Chronic Ischemia Induces Regional Axonal Damage in Experimental Primate Optic Neuropathy

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Objectives: To evaluate the effects of chronic optic nerve ischemia in a nonhuman primate model and to evaluate the regional variability of axonal loss.

Methods: Unilateral ischemic optic neuropathy was induced by administration of endothelin-1 to the retrobulbar space via osmotic pumps in 12 primates for 6 to 12 months. The transversely cut sections were stained and divided into 16 regions. Average axonal density in each region was quantified and compared with the untreated contralateral control eyes.

Results: Mean axonal density was 208310/mm² and 220661/mm² in treated and control eyes, respectively (P = .03, 1-tailed paired t test), for the entire group. Two-way analysis of variance showed a significant effect of endothelin-1 on overall axonal density for the experimental group (P < .001). Among the nerves with significant axonal loss, the mean axonal loss was 11.6% (4%–21%). Regional mapping of the damage showed the axonal loss varied in the damaged nerves; the damaged regions often clustered within specific quadrants.

Conclusion: Chronic ischemia induced by local administration of endothelin-1 causes significant loss of optic nerve axons with varying regional susceptibility.

Clinical Relevance: Localized damage occurs in other types of optic neuropathy, such as glaucoma, and may result from regional differences in anatomy, metabolism, or vasculature of the primate optic nerve.

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VASCULAR ABNORMALITIES are thought to contribute to the development of glaucomatous optic neuropathy and have been investigated for more than 150 years. A variety of experimental and clinical observations demonstrate circulatory aberrations of the anterior optic nerve, the peripapillary region,1-3 the choroid,4,5 and the retrobulbar vasculature6-11 in eyes with glaucomatous optic neuropathy.7,11-14 These apparent alterations in the circulation of the glaucomatous optic nerve implicate dysfunction of vascular regulatory mechanisms. Further support for vascular dysregulation has been suggested by the finding of elevated levels of endothelin-1 (ET-1), a potent vasoconstricting peptide, in the plasma10,15-17 and aqueous humor18,19 of patients with glaucoma.

To further investigate the consequences of chronic ischemia, we developed a model of sustained optic nerve ischemia. This model uses the continuous administration of ET-1 to the perineural region of the optic nerve in the retrobulbar space and has been described in rabbits,20 rats,21 and nonhuman primates.22 These experiments allow the direct in vivo observation of optic nerve structure and function during periods of chronic ischemia. Comparisons to human glaucomatous optic neuropathy and experimental optic neuropathy following chronic elevation of intraocular pressure (IOP) are also possible. In the rabbit model, a reduction of blood flow to the anterior optic nerve of approximately 38% was achieved, resulting in loss of optic nerve axons, loss of neuroretinal tissues of the optic nerve, and enlargement of the optic nerve cup.22,23 Similarly, the nonhuman primate model showed approximately 36% blood flow reductions to the anterior optic nerve and significant loss of optic nerve axons after 3 to 6 months of continuous ischemia.24,25 Although axonal loss in these initial studies appeared in all regions of the optic nerve, visual inspection of the optic nerve sections revealed regional differences in the loss of axons. This finding has led to a more thorough examination of regional axonal changes. Because nonhuman primates have a vascular architecture and tissue properties within the anterior op-
tic nerve that are similar to the human eye, primate models are often used to provide meaningful comparisons to human glaucoma.

This study examined the susceptibility of the optic nerve retinal ganglion cell axons to chronic ischemia in the nonhuman primate eye. Axonal loss within the optic nerve is assessed histologically and compared between eyes with chronic ischemia from ET-1 treatment and untreated contralateral control eyes. We further evaluate changes of axonal density in various subregions of the optic nerve following experimental chronic ischemia to examine regional differences in the axonal loss.

METHODS

ET-1 INDUCED OPTIC NERVE ISCHEMIA MODEL

Twelve adult female rhesus (Macaca mulatta) monkeys, weighing 4 to 8 kg and between the ages of 8 and 19 years, were used in accord with the Association for Research in Vision and Ophthalmology Statement on the use of animals in ophthalmic and vision research. At the beginning of the study, animals underwent a complete eye examination, which included slitlamp examination, IOP measurements, and dilated funduscopy examination followed by stereoscopic optic nerve photography. For these examinations, animals were sedated with ketamine (Ketaset; Fort Dodge Animal Health, Fort Dodge, Iowa) administered intramuscularly (15 mg/kg) and were intubated. Anesthesia of the animals is achieved with inhalation of 3% isoflurane in oxygen. Blood oxygen saturation and pulse rate are monitored via peripheral oximetry and core body temperature was maintained with a heating pad.

The surgical procedures for the implantation of the ET-1 minipumps in primates has been described previously. The untreated contralateral eye served as the control. In brief, an osmotic minipump (Alzet model 2004; Durect Corp, Cupertino, Calif) preloaded with approximately 230-µL ET-1 (Peptides International, Louisville, Ky) solution (55µM/mL) was implanted subcutaneously superior to the lateral rim of the orbit of the right eye. The outlet of the pump was connected to one end of a silicone delivery tube. The tube was passed subcutaneously into the superotemporal orbit and under the bulbar conjunctiva and the Tenon capsule. The tube was placed beneath the superior rectus muscle, and the end of the tube was secured in the retrobulbar space adjacent to the superonasal aspect of the optic nerve. The tube was fixed in place using a 9-0 nylon suture through the sclera. The minipump continually delivered the ET-1 solution to the retrobulbar region at a dose of 0.34 pg/d with a constant flow rate of 6 µL/d over 4 weeks. At the end of each 4-week period, the minipump was replaced with a new ET-1 loaded pump at the original site of placement; the patency of the tube was ensured by irrigation of balanced salt solution (Alcon Laboratories Inc, Fort Worth, Tex), prior to the placement of the new pump. Tubes with a blocked opening due to scar tissue growth were replaced. Otherwise, the tube was left in place and the new pump attached. Sham implantation of minipumps loaded with vehicle without ET-1 demonstrated no short-term effect on blood flow and other factors examined in a previous report. An additional sham animal was included in the present study, but unfortunately, it died of unrelated causes 3 months following pump implantation; tissue was unavailable for analysis. In addition, both optic nerves of a control animal with normal eyes (no pump implantation and normal findings on ocular examination) were analyzed to assess the normal intereye variability of axonal density.

The duration of ET-1–induced ischemia was approximately 6 months (mean [SD], 23 [2] weeks) in 8 monkeys, 9 months (38 weeks) in 1 monkey, and 12 months (52 [1] weeks) in 3 monkeys (Table 1). During this period, a variety of in vivo measurements were made in each animal at baseline prior to pump implantation and bimonthly during the study. These tests included IOP measurements in both eyes measured with tonometry (Tonopen; Pat Leonard Surgical Inc, Shawnee, Kan) immediately after the animals were anesthetized with 15 mg/kg of intramuscular ketamine hydrochloride. Three measurements were made for each eye and a mean IOP was recorded. Optic nerve appearance was monitored with stereoscopic optic nerve photography (model 3-DX; Nidek Co Ltd, Tokyo, Japan). Photographs of the optic nerves were evaluated in a masked fashion for indications of acute anterior ischemic optic neuropathy, such as focal or global pallor, edema, or hemorrhages.

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<th>Untreated Contralateral Control Eye</th>
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Mean (SD) 13.5 (4.2) 7.75 (2.7) 208 310 (33 579) 220 661 (35 859) 0.95 (0.10) .03
minipump and compared with a freshly prepared control ET-1 solution (3 tests for each sample). Using high-performance liquid chromatography (model 1100; Hewlett Packard, Palo Alto, Calif) the test samples and control samples were analyzed. Compared with the control sample, the percentage of ET-1 detected in the samples at the end of 1 through 4 weeks were 110%, 83%, 83%, and 74%, respectively.

**OPTIC NERVE TISSUE SAMPLING AND STAINING**

At the end of the experiments, the animals were euthanized with an intravenous injection of 100 mg/kg pentobarbital sodium and phenytoin sodium (Euthasol; Delmarva Laboratories Inc, Midlothian, Va). Perfusion fixation was accomplished using a 4% formaldehyde solution via the carotid arteries. Both eyes were immediately enucleated and fixed in a 4% formaldehyde solution for an additional 2 to 3 hours. The retrobulbar optic nerves were transected (approximately 2-3 mm behind the globe), and a 0.5-mm-thick section was obtained and processed for resin sectioning. All of the optic nerve tissue sections were fixed in 5% glutaraldehyde in a phosphate buffer (pH 7.4) for 1 hour and then rinsed in phosphate-buffered saline solution before being postfixed in 2% osmium tetroxide for 3½ hours. The tissue was rinsed again, dehydrated in an ethanol-acetone series, and embedded in epoxy resin (Epon 812; Epon-LKB Instrument, Gaithersburg, Md). Semithin sections (1 µm) were cut and mounted on slides. The adjacent stump of each optic nerve still attached to the globe immediately opposite to the cut surface was marked with colored tissue dye (Tissue Marking Dyes; Triangle Biomedical Sciences Inc, Durham, NC) to preserve the orientation in situ for later anatomical orientation of each section.

The sections on the slides were stained with 1% phenylenediamine (in 1:1 methanol/isopropanol) for 20 minutes, rinsed 2 to 3 times with isopropanol, and air-dried. If staining was incomplete with phenylenediamine, at a temperature of approximately 80°C, adjacent sections were stained with 1% toluidine blue in the phosphate-buffered saline solution (pH 7.0-7.4) for 3 minutes followed by adding a few drops of Sorensen’s buffer for another 2 minutes. The slides were rinsed with distilled water and air-dried. The orientation of the optic nerve sections was determined by matching certain landmarks (such as blood vessels and contour lines of axonal bundles) of the sections with the adjacent optic nerve stumps from where the sections were cut.

**OPTIC NERVE RETINAL GANGLION CELL AXON DENSITY ESTIMATES**

The methodological details of the retinal ganglion cell (RGC) axonal density quantification are described in a previous publication.29 In brief, with an image analysis system (Bioquant; R&M Biometrics Inc, Nashville, Tenn), a composite of the entire optic nerve area was determined by comparison of ET-1–treated and control eye group averages for overall axonal density using a 1-tailed paired t test

For statistical analysis and assessment of regional axonal loss, the optic nerves were divided into 16 subregions consisting of an inner and outer segment within each of 8 wedge-shaped, radial sectors as shown in Figure 1 (ST, TS, TI, IT, IN, NI, NS, SN with T indicating temporal; N, nasal; S, superior; and I, inferior). All 16 subregions had approximately equal areas. As mentioned earlier, the axonal density for each region was calculated as the average of all grid-square samples within the region. Similarly, the total overall axonal density for an entire optic nerve was calculated as the average of all density samples (ie, all counted grid squares) for each optic nerve.

The effect of ET-1–induced chronic ischemia was evaluated by comparison of ET-1–treated and control eye group averages for overall axonal density using a 1-tailed paired t test (n=12 pairs). Two-way, matched-pair analysis of variance (ANOVA) (treatment × animal) was used to further evaluate the effect of ET-1 treatment on the overall axonal density, while controlling for variation due to differences between individual animals. Bonferroni-corrected post hoc tests were used to evaluate the significance of ET-1 effects for individual animals (pairs of eyes). Potential regional effects of ET-1–induced ischemia were first evaluated across the whole group (n=12) using 2-way, matched-pair ANOVA (treatment × region). Regional effects of ET-1 treatment were further explored within individual animals using a similar 2-way, matched-pair ANOVA (treatment × region). This analysis was possible because the variance of each observation (mean of each optic nerve region) could be calculated based on the numerous samples obtained (grid squares counted) in each optic nerve region. For this regional analysis of each individual animal, the effect of ET-1 treatment was considered statistically significant at P<.0042; thus, α was adjusted for 12 comparisons. If there was also a significant interaction between ET-1 treatment and optic nerve region, Bonferroni-corrected post hoc tests were used to evaluate the significance of axonal density differences among individual pairs of regions (ET-1 vs control eye).

Finally, the optic nerve areas were compared using a paired t test.
to assess for optic nerve swelling or shrinkage. Intraocular pressure measurements from baseline, the midpoint, and the end of the experiment were analyzed with repeated-measures ANOVA.

RESULTS

IOP FINDINGS

The mean (SD) IOP for the 12 ET-1–treated eyes at baseline (pretreatment) was 15.5 (4.3) mm Hg. At the midpoint of each animal’s experimental period, the average IOP in ET-1–treated eyes was 17.1 (3.0) mm Hg. At the final examination in the ET-1–treated eyes the IOP was 14.9 (4.5) mm Hg. The average IOP in the group of untreated contralateral control eyes was 15.1 (3.6) mm Hg at baseline, 16.7 (3.8) mm Hg during the experimental period, and 14.6 (4.0) mm Hg at the final examination. There was no significant difference between the IOPs in the control and ET-1–treated eyes (P = .61). Likewise, the IOP did not differ significantly between baseline and final measurements in either the control eyes or the ET-1–treated eyes (P > .05, ANOVA).

IN VIVO OPTIC NERVE APPEARANCE

The stereophotographs of the optic nerves were examined and compared between baseline and subsequent examinations. There was no evidence of pallor, edema, or other changes commonly associated with anterior ischemic optic neuropathy in any of the photographs (Figure 2). Owing to the few animals and the high variability associated with clinical assessment of stereoscopic optic nerve photographs for progressive change, we have excluded such an analysis from this article. However, automated optic nerve head analysis (Heidelberg Retina Tomographs; Heidelberg Engineering, Dossenheim, Germany) was performed and is being analyzed.

LIGHT MICROSCOPY: OPTIC NERVE AND RGC AXON SIZE

Under the light microscope, the optic nerves of the ET-1–treated eyes showed a variety of morphological changes in the RGC axons and connective tissues com-
pared with the untreated contralateral eyes (Figure 3).

These changes included axonal demyelination, axonal swelling, axonal shrinkage, and axonal fragmentation. These changes may represent the various stages in the continuum of axonal degeneration. Connective tissue changes included increased thickness of the perineurium and enlarged extra-axonal spaces associated with the loss of axons. In areas with more severe damage, neuroglial cells appeared to be larger and increased in number and axons appeared to be significantly larger. Figure 3 shows comparisons between 2 different optic nerve regions in 2 different individual animals (monkeys 2 and 6). The optic nerve subregions were chosen because they represent areas of significant axonal loss in the ET-1–treated eyes compared with the untreated contralateral eyes (see the “Regional RGC Axonal Density Comparison” subsection). The mean and standard deviation of the axon sizes is given for each region in Figure 3. On average, in regions of axonal damage in the ET-1–treated eyes, more of the RGC axons appeared swollen, the variability of the axon size was greater, and the optic nerves were larger. The mean area of the optic nerves in the ET-1–treated eyes was 6.27 (0.96) mm² while the area of the untreated contralateral control eye was 5.91 (0.89) mm². This represents an overall increase of 6% in the size of the optic nerves in the ET-1–treated eyes (P = .03, 1-tailed paired t test). However, the contribution of optic nerve swelling to axonal density calculations is not uniform, as some ET-1–treated optic nerves were larger and some were unchanged. In addition, axon sizes appeared larger overall in the ET-1–treated eyes, meaning that axonal enlargement and extracellular space enlargement both contribute to the overall increase in the size of the optic nerves. As an example, monkey 6 (Figure 3) had significant overall, as well as regional, axonal loss without optic nerve swelling (ET-1–treated eye optic nerve size, 7.3 mm²; untreated contralateral control eye, 7.2 mm²), while monkey 2 had significant regional and overall axonal loss with optic nerve enlargement (ET-1–treated eye optic nerve size, 7.4 mm²; untreated contralateral control eye, 6.3 mm²).

**COMPARISON OF AXONAL DENSITY BETWEEN ET-1–TREATED EYES AND UNTREATED CONTRALATERAL CONTROL EYES**

**Global RGC Axonal Density Comparison**

Following 6 to 12 months of continuous ET-1 administration in 1 eye, the group mean axonal density for the entire optic nerve among the 12 ET-1–treated eyes was 208310/mm², while the untreated contralateral control eye group mean was 220661/mm² (t = 2.04, P = .03 1-tailed paired t test; Table 1). On average, this change represents a 5.1% (10.0%) decrease of axonal density owing to ET-1–induced ischemic ischemia. However, Table 1 also shows that the effect of ET-1 treatment was not uniform across all 12 animals; individual animals varied greatly in response to chronic ischemia. There was no significant effect of duration of exposure to ET-1 treatment (Kruskal-Wallis statistic = 0.04, P = .98). Admittedly, the number of animals treated for 9 and 12 months is small and may have prevented detection of an effect. Axonal density also varied substantially among untreated contralateral control eyes ranging from 170622 to 286825 axons/mm² (220661 [35855] axons/mm²). Therefore, a 2-way ANOVA (treatment × animal) was applied and Bonferroni-corrected post hoc tests were used to evaluate the effect of ET-1 on overall axonal density for the whole experimental group and for each individual, respectively (Table 1). This allowed for control of the variation owing to differences between individual animals. As expected, axonal density varied significantly between individual animals (F = 129.9; P < .001). The ET-1 treatment effect was significant for the group as a whole (F = 311.2; P < .001), and there was a significant interaction between treatment and animal effects (F = 31.4, P < .001). Bonferroni-corrected post hoc testing revealed that 9 of the 12 animals had significant axonal density differences between their ET-1–treated and untreated contralateral control eyes, but 2 of these had a greater density in the ET-1–treated eye (Table 1). Significant axonal loss was seen in 7 animals and the loss varied from 4% to 21%, with a mean (SD) loss of 11.6% (6.7%).

**Regional RGC Axonal Density Comparison**

Several studies have demonstrated regional susceptibility within the optic nerve in glaucoma and other forms of optic neuropathy in human, as well as in experimental glaucoma in nonhuman primates and rodents. In visual evaluation of most optic nerve cross sections, it was immediately evident that various subregions sustained more damage than others. To evaluate the potential regional susceptibility in this experimental model of chronic ischemia, the axonal density was also assessed for 16 optic nerve subregions (see the “Methods” section). Figure 4 shows regional differences in axonal densities for the group of 12 experimental animals, ET-1–treated vs untreated contralateral control eyes. Regional...
analysis showed a significant group effect of ET-1–induced ischemia \( (F = 19.9; \ P < .001) \), but there was no significant interaction observed between treatment and region \( (F = 1.2; \ P > .05) \). As most of the variance (71%) in axonal density was attributable to differences between individual animals, comparisons between the 2 eyes (ET-1–treated and untreated contralateral control) of each animal would allow a better measure of regional axonal density change.

Table 2 lists that regional losses in axonal density were observed in 8 of 12 ET-1–treated eyes relative to the corresponding region of the optic nerve from each animal’s untreated contralateral control eye. Two of the 12 (monkeys 3 and 4) experimental eyes had differences in regional axonal density where the density was actually significantly greater in the ET-1–treated eye. The remaining 2 experimental eyes (monkeys 5 and 7) had no significant effect of ET-1 treatment and, thus, analysis of regional differences (treatment \( \times \) region interactions) was not applicable.

Figure 5 shows the spectrum of regional effects observed across the group. The pair of pie charts in each panel shows data for a single animal. The numbers within each region in the left column represent the ratio of axonal density in the ET-1–treated eye relative to the density in the corresponding region of the optic nerve from the fellow control eye (gray shaded subregions, ET-1 axonal density \( < \) control). Most common areas of axonal loss were found in clusters of contiguous subregions. Panels D, F, and G demonstrate significantly lower axonal densities within isolated subregions of the control eyes (gray shaded subregions, ET-1 axonal density \( > \) control).
treated contralateral control eye. For example, panels D through G demonstrate significantly lower axonal densities within single subregions of the untreated contralateral control eyes (gray-shaded subregions, ET-1 axonal density > control). In contrast, regions where the axonal density in the ET-1–treated eye was significantly reduced compared with the axonal density in the corresponding region of the untreated contralateral control eye was common. Panels A through F each show that significant loss of axonal density occurred in multiple subregions of ET-1–treated eyes. Most common areas of axonal loss were found in clusters of contiguous subregions. In panel B, there is significant loss in both the superonasal and inferotemporal regions, whereas panels A and C through F show more focal deficits in clusters of 2 to 7 subregions.

INTEREYE VARIABILITY IN A CONTROL ANIMAL

To further investigate the variability of axonal density in healthy eyes and between the 2 eyes of a healthy primate, 2 eyes of a single monkey without minipump implantation were examined and analyzed in an identical statistical fashion. Figure 6 shows the relative axon densities in the various regions between 2 normal eyes of the single monkey. The probability plot derived by identical statistical methods as was used for the ET-1–treated monkeys. As expected, there was some variability of the intereye ratio of axonal density for various optic nerve regions, although none of the intereye regional differences were statistically significant (F = 0.1, P = .79). The effect of region was statistically significant (F = 11.3, P < .001), but the effect of eye was not statistically significant (F = 0.1, P = .79). The global axonal density difference between these 2 healthy eyes was less than 1% and not significantly different (OD, 233,118 axons/mm²; and OS, 221,470 axons/mm²; P = .78, paired t test).

Using an established model of optic nerve ischemia that is based on ET-1 administration to the retrobulbar space in the primate eye, we demonstrate that chronic ischemia results in significant damage of the optic nerve without elevation of IOP. Eyes in which ischemia was induced for 6 to 12 months showed significant decrease in axonal density. While the axonal loss was significant overall for the population, the damage represents early loss with an average of approximately 5%. However, some individuals have global loss exceeding 15% to 20%, with even greater amounts of regional loss. This ischemia-induced axonal loss was commonly focal in nature, but the location of loss within the optic nerve varied among individual animals. The axonal loss was often seen in clusters of contiguous subregions of the optic nerve of an individual animal. Clinical findings typical for anterior ischemic optic neuropathy, such as generalized or focal optic nerve pallor, optic nerve and retinal edema, and telangiectatic vessels, were not seen. Although, on average, the retrobulbar optic nerve area was increased in the ischemic eyes owing to axonal enlargement and extracel-

<table>
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Table 2. Effect of Endothelin-1 (ET)–Induced Chronic Ischemia on Axonal Density by Optic Nerve Region for Each Animal (ANOVA Table)

Abbreviations: ANOVA, analysis of variance; NA, not applicable.
ular space enlargement from axonal loss, there was no clinical evidence of anterior optic nerve swelling. This finding may imply a difference between severe acute ischemia associated with anterior ischemic optic neuropathy and milder, chronic ischemia associated with the hemodynamic alterations produced in our model.

Variability in the response to ischemia was seen both between animals and among regions of the optic nerve. These findings suggest that optic nerves differ in their susceptibility to ischemia and that individual optic nerves have varied regional susceptibility. Regional axonal loss and structural damage within the optic nerve is found in various forms of optic neuropathy, including glaucoma. The findings of the present study pose an important question: Why are certain optic nerves and certain optic nerve regions of an individual optic nerve more susceptible to damage from noxious stimuli?

Presumably, disparate insults such as elevated IOP and chronic ischemia may both result in regional or focal axonal injury. However, before considering potential hypotheses for the regional loss of axons in this model, one should consider if the model itself is responsible for a particular pattern of loss. The delivery tubes for the ET-1 within the retrobulbar space were located to the nasal or superonasal side of the optic nerve in all animals. Although there is no way to know if the decrease in blood flow to the optic nerve was uniform, the highly varied susceptibility to damage is somewhat surprising. If the location of the delivery of the ET-1 is an important factor in the pattern of axonal loss, one would anticipate a greater loss of axons in the superonasal region owing to the relatively greater exposure. However, this pattern was not uniformly seen. Instead, Figures 3 and 5 demonstrate that regional susceptibility was varied among individuals. Varying the placement of the delivery tube in future experiments will provide additional information regarding this matter.

Preferential superior and inferior regional damage of the optic nerve in human glaucoma is seen clinically as the development of localized neuroretinal rim notches, optic disc hemorrhages, and visual function defects. Jonas et al and Mok et al showed glaucomatous optic nerve loss occurs in all neuroretinal rim sectors of the optic nerve, but in early glaucoma the most pronounced loss is in the inferotemporal rim. Preferential regional damage in glaucoma has traditionally been attributed to differential mechanical forces and explained by anatomical features at the level of the anterior lamina cribrosa. That is, in the superior and inferior regions, there is less connective tissue around lamina cribrosa pores and the pores are large compared with the nasal and temporal regions. In addition, lamina cribrosa pores in the peripheral region are longer than the central pores. Recent biomechanical modeling of posterior segment of the eye and optic nerve suggests disproportionate stress and strain within the optic nerve may contribute to regional loss of axons. These anatomical features may cause the axons passing through the lamina cribrosa within these regions to be more vulnerable to mechanical stress, such as an increase in IOP.

Realization that both mechanical insults preferentially damage certain regions of the optic nerve and that a chronic ischemic insult produces regional damage, forces a reexamination of the topic. Several hypothetical explanations based on regional differences in the anatomical, metabolic, and/or vascular profiles of the anterior optic nerve can be considered. First, regional anatomical differences that exist within the lamina cribrosa, such as differences in the constitutive glial cell populations and in the retinal ganglion cell types, may account for varied susceptibility. Second, the metabolic demand may vary between different regions of the optic nerve. Under normal physiological conditions the metabolic demands of the certain regions of the optic nerve are accommodated for, but when perturbed, the system is overwhelmed and high metabolic regions would be more vulnerable to insults, such as perfusion deficits or localized mechanical stress. Third, differences in the vascular anatomy, such as the number and caliber of supply arteries or the distribution of capillaries within the optic nerve, may play a role in regional variations. Perhaps the total number of capillaries subserving a set number of RGC axons in various regions of the optic nerve is borderline but becomes inadequate and exhibits differential susceptibility when stressed owing to differential regional autoregulatory capabilities. Regional functional inadequacy may not be accompanied by a structural decrease in the vasculature, as demonstrated previously. Instead of a measurable decrease in the number of capillaries, loss of autoregulatory capacity may be the underlying deficiency.

The existence of regional watershed zones has been suggested to be important in the development of regional damage in the human glaucomatous optic nerve. Approximately 60% of these watershed zones pass through the temporal half of the optic nerve in eyes with glaucoma. Because the area within a watershed zone is comparatively less perfused, especially during hemodynamic stress, these regions are more vulnerable to ischemic damage. Given the similar optic nerve structure and vasculature of nonhuman primates to the human eye, the varied severity and regional location of axonal damage seen in this experiment may be owing to the result of uneven perfusion of the optic nerve caused by watershed zones. This hypothesis might also explain the variability in the response between different animals. In the future, the addition of serial in vivo angiography before and after the onset of endothelin treatment would allow testing the hypotheses in this primate model.

Fourth, differential regional response or sensitivity to the vasoconstrictive effects of ET-1 treatment could also explain the findings of this study. This model assumes that the axonal damage is the direct result of ischemia, which was induced by ET-1. Endothelins are a family of short-chain polypeptides that have been identified and described over the last 2 decades. As the most potent vasoconstrictor known, ET-1 was selected in this model to create significant vasoconstriction over a prolonged period. This peptide affects the vasculature through 2 major receptors, ET A and B receptors. Recent studies have indicated that the ET B receptor, which is largely located on glial cells in the central nervous system, can also participate in the pathological mechanism of neuronal damage. This concept may be particularly interesting because patients with primary open-angle glau-
We have demonstrated a significant loss of RGC axons in the primate optic nerve following prolonged, chronic ischemia. This ischemia-induced, axonal loss was commonly focal in nature, but variable in location within the optic nerve of individual animals. As well, there was a significant variability in the response of different animals to the ischemic insult. Preferential regional loss of optic axons may result from regional differences in the anatomy, metabolism, or vasculature of the optic nerve. These observations warrant further investigation leading to a more complete understanding of the mechanisms of ischemic neuronal damage and their potential role in the development of glaucomatous optic neuropathy.

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