 births.
Fig. 1. Photomicrographs of a section through the optic disc region of the microphthalmic eye.  
la, Retina has not developed normally. Although for the most part the retinal cells are undifferentiated, there is some suggestion that both the inner and outer plexiform layers are beginning to appear. b, In addition, a few ganglion cell bodies (arrow) can be seen. (Cresyl violet; a ×6, b ×63.)
Fig. 2. Top, Camera lucida drawings of coronal sections through the left lateral geniculate nucleus. The nonshaded regions show the projection of the ipsilateral retinogeniculate axons, as determined by the distribution of silver grains. Bottom, Plots of relative grain density (closed circles) and cell area (open circles) for three locations shown on section 37. The grain density measurements were made with a Leitz-Tectur-Analyse System. Relative grain density at background levels was 100. Cell-area measurements were averaged over all cells in a given 1000x field of view.

In order to study the retinogeniculate projections of the normal eye, we injected 500 μCi of H³-leucine into the vitreal chamber of the left (normal) eye. Twenty-four hours later the animal was anesthetized and perfused through the heart with formol-saline. Gross examination of the brain showed that the right optic nerve was present but severely atrophied. Other than this, the brain appeared normal on gross examination. Blocks of brain tissue containing the lateral geniculate nuclei were embedded in paraffin, sectioned coronally, at 14 μ, and processed routinely for autoradiography. In addition, both eyes were embedded in celloidin and cut (40 μ) in the parasagittal plane. Every third and fourth section was stained with cresyl violet and Mallory triple stain, respectively.

Results. Fig. 1, a, shows a section through the optic disc region of the microphthalmic eye. Besides being less than half the size of the normal eye, the microphthalmic eye has developed quite abnormally. The abnormal development is most evident in the retina, which only covers a small part of the optic cup. The higher power photograph in Fig. 1, b, shows that cell differentiation in the retina is at a very early stage. Although a few ganglion cells (arrow) can be seen in Fig. 1, b, we were unable to further classify the other retinal cells. There is a suggestion, however, that the inner and outer plexiform layers are developing. The left eye appeared normal both in terms of its size and postnatal development.²

Fig. 2 shows a series of camera lucida drawings of coronal sections through the lateral geniculate nucleus ipsilateral to the normal eye. Only laminae A and A1 are shown, and the interlaminar zones have been included only where they could be defined with reasonable certainty. In all instances the interlaminar regions were located on sections not processed for autoradiography. In this way the distribution of silver grains could not affect the way in which the laminar patterns were...
Fig. 3. a, Bright-field photomicrograph of the lateral aspects of section 37 (Fig. 2). Approximate locations used for making grain-density and cell-area measurements are shown as well as the A-A1 interlaminar zone (IL), the dorsal and ventral borders of which are defined by the open arrows. b, Dark-field photomicrograph of same area shown in a. The interlaminar zone is again shown (arrows). As can be seen, the distribution of silver grains (white) extends well into lamina A. (Cresyl violet; ×63.)

drawn. Adjacent autoradiographs were then superimposed on the drawings, and the distribution of silver grains was plotted. In these drawings the shaded regions represent areas which did not receive retinal projections from the normal eye, as determined by the distribution of silver grains. In addition, the cell bodies in these regions were quite shrunken. Although the A-A1 interlaminar zone is difficult to define, there are some cell-free areas separating the two laminae. Where such regions can be defined, it is possible to show that some cells in the ventral-most aspects of lamina A are not reduced in size. When the distribution of retinogeniculate fibers is studied, these cells can be seen to lie in an area that is entered by retinal axons. Although this abnormal projection is most marked in the more lateral aspects of the section, including the monocular segment, such projections also occur in more medial parts of the section. Both of these findings are illustrated graphically at the bottom of the figure. Grain-density and cell-area measurements were made at three locations shown on drawing 37. The graph shows that both the density of silver grains and the size of cell bodies remains about the same for measurements made in lamina A1 (location 1) and just inside the ventral edge of lamina A (location 2). However, measurements made in the more dorsal aspects of lamina A (location 3) show both grain density and cell size to be reduced considerably.

Fig. 3, a, shows a bright-field photomicrograph of the region over which the cell area and grain density measurements were made. The approximate points at which these measurements were made are shown. Fig. 3, b, shows a dark-field photomicrograph of the same region. The A-A1 interlaminar zone (IL) is defined by the open arrows. Fig. 3, a, shows quite clearly that some of the cells along the ventral aspects of lamina A appear normal in size. However, more dorsally placed cells are shrunken. With the use of various blood vessels as landmarks in making comparisons between Fig. 3, a and b, it is possible to show that the regions of surviving large cells in lamina A...
receive abnormal, ipsilateral retinogeniculate projections.

Although we have limited our detailed analyses to the "A" laminae, it was evident that similar examples of abnormal retinogeniculate projections could be seen in the more ventral "C" laminae.

Discussion. There are at least two ways in which the present findings can be interpreted. The abnormal retinogeniculate projections described here may represent a congenitally induced example of axonal sprouting. Translaminar growth (sprouting) of retinogeniculate axons following early removal of one eye has been reported previously.3-5 The severity of cell shrinkage and pattern of abnormal growth seen here are very similar, if not identical, to those seen in kittens having one eye removed shortly after birth.4 Certainly it is not possible to conclusively demonstrate that the extent of abnormal growth is greater here, even though it is obvious that the microphthalmic eye stopped developing quite early. Our findings and those of others4 5 suggest that the over-all extent of abnormal growth is limited by some factor other than the duration of normal growth remaining following the removal (or halt in development) of one eye.

A second possible interpretation concerns the development of laminar patterns in a lateral geniculate nucleus receiving a normal retinal projection from only one eye. In the microphthalmic cat the segregation of cells into separate, well-defined laminae is only partially complete. In light of this it is interesting to relate the formation of laminar patterns in the geniculate to the distribution of abnormal growth. In this and previous4 studies, the regions of the geniculate that exhibited the most extensive abnormal growth were also the regions with the least-defined interlamellar zones. Rakic5 has shown that a close relationship exists between the formation of laminae in the monkey geniculate and the segregation of retinogeniculate afferents. In the fetal monkey the projections from both eyes initially overlap in the geniculate. As the retinal afferents from the two eyes segregate, the geniculate laminae emerge. If one eye is removed during this process of segregation, the formation of separate cellular laminae is halted. If a similar situation exists for the cat, then what we have termed abnormal growth here and elsewhere4 translaminar growth could simply represent the proportion of early, nonsegregated growth that had not retracted at the time one eye was removed or stopped developing. If it is assumed that the process of cellular segregation occurs gradually as more and more fibers retract to end within a given lamina, one would expect to find the cellular laminae well defined in regions with little or no abnormal growth and poorly defined in regions with extensive abnormal growth. Although such a hypothesis is certainly compatible with the data presented here, a question still remains as to why the extent of abnormal growth (i.e., the lack of retinal afferent segregation) is not greater, given the point at which the microphthalmic eye stopped developing.

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Key words: microphthalmia, lateral geniculate nucleus, abnormal projections, cat

REFERENCES


The pH dependency of sodium and chloride transport in the isolated human cornea.

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Sodium and chloride net transport in isolated human cornea preparations were found to be pH dependent. Sodium net transport was directed from aqueous humor to tear side at pH 7.6 and 8.6. At pH 7.4, net sodium flux did not differ significantly from zero, and at pH 7.0, net sodium transport was directed toward the aqueous humor side. Chloride net flux at pH 8.6 and 7.6 was also directed from aqueous humor to tear side. Acidification of the bathing solution to pH 7.4 and 7.0 was followed by a decrease in chloride net fluxes to values not significantly different from zero.

Corneal hydration regulates corneal transparency and is maintained by means of active ion transport processes located in the endothelium as well as in the epithelium. Experiments suggesting the pH dependency of ion transport mechanisms in corneas have been published earlier, for frog, rabbit, monkey, and human corneas. The aim of the experiments presented here was to measure the transcorneal Na⁺ and Cl⁻ fluxes and to study the influence of pH upon them. Significant net Na⁺ and Cl⁻ fluxes which were both stimulated by alkalinization could be detected.

Methods. Human corneas were dissected out with a triphine 2 to 5 hr after death. The corneas were then transferred to a Ussing-Zerahn-type chamber modified by Zadunaisky for measurements in the cornea. In some experiments the bulbus was enucleated and the preparation completed in a chamber with a suction ring described by Klyce, Neufeld and Zadunaisky. The experiments were performed under short-circuit conditions at 33°C. Resistance was calculated as potential difference over short-circuit current (PD/SCC) or as ΔPD/ΔI. Corneas with a resistance lower than 300 ohm cm² were discarded. Two types of Ringer’s solutions with different buffer substances were used. Ringer’s solution A was made with bicarbonate—the composition was 118.5 mM NaCl, 4.1 mM KCl, 0.7 mM MgSO₄, 7 mM KH₂PO₄, 1.2 mM K₂HPO₄, 1.7 mM CaCl₂, 26.2 mM NaHCO₃, and 5.1 mM glucose. The Ringer’s solution throughout the experiments was equilibrated on both sides of the cornea with air, resulting in a pH of 8.6, or with a mixture of 5% CO₂ and 95% air, resulting in a pH of 7.6. Replacement of the polyethylene tubing, which is CO₂ permeable, by nylon tubing (Technicon), resulted in a pH of 7.4. Ringer’s solution B contained phosphate and TRIS as buffer systems. KOH at 1.0 M and 100 mM TRIS-(hydroxymethyl)-aminomethan were titrated with orthophosphoric acid to pH 7.0. The composition was 103.5 mM NaCl, 20.3 mM Na₂SO₄, 0.5 mM MgSO₄, 1.4 mM CaCl₂, 13.2 mM glucose, 2.5 mM K₂HPO₄, 1.7 mM KH₂PO₄, 3.1 mM (TRIS)2HPO₄, and 2.1 (TRIS)H₂PO₄. Measurements of pH were made with Astrup micro equipment or with the Ingold pH probe type 405-M5 and Knick pH-meter type 350. Isotopic flux measurement were performed with ²²NaCl carrier free in H₂O and with H³°Cl. The latter was neutralized with NaOH, and the resultant NaCl substituted for an appropriate amount of NaCl in the Ringer’s solution. Beta radiation of ²²Na or ³°Cl was detected with a liquid scintillation counter (Packard Tri-Carb scintillation spectrometer). In some experiments, Na⁺ and Cl⁻ fluxes were measured simultaneously, using the gamma radiation of ²²Na (Packard Auto-Gamma spectrometer) for calculating Na⁺ fluxes. Flux measurements were made during periods of 30 min after an incubation period of at least 45 min. A more detailed description of methods has been given elsewhere. All experiments were made with paired corneas. The t-test was used to calculate significances.

Results. In Table I, unidirectional and net fluxes of ²²Na and ³°Cl at various pH values and resistance are shown. Table II summarizes PD and SCC data. Na⁺ net flux vs. pH is plotted in Fig. 1. At pH 7.6 and 8.6, Na⁺ net flux is directed from aqueous humor to tear side, and at pH 7.0 from tear to aqueous humor side. At pH 7.4, Na⁺ net flux did not differ significantly from zero. The intercept of the linear regression line with the abcissa occurs at pH 7.38. This means that at pH values lower than 7.38, the Na⁺ net flux across the isolated human cornea preparation is directed toward the anterior chamber, and at pH values greater than 7.38, it is directed toward the tear fluid. The correlation coefficient r = 0.634 was significantly different from zero (p < 0.001). In