Effect of intraocular pressure on rapid axoplasmic transport in monkey optic nerve

Douglas R. Anderson and Anita Hendrickson

Tritiated leucine was injected into the vitreous of owl monkeys (Aotus trivirgatus) to make it available to retinal ganglion cells for protein synthesis. Intraocular pressure was maintained for eight hours at selected levels between 15 and 105 mm Hg. Autoradiography and scintillation counting were used to follow the movement of the labeled protein along the axons from the retinal ganglion cells to the lateral geniculate nucleus (LGN) by axoplasmic transport. At normal levels of intraocular pressure, label was distributed continuously along the retina, optic disc, and nerve, and radioactive label reached the LGN by eight hours. When the intraocular pressure was moderately elevated there was partial obstruction of axoplasmic transport in the region of the lamina cribrosa, but some label reached the optic nerve and LGN. When intraocular pressure was elevated to within 25 mm Hg of mean blood pressure, there was complete obstruction of transport at the lamina cribrosa and label did not reach the LGN; retinal synthesis and transport along the axons to the optic nerve head continued. When the intraocular pressure approached or exceeded mean blood pressure, retinal synthesis stopped, as evidenced by absence of label in both the retina and disc. Our conclusions are that: (1) axoplasmic transport is affected by intraocular pressure, (2) there is a selective effect deep in the optic nerve head at the lamina cribrosa, and (3) a partial effect can be detected even at moderate elevations of intraocular pressure. Whether the obstruction is mechanical or is secondary to reduced blood flow has not been determined, and the exact relevance of these findings to the pathogenic mechanisms of glaucomatous cupping is not yet clear.

Key words: axoplasmic transport, glaucomatous cupping, intraocular pressure, experimental glaucoma.

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The pathogenic mechanism involved in glaucomatous cupping of the disc has not completely been elucidated. As recently reviewed in detail,1-2 it has been shown that if the intraocular pressure is high

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enough, blood circulation in the optic disc (and the rest of the eye) is affected. However in chronic glaucoma the intraocular pressure is often only slightly elevated, and it is not clear whether such moderate elevation of the intraocular pressure significantly impairs circulation of the optic disc. A review of available data fails to prove whether glaucomatous damage to the optic nerve is due to reduced blood flow, or whether axons and glia of the optic nerve might be affected directly by intraocular pressure and some other biologic phenomenon might be the critical physiologic function affected by elevation of intraocular pressure.

In this context, we became interested in determining to what extent intraocular pressure might influence the biologic phenomenon of axoplasmic transport in the nerve fibers of retinal ganglion cells, especially where they traverse the optic nerve head and exit from the eye. As recently reviewed, axoplasmic transport is now a well established phenomenon in the visual system of amphibia, fish, birds, and mammals, including monkeys, as well as other neuronal pathways. Axoplasmic transport includes movement of organelles and metabolic substances, including proteins, along the length of the axon at various speeds. For example, following an intravitreal injection of tritiated amino acid, the retinal ganglion cell synthesizes radioactively labeled protein which is then transported from the eye along the optic nerve. In the rabbit visual system the most rapid component of this transport moves 150 to 250 mm. per day, and in the rhesus monkey it covers the distance from retina to the lateral geniculate nucleus (LGN) within six hours. Our experiments, which had an eight-hour survival time, dealt with the most rapid component of axoplasmic transport along with the slightly less rapid components (phase II), but not the slower components.

Axoplasmic transport is blocked by anoxia, inhibitors of oxidative phosphorylation, inhibitors of glycolysis, anti-mitotic agents, local anesthetics, and mechanical compression (ligation). In this study we used owl monkeys to study whether variations in intraocular pressure will affect the rapid phases of axoplasmic transport, and if there is a blockage, where it occurs.

Materials and methods

Owl monkeys (Aotes trivirgatus) were anesthetized with phencyclidine (Sernylan) and pentobarbital (Nembutal). Their femoral arteries were cannulated with heparinized saline-filled polyethylene tubing to record systemic blood pressure with a pressure transducer. Intraocular pressure (IOP) was controlled manometrically by means of a saline reservoir connected via polyethylene tubing to a 27-gauge needle inserted obliquely through the peripheral cornea with the tip lying between lens and iris on the opposite side. The IOP was set by varying the height of the reservoir so that the desired perfusion pressure (PP) was obtained, with perfusion pressure taken to be the difference between IOP and mean femoral arterial pressure. Mean arterial pressure was calculated as diastolic blood pressure (D) plus one-third the difference between systolic and diastolic pressure (S). Thus

$$PP = [D + \frac{1}{3}(S-D)] - IOP.$$ 

The femoral blood pressure was continually monitored and the height of the saline reservoir adjusted so that the PP remained constant over the eight-hour period of the experiment.

To inject the tritiated (3H) leucine (L-leucine-4, 5-3H(N), 30 to 50 Ci per millimole, New England Nuclear), the conjunctiva was disinserted from the limbus on the temporal side of the eye. A 30-gauge needle was inserted into the vitreous through the bared sclera over the pars plana, and the tip of the needle positioned close to the surface of the retina in the posterior pole with a binocular indirect ophthalmoscope. The reservoir of saline was lowered to decompress the eye and the 3H-leucine injected. In 27 eyes, 0.1 ml containing 100 μCi of 3H-leucine was injected, while two eyes for electron microscopic (EM) autoradiography received 0.3 ml containing 300 μCi of 3H-leucine. The needle was withdrawn and the puncture site sealed with a drop of e-cyanoacrylate glue. The corneal needle was also stabilized with e-cyanoacrylate. When the glue was set (5 to 10 seconds), the blood pressure was checked and the saline reservoir was elevated to the height necessary to achieve the desired PP. The time from 3H-leucine injection to PP stabilization was less than five minutes. Throughout the day the height of the saline reservoir was adjusted if the
blood pressure varied so that the PP was kept constant. Significant adjustments were required in very few of the animals.

Eight hours (± ½ hour) after ³H-leucine injection, the monkey was perfused via the femoral catheter with 4 per cent paraformaldehyde fixative (phosphate-buffered, pH 7.3). Fixative passed retrograde through the aorta to enter the major aortic branches, including the carotid arteries, but was prevented from entering the heart by the aortic valve. The tip of the heart was cut off to allow drainage of blood and fixative returning to the heart via the venous system. The saline reservoir was lowered after the perfusion had begun in order to lower intraocular pressure and promote adequate perfusion of fixative through the optic nerve and disc.

The eyes and dorsal lateral geniculate nuclei (LGN) were embedded in paraffin, sectioned at 10 μm, and coated for light microscopic autoradiography by dipping in Kodak NTB2 emulsion.22 The eyes were exposed for three days and the LGN for four weeks after which the sections were developed in Kodak D19 for two minutes and fixed in Rapid Fix for four minutes. Sections from all eyes were stained with cresyl violet after developing, but some eye sections were also stained before coating with luxol-fast blue.22

For electron microscopic (EM) examination, two monkeys (265 and 266) were maintained in a slightly different fashion. In monkey 265, one eye was kept at a PP of 25 and injected with 300 μCi of ³H-leucine while the other was at a normal intraocular pressure of 10 mm Hg and was not injected. In monkey 266, both eyes were at a PP of 25, but only the left eye was injected with 300 μCi of ³H-leucine. All four eyes were prepared for EM after 4 per cent paraformaldehyde perfusion by cutting the optic disc into four pieces along the long axis of the optic nerve. The tissue pieces were postfixed in 2.5 per cent phosphate-buffered osmium tetroxide for one hour, dehydrated in ethanol, and embedded in epoxy resin. Grids containing thin sections were coated by a modification of the loop method23 with Ilford L4 emulsion and exposed for 6 and 12 weeks. The grids were developed in either D19 or Microdol X for two minutes, fixed in Rapid Fix for one minute, and poststained in lead citrate for 10 minutes. For standard EM, thin sections of control and elevated IOP eyes were double-stained in uranyl acetate and lead citrate.

To minimize bias, light microscopic autoradiographic slides from the first 23 eyes were studied in a “blind” fashion by one of us, so that the person interpreting the light autoradiographs of eyes and LGN did not know the perfusion pressures that had been used. After this series was finished and the range of effective pressures known, eight eyes were done at pressures either not covered in the first series or deemed especially important. To allow direct comparison, the last two animals (272 and 274) had both eyes injected with ³H-leucine with one eye at PP = 25 and the other eye at PP = 100. At the conclusion, autoradiographic light microscopic slides from all animals were examined again in a blind manner by the other investigator as an independent “blind” evaluation.

Grain counts were done on the LGN of the two animals (272 and 274) that had both eyes injected with one eye PP = 100 and the other eye at PP = 25. For comparison, similar counts were done on the right LGN of monkey 210. Adjacent parvocellular layers from both nuclei in the same section were counted at x1,000; medial, central, and lateral regions of 13,600 μ² were counted for layers three and four on two different sections. Scintillation counts were done on selected eyes by mounting 12 paraffin sections on glass slides, melting off the paraffin, and then scraping off the optic disc and retina with a razor blade. Six sections were pooled for each sample, thereby obtaining duplicate counts from each specimen.

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Fig. 1. Low-power autoradiograph of monkey 210 for orientation. The field includes the prelaminar optic nerve head (A), the region of the scleral trabecula of the lamina cribrosa (B), and the beginning of the orbital portion of the optic nerve. The field included in Fig. 2 is indicated. (Luxol fast blue-cresyl violet, x240).
Table I. Incorporated radioactive label eight hours after injection of tritiated leucine into the vitreous

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Femoral arterial blood pressure (mm. Hg)</th>
<th>Mean femoral blood pressure (mm. Hg)</th>
<th>Intraocular pressure (mm. Hg)</th>
<th>Perfusion pressure (mm. Hg)</th>
<th>Retina</th>
<th>Accumulation at lamina cribrosa</th>
<th>Retrobulbar optic nerve</th>
<th>Lateral geniculate body</th>
<th>Leakage into orbit</th>
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from the 12 sections. The tissue was digested overnight at 55° C. in 1 ml. Hyamine hydroxide and counted using PPO-POPOP toluene-ethanol solution.

Results

Normal axoplasmic transport. At normal and slightly elevated levels of intraocular pressure (PP > 60) the incorporated label had reached the lateral geniculate body by eight hours (Table I). The label was distributed through the optic nerve head and intraorbital optic nerve without any accumulation in a specific location (Figs. 1 and 2). The orbital portion was more lightly labeled, presumably because the presence of septa and myelin results in fewer axons per area.

Partial obstruction of axoplasmic transport. With slight elevation of IOP to achieve a perfusion pressure (PP) of 60 mm. Hg, there was partial obstruction of axoplasmic transport, and this was more definite at a PP of 45 and 35. The obstruction was evidenced by heavier accumulation of radioactivity in the optic nerve head in the region of the scleral trabecula forming the lamina cribrosa (Fig. 3). However, the obstruction was only partial, since radioactive label was seen along the length of the optic nerve and in the LGN.

Total obstruction of axoplasmic transport. When intraocular pressure was elevated to the point that it was only 25 mm. Hg below mean femoral blood pressure (PP = 25), the label accumulated in heavy amounts in the region of the lamina cribrosa (Fig. 4), but little or none
reached the orbital portion of the optic nerve or LCN (Table I). It was obvious, even by light microscopy, that the high radioactivity was in the nerve fiber bundles and not in the glial columns. Scintillation counts in the animals receiving an injection in both eyes reflect the accumulation in the optic nerve head at PP = 25 by showing twice as many counts in the optic disc compared to the normal pressure (Table II).
Table II. Scintillation counts of eyes injected with ³H-leucine

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Eye</th>
<th>Intraocular pressure</th>
<th>Disc (counts/min.) average</th>
<th>Retina (counts/min.) average</th>
<th>Ratio retina/disc</th>
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<tr>
<td>272</td>
<td>Right</td>
<td>Normal</td>
<td>(209, 202) 206</td>
<td>(2890, 2831) 2861</td>
<td>13.89</td>
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<tr>
<td>272</td>
<td>Left</td>
<td>High</td>
<td>(716, 733) 723</td>
<td>(4728, 4110) 4419</td>
<td>6.11</td>
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<td>274</td>
<td>Right</td>
<td>Normal</td>
<td>(142, 158) 150</td>
<td>(2066, 1873) 1970</td>
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<tr>
<td>274</td>
<td>Left</td>
<td>High</td>
<td>(397, 356) 377</td>
<td>(3393, 3819) 3606</td>
<td>9.56</td>
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Table III. Incorporated radioactive label eight hours after injection of ³H-leucine into the vitreous

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<th>Perfusion pressure</th>
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<td>++</td>
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*Results enclosed in a box are from animals in which there was significant synthesis in the retina.

These findings signify severe blockage of axoplasmic transport in the region of the lamina cribrosa.

Protein synthesis by retinal ganglion cells continued at this pressure, and the cell bodies of the ganglion cells were prominently labeled (Fig. 5). The nerve fiber layer seemed proportionately more heavily labeled than in eyes with higher PP, since at PP = 25 the nerve fiber layer was as heavily labeled as the outer retinal layers, while in the other specimens (with higher PP) the nerve fiber layer was usually more lightly labeled than the outer retinal layers. This proportionately heavier labeling of nerve fiber layer could represent partial interferences with axoplasmic transport in the inner retina in addition to obvious total obstruction at the lamina cribrosa. Scintillation counts of retinal and disc samples in animals receiving an injection in both eyes (Table II) tended to confirm that there is a heavier retinal label at the higher intraocular pressure accompanying
the proportionately greater increase in disc label.

From Table I it can be seen that, in spite of heavy retinal labeling, the obstruction at the lamina cribrosa at a PP of 25 to 35 reduced the label reaching the LGN in some of the animals and eliminated it completely in most. None of the animals with a PP of 20 or less showed label in the LGN.

We studied the ultrastructural alterations of the optic nerve head at a perfusion pressure of 25 mm. Hg in monkey eyes 265 and 266. Control observations were made on the opposite eyes of monkey 265 (which had not been injected and had a normal pressure) and 210 (which had received a leucine injection but had a normal perfusion pressure). These control specimens showed a normal primate optic disc ultrastructure (Fig. 6). 24, 25

In the eyes with a PP of 25 mm. Hg, the prelaminar region was not strikingly different from the prelaminar region of eyes with normal IOP, with the possible exception that axonal microtubules were either less well preserved or somewhat reduced in number in some axons. However, there was a striking difference (Fig. 7) at the posterior boundary of the optic nerve head (i.e., the scleral portion of the lamina cribrosa), where almost every axon was dilated due to an accumulation of mitochondria and vesicles of smooth endoplasmic reticulum. Some of the dilated regions had small accumulations of normal appearing organelles. The more severely involved axons also had numerous myelin figures and distorted mitochondria. Some of the axons had enormous club-shaped areas several micrometers across containing vesicles and dilated cisternae of smooth endoplasmic reticulum. Interspersed among the vesicles were normal mitochondria,
Fig. 6. Electron micrograph of region of the lamina cribrosa in the normal eye of monkey 265. The nerve fibers (nf) have a normal appearance as they pass an opening in a scleral trabeculum (st) (×8,500).

Multivesicular bodies, glycogen granules, and myelin figures. Many of the myelin figures appear to originate from mitochondria since frequent instances are seen of mitochondria with disorganized, replicated cristae, dark matrix, or loops of membrane surrounding the outer membrane. Whether the smooth endoplasmic reticulum also gives rise to myelin figures could not be determined. These morphologic findings are very similar to the findings in other nerves when axoplasmic transport is interrupted by ligation or drugs. In this region of congested axons, neurofilaments were regularly seen. Microtubules were more difficult to identify, but
were clearly present in many axons including some of the congested ones, but it would take careful counts corrected for volume differences to show whether or not there was a quantitative change in either axonal microtubule or neurofilament number.

In electron microscopic autoradiographs of optic disc at a PP of 25 mm. Hg, silver grains were found over optic axons, and not over astroglia, connective tissue, or vascular spaces. The optic axons were labeled in the prelaminar region, but the greatest grain density was over the congested axons of the posterior half of the lamina cribrosa (Fig. 8). In this area, the
silver grains were associated with the regions of the cell containing mitochondria and smooth endoplasmic reticulum, while the neurofilament-microtubule-rich regions were only lightly labeled. The greatest grain density was over the smooth endoplasmic reticulum vesicle-filled "clubs" which were almost completely hidden by silver grains after 12 weeks exposure. Axon labeling continued a short distance into the optic nerve and then dropped off sharply.

Reduced retinal synthesis. When IOP was elevated so that the PP was less than 25 mm Hg, there was practically no incorporation of radioactive leucine in the retina, nor was there label in the optic nerve or the lateral geniculate body. This indicates that at these pressures retinal ischemia prevented protein synthesis in the ganglion cells, and the radioactive leucine did not become incorporated into transportable material. In some animals the orbital tissues were labeled with incorporated tritiated leucine. This occurred particularly in some of the animals with high intraocular pressure whose orbits had become progressively swollen from the scleral puncture site during the eight hours. This leak not only washed some of the leucine from the vitreous into the orbit, but also caused the intraocular pressure to be somewhat lower than the height of the reservoir could indicate. This resulted in a corresponding lessened effect on axoplasmic transport so that some of these animals showed a slight degree of protein synthesis.
Discussion

The study of axoplasmic transport includes two events. The first is the uptake and incorporation of the labeled amino acid into protein by synthesis within the nerve cell body. The second event is movement of labeled protein down the axon, or axoplasmic transport itself. With regard to the first event, only the nerve cell body contains the necessary protein synthetic machinery; neither axon nor synaptic terminal can synthesize significant amounts of protein. At this step any factor inhibiting protein synthesis (such as anoxia or protein-synthesis inhibitors) will prevent the appearance of labeled protein. In our experiment, this synthetic step appears to be involved in the eyes having a PP of 20 mm. Hg or lower. With the exception of monkey 245, the retinas show very light labeling, with virtually nothing in the optic disc. This range of pressures presumably affected the inner retinal artery circulation, creating an anoxic block of protein synthesis.

Once the nerve cell body has synthesized labeled protein and it has reached the axon (5 to 30 minutes), the nerve cell body is no longer necessary because transport at any point within the axon apparently is dependent only on local conditions at that point along the axon. The results of our experiment indicate that IOP has an affect on transport along the axon, and that the greatest effect is felt deep in the optic nerve head at the level of the sclera, especially when PP approaches 25 mm. Hg.

It is of interest not only that the laminar portion of the optic nerve head selectively suffers more than other regions at severe elevations of IOP, but that there is some apparent partial effect at moderate elevations in pressure. As reviewed in detail elsewhere, nearly all previous experiments have failed to demonstrate effects of IOP on any physiologic phenomenon in the optic nerve head except at extreme elevations of pressure. It is not clear whether the incomplete block noted is due to a partial effect on some axons, or is due to a total effect on some axons and no effect on others.

The question immediately arises whether the interruption of axoplasmic flow is due to mechanical compression of the axons, or whether it is due to ischemia, since it is known that either localized ischemia of an axon segment or ligature will interrupt axoplasmic flow in that segment. One might hope to make the separation between ischemic effects and mechanical effects by noting whether the results depend more upon PP or upon the absolute level of IOP. As shown in Table I, both retinal synthesis and blockage at the lamina cribrosa correspond fairly well with PP. If the results are rearranged according to IOP (Table III), it is clear that the first event (retinal synthesis of protein) does not correspond as well to IOP as it did to PP. Hence, it is probable that reduction of protein synthesis is an ischemic effect (dependent upon PP). The second event (axoplasmic transport itself) can be judged only from those animals in which retinal synthesis occurred, since blockage of transport cannot be judged in those animals that had no retinal synthesis. Considering only the eyes that had significant retinal synthesis (as indicated by enclosure in a box in Table III), the accumulation of label at the lamina cribrosa corresponds to the absolute level of IOP nearly as well as it did to PP (Table I), except for monkey 269. Since no valid conclusion can be based on a single animal, the data of these experiments are equally consistent with a mechanical or an ischemic mechanism for blockage at the lamina cribrosa, and further experiments will be necessary to determine the mechanism of obstruction.

It is important to keep in mind that the phenomenon studied in these experiments is only the more rapid phases of axoplasmic transport, which is highly heterogeneous and consists of numerous enzymes and membrane proteins. We have not studied the slower moving components which might also be affected by IOP. In addition, the experimental design did not
study retrograde axoplasmic transport\textsuperscript{27-31} (from the synapse in the LGN toward the cell body in the retina), which occurs at about half the rate of orthograde rapid transport (75 to 150 mm. per day) and is also affected by ligature, anoxia, and antimitotic agents.

We cannot yet conclude that any component of axoplasmic transport is the critical physiologic function which—if interrupted—results in axonal death. Apparently, many types of substances are transported, and presumably many physiologic processes would be affected; but from direct evidence it is not known to what degree the cell body, axon, or synaptic terminal are dependent on the materials supplied by either orthograde or retrograde transport. Among the slight evidence on this question is that a significant amount of rapid orthograde transport never reaches the terminal, but apparently is utilized in the axon as a source of renewal of axon protein.\textsuperscript{22} However, when rapid axoplasmic transport is chemically blocked for days or weeks in the pigeon retina, there is no evidence for neuronal degeneration even after six weeks of blockage.\textsuperscript{17, 33} There appears to be only relatively minor changes in the synaptic terminal such as swelling of synaptic vesicles; in this experiment, a prominent change was a failure of synaptic transmission which appeared one to three days after blocking rapid transport by intravitreal colchicine.\textsuperscript{34} Also pertinent to the role of orthograde transport is that in the newt limb after axoplasmic transport of one major nerve was blocked by colchicine, the other nerves no longer "recognized" its presence and sprouted to fill its territory in the same manner as when the nerve was sectioned.\textsuperscript{35} These and other lines of evidence suggest that rapid orthograde transport helps maintain synaptic transmission and that some material may also be liberated from the axon terminal.\textsuperscript{1, 22, 33, 36} Considering the opposite direction, retrograde rapid transport\textsuperscript{27-31} appears to be a mechanism for breakdown of worn-out synaptic proteins since much of this material ends up in the lysosomes of the nerve cell body. It could also be a means of information feedback from synapse to cell body and, in addition, may be an avenue for virus to infect the nerve cell.

Since so little is known about the physiologic roles of axoplasmic transport, it is certainly not clear how, and to what degree, interruption of axoplasmic transport in either direction would affect the neuron, much less the glial cells (which are prominently affected in glaucoma\textsuperscript{2}). Nor do we know that axoplasmic transport is a physiologic function that can be chronically affected in a partial but cumulative manner to explain how prolonged mild elevations of IOP can have little initial effect on function but ultimately damage the axon. It must also be considered that the various components of axoplasmic transport may only be some of many physiologic functions affected primarily or secondarily by elevation of IOP, and which, if any, of these are in the chain of critical events leading to cell death remains to be determined.

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