Pressure-induced Fast Axonal Transport Abnormalities and the Anatomy at the Lamina Cribrosa in Primate Eyes

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In ten owl monkey eyes (Aotus trivirgatus) the location of pressure-induced (perfusion pressure 35 mmHg) axonal transport abnormalities was determined by the examination of serial step cross-section tissue radioautographs from the optic nerve head. The degree of the local transport interruption did not correlate with the fiber bundle cross-section area, the shape of the laminar pores or the density of the inter-bundle septa in that region. Invest Ophthalmol Vis Sci 24:343-346, 1983

In the primate eye, an elevated intraocular pressure (IOP) interrupts the optic nerve fast axonal transport at the lamina cribrosa. It is unclear to what extent this pressure-induced transport interruption is analogous to optic nerve disease seen in human eyes with elevated pressure. Nevertheless, this transport blockade can be useful as an experimental marker of pressure-induced optic nerve damage.

This transport abnormality may involve any region of the nerve cross-section, but it occurs most often in the superotemporal and the inferotemporal sectors of the lamina cribrosa. These same regions of the lamina are characterized by relatively large fiber bundle pores and thin inter-bundle septa. We have hypothesized that these differences in the local anatomy at the lamina cribrosa determine the relative vulnerability of the individual fiber bundles to a pressure insult.

However, this apparent correlation between the anatomy at the lamina and the location of the transport interruption may be spurious. In the cat eye, the pressure-induced transport interruption involves the temporal nerve more than the nasal nerve. These observations would appear to support our hypothesis. However, in the individual experimental eyes, there is no correlation between the location of the transport interruption and the anatomy at the lamina cribrosa. The present report describes a similar direct comparison between the location of the transport abnormality and the anatomy at the nerve head in owl monkey eyes.

Materials and Methods

Pressure Model

Ten eyes were studied after a 4-hr interval of elevated IOP (perfusion pressure 35 mmHg). The animals (Aotus trivirgatus) were anesthetized with an intramuscular injection of ketamine (10 mg/kg). This anesthesia was maintained by periodic intravenous injections of 0.1 cc pentobarbital (100 mg/cc). The systemic blood pressure was monitored by means of a pressure transducer attached to a heparinized polyethylene catheter inserted into the right femoral artery. The intraocular pressure was manipulated by adjusting the height of a fluid reservoir attached, via polyethylene tubing, to a #25-gauge needle inserted into the anterior chamber of each experimental eye.

The IOP was reduced to atmospheric pressure and 0.1 cc tritiated leucine (L-leucine-4, 5-3H(N), 30 to 50 Ci/m mole) was injected, through a pars plana stab incision, into the vitreous cavity of each eye. The injection site was sealed with ethyl cyanoacrylate. Thereafter, the IOP was elevated such that the ocular perfusion pressure (the mean systemic blood pressure less the IOP) was 35 mmHg. Adjustments in this pressure level were made at ½-hr intervals to compensate for fluctuations in the systemic blood pressure.

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Supported in part by the National Society for the Prevention of Blindness, Inc., New York, New York, and Research to Prevent Blindness, Inc., New York, New York. Additional support was obtained from Research Grants EY-01931 and EY-04193 from the National Eye Institute, Bethesda, Maryland.

Submitted for publication March 29, 1982.

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Fig. 1. A cross-section of the lamina cribrosa from an owl monkey eye. The nerve has been divided into three regions. Zone I encompasses that portion of the nerve with the greatest density of exposed emulsion. Zone II has an intermediate amount of the leucine accumulation and Zone III has a minimal amount of the transport abnormality. On adjacent (2 μm) tissue sections the mean fiber bundle size and the density of the inter-bundle septa in each of these three zones were measured by computer assisted planimetry (paraphenylene diamine, ×50).

Specimen Preparation

After 4 hrs of this pressure insult, the animals were killed by an intravenous injection of sodium pentobarbital. The tissue specimens were fixed in situ by a retroarterial (abdominal aorta) infusion of 200 cc of normal saline, followed by 200 cc of 4% gluteraldehyde fixative (perfusion pressure 200 mmHg). A right ventricular cardiac incision was made to allow the egress of blood, saline, and fixative. After fixation, the eyes were enucleated with the retro-orbital portion of the optic nerve cut flush with the globe. Additional fixative was injected into the vitreous cavity. Twenty-four hours later, the specimens were opened by a coronal incision and a 6-mm², full-thickness tissue specimen containing the optic nerve head was excised from the posterior eye wall. The nerve head was divided in half by a horizontal or a vertical incision. After postfixation in 1% osmium tetroxide, each specimen was dehydrated by serial passage through alcohol, propylene, and xylene solutions and embedded in epoxy resin for sectioning.

Tissue Radioautography

With the plane of the section parallel to that of the retinal surface, a pair of thick sections (2 μm) was cut at successive 100-μm step-levels through the optic nerve head. All of the sections were heat impregnated on glass slides, and the first of each pair was coated with liquid photographic emulsion. The coated slides were incubated in the dark at 4°C for two weeks after which the emulsion was developed. An examination of these step sections by light microscopy identified those regions of the nerve cross-section with an interruption in optic nerve transport. A single section from the level of the scleral lamina cribrosa was selected from each eye for further examination. In these tissue radioautographs, the density of the exposed emulsion grains was used to estimate the amount of the focal leucine accumulation. Each nerve cross-section was divided into three regions, identifying those areas of the cross-section with a maximal, moderate, or a minimal amount of the transport interruption for that specimen (Fig. 1).

Fiber Bundle Dimensions

The second of each pair of sections was stained with paraphenylene diamine and examined by phase contrast light microscopy. For each eye the paired section from the level of the scleral lamina cribrosa was photographed and reproduced at a magnification of 500X. The laminar pore cross-section area and the ratio of the major and the minor axis diameters for each fiber bundle were measured on these photographs by computer-assisted planimetry. The outlines defining the areas of the nerve with maximal, moderate, or minimal transport abnormality (from the adjacent tissue radioautographs) were duplicated on these stained sections. The mean fiber bundle cross-section area and the mean ratio of the major and the minor axis diameters of the laminar pores were calculated for each of these areas (maximal, moderate, and minimal block) in each eye.

Inter-bundle Tissue Density

The regional density of the inter-bundle septa was defined as the ratio of the area occupied by these trabecula to the total sector cross-section area. The area of the inter-bundle septa was calculated as the total sector cross-section area less the summed areas of the laminar pores in that sector. The density of the inter-bundle tissue in each of the three regions (maximal, moderate, and minimal block) was recorded for each eye.

Pooled Data from Ten Experimental Eyes

The mean values for the laminar pore cross-section area, the ratio of the major and the minor axis di-
ameters, and the density of the inter-bundle septa were calculated for the three nerve head regions (maximal, moderate, and minimal transport interruption) by averaging the mean values for each region from each of the ten eyes.

Data Analysis

The Student's t-test for statistical significance was employed in all comparisons to determine whether the observed differences in the means were significant at the 0.05 confidence level.

Results

Tissue Radioautography

In each of the ten experimental eyes, the density of the exposed emulsion grains was greater in the step sections from the level of the lamina cribrosa than in the specimens from the more anterior and posterior optic nerve. This pattern was consistent with an accumulation of the labeled material at the lamina cribrosa. The density of the leucine labeling varied across the nerve cross section.

Fiber Bundle Dimensions

In six eyes, the regions of the nerve with more transport abnormality had smaller fiber bundles (P < 0.05). In three eyes, the regions of the nerve with more transport block had larger fiber bundle pores (P < 0.05). In one eye the mean cross-section area of the fiber bundles was the same in all three regions (maximal, moderate, or minimal block) of the nerve head. The shape of the laminar pores (the ratio of the major and the minor axis diameters) was equivalent in all three regions (maximal, moderate, and minimal block) of the nerve head.

Inter-bundle Tissue Density

In five eyes, the regions of the nerve cross-section with greater transport interruption had less inter-bundle tissue while in three eyes the exact opposite pattern was seen (P < 0.05). In two eyes, the density of the inter-bundle septa was the same in all three nerve head regions.

Pooled Data from Ten Experimental Eyes

In the ten eyes, the mean fiber bundle cross-section area was smaller and the density of the inter-bundle septa was less in those areas of the nerve with more of the transport abnormality (Table 1). These differences, however, were not significant at the 0.05 confidence level.

Table 1. Anatomic measurements at the lamina cribrosa and focal involvement by pressure-induced axonal transport interruption

<table>
<thead>
<tr>
<th>Degree of transport interruption</th>
<th>Maximal</th>
<th>Moderate</th>
<th>Minimal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inter-bundle tissue density</td>
<td>42.6 ± 0.9</td>
<td>44.1 ± 2.9</td>
<td>45.5 ± 2.5</td>
</tr>
<tr>
<td>Cross-section area lamina pores</td>
<td>20 ± 3</td>
<td>25 ± 4</td>
<td>27 ± 4</td>
</tr>
<tr>
<td>Ratio major to minor axis</td>
<td>1.7 ± 0.5</td>
<td>2.7 ± 0.5</td>
<td>2.4 ± 0.5</td>
</tr>
</tbody>
</table>

* ×10^2 μm².

Discussion

In both owl (Aotus trivirgatus) and rhesus (Macaca mulatta) monkey eyes, pressure-induced optic nerve transport interruption preferentially involves the supertemporal and inferotemporal regions of the lamina cribrosa. In both rhesus monkey and human eyes these same regions of the nerve have larger fiber bundle dimensions and finer inter-bundle septa. These observations suggest that this anatomy at the lamina cribrosa may determine local vulnerability to a pressure insult. In this experiment, however, I was unable to demonstrate any significant correlation between the degree of focal, pressure-induced transport abnormality and the size and shape of the nerve fiber bundles or the density of the inter-bundle septa.

Could differences in the optic nerve head anatomy between these several primate species account for this unexpected result? The distribution in fiber bundle size and tissue density at the nerve head in the owl monkey has not been defined. On the other hand, we do know that the temporal nerve sectors of the owl monkey eye are preferentially involved by the pressure-induced transport interruption. This increased vulnerability of the temporal nerve is apparently not related to anatomic parameters, at least as defined in this study.

Only ten eyes were examined in this report. This sample size may have been too small to demonstrate a correlation between the anatomy at the lamina and the vulnerability to the pressure insult. On the other hand, the present data do not suggest that an examination of additional eyes would be useful. There was more block in the regions of the nerve with finer...
inter-bundle septa and smaller fiber bundle dimensions, however, the variation from eye to eye was so extreme that, even if the nerve anatomy does play a role in defining the local vulnerability to the pressure insult, it cannot be a very great one.

This analysis also examined only one pair of the multiple cross-sections prepared from each of the ten eyes. Perhaps an examination of the remaining paired sections would detect a correlation between the anatomy at the lamina and the location of the transport interruption. On the other hand, the paired sections that were examined were from the level of the scleral lamina cribrosa, the presumed site of the axonal obstruction. Furthermore, in any given eye the details of the anatomy and the locations of the transport interruption at the lamina cribrosa are relatively constant at various levels throughout the optic nerve head thickness. Consequently, although any paired sections are likely to be representative of the entire specimen, the sections from the level of the scleral lamina seem to be most appropriate for study.

Finally, the relative degree of the focal transport interruption in these eyes was defined by a qualitative rather than a quantitative estimate of the density of the exposed emulsion grains in cross-section tissue radioautographs. An examination of multiple cross-sections from each eye was used to distinguish transport blockade from other causes of focal increase in leucine labeling, whereas the examination of individual cross-sections was used to define those regions of the lamina with maximal, moderate or minimal block for any particular specimen. The limitations of this technique have been discussed in more detail elsewhere; however, I do not feel that a quantitative estimate of the density of the exposed emulsion would have improved this analysis.

In summary, this radioautographic examination of the pressure-induced transport blockade has some important theoretical and practical shortcomings. Nevertheless, the present data indicate that, in the owl monkey eye, the location of the pressure-induced axonal transport interruption is not determined by the density of the inter-bundle septa or the dimensions of the nerve fiber bundles.

**Key words:** optic nerve, axonal transport, glaucoma, radioautography

**References**