Protection of Retinal Ganglion Cells and the Optic Nerve During Short-term Hyperglycemia in Experimental Glaucoma

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**Objective:** To evaluate the neuroprotective effect of short-term hyperglycemia on the retinal ganglion cell body and axon in a rat model of experimental glaucoma.

**Methods:** Using a well-described limbal laser technique, unilateral ocular hypertension was induced in 2 groups (26 per group) of Sprague-Dawley rats. One group remained normoglycemic; the other was rendered hyperglycemic by means of an intraperitoneal injection of streptozocin. After 2 weeks of elevated intraocular pressure, axonal and retinal damage profiles were compared using several histological techniques. Immunohistochemical changes in the retina and optic nerve were also assessed.

**Results:** We found convincing evidence of delayed axonal degeneration and retinal ganglion cell death in hyperglycemic rats. Axon loss was reduced by about 50% 2 weeks after induction of ocular hypertension. Survival of retinal ganglion cell perikarya increased to a similar extent in hyperglycemic rats.

**Conclusions:** The optic nerve and retinal ganglion cells are partially protected by short-term hyperglycemia in this rat model of experimental glaucoma. Energy substrate availability may therefore play a role in glaucomatous optic neuropathy.

**Clinical Relevance:** Our findings, to some extent, support the claims of the Ocular Hypertension Treatment Study, in which diabetes appeared to protect against the conversion to glaucoma. Targeted manipulation of neuronal energy metabolism may delay optic nerve degeneration and may represent a novel neuroprotective strategy for neurodegenerative diseases of the visual system such as glaucoma.

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**GLAUCOMA REFER TO A** family of ocular conditions with multifactorial causes united by a clinically characteristic optic neuropathy. The pathogenesis of glaucoma remains unclear, but there is considerable evidence that inadequate blood supply to the optic nerve (ON) head may be involved, in at least some types of glaucoma. Given that vasculopathy is a hallmark of diabetes and that compromised retinal and ON blood flow is a feature of glaucoma, it seems reasonable to suggest that diabetes might exacerbate glaucoma; however, despite decades of searching, there remains, at best, conflicting evidence regarding a clinical or an epidemiological association between these 2 diseases. In large-scale clinical trials, no association was found in the Framingham, Barbados, or Baltimore Eye studies. In the Beaver Dam Study, glaucoma was weakly associated with older-onset diabetes only. A weak association was also reported in the Los Angeles Latino Eye Study, whereas the strongest reported association was documented in the Blue Mountains Eye Study. This lack of convincing epidemiological association seems odd, particularly when considering the fact that studies using intraocular pressure (IOP) in their definition of glaucoma were biased toward finding an association based on corneal biomechanical changes in persons with diabetes. There is increasing evidence that hyperglycemia causes cross-linking and is associated with increased central corneal thickness, which results in augmented stiffness of the cornea. This means that IOP measurements taken by methods that depend on these corneal variables, such as Goldmann applanation tonometry, overestimate the actual IOP; hence, there is an inherent measurement bias. Data from the Ocular Hypertension Treatment Study are even more curious. In that study, the presence of diabetes protected against the conversion of ocular hypertension (OHT) to glaucoma. In fact, this...
finding was one of the strongest associations reported, and it has subsequently been incorporated into risk calculators for clinical use.15 It is possible that the finding is spurious; however, Quigley16 has recently outlined reasons why diabetes may be protective.

Glucose is an essential energy substrate for central nervous system tissues, including the retina and ON, but it has become dogma within clinical medicine that elevated serum glucose levels (hyperglycemia) are deleterious to neurons, including those in the retina, under conditions of energy deprivation.17-19 Contrary to this viewpoint, we hypothesized that providing glucose to retinal neurons in vivo during acute20 and sustained21 periods of ischemia affords a robust neuroprotective effect, arguably one of the strongest and most durable demonstrations of retinal preservation. Recent studies further showed that hypoglycemia exacerbated ischemic retinal injury,22 that retinal neuronal cells in vitro preferentially metabolized glucose compared with lactate,23 that intracellular lactate delivery was not protective,24 and that inhibition of lactate transport did not exacerbate ischemic retinal injury in vivo.25 Together, these findings indicate that glucose and not lactate is responsible for the protective effect.

To date, little research has investigated the effect of diabetes in models of experimental glaucoma. Herein, we provide evidence that retinal ganglion cell (RGC) axons and their perikarya are partially protected by hyperglycemia.

**METHODS**

**OUTCOMES**

Primary outcomes of the study were RGC loss and axonal injury. Complementary outcomes included measurement of microglial and macroglial activation, RGC stress, and neurofilament breakdown.

**STUDY PLAN**

In preliminary experiments,25 we demonstrated that 2 weeks of elevated IOP caused measurable loss of RGCs and their axons, together with activation of retinal and ON glia. Furthermore, we found that 2 weeks of hyperglycemia in normotensive rats caused no significant loss of RGCs (data not shown). Hence, the 2-week point was chosen for the present study. This time frame is short enough for diabetes per se not to cause any confounding RGC death26 but long enough to allow measurable retinal and axonal damage resulting from chronic OHT.

Rats were randomly divided into 2 groups (26 rats per group). One group retained physiologic serum glucose levels (normoglycemic [NG-OHT]) and the other was rendered hyperglycemic (HG-OHT). Ocular hypertension was then induced in the right eye of each animal by laser photocoagulation of the trabecular meshwork using a slightly modified protocol25 of the method described by Levkovitch-Verbin et al.27 After brief anesthesia with isoflurane, IOPs were measured bilaterally at baseline and on days 1, 3, 5, 7, 8, 10, and 14 using a rebound tonometer (TonoLab; Icare Finland, Espoo, Finland). To characterize IOP profiles for each rat peak IOP, IOP exposure (positive IOP integral) and the IOP integral were calculated using the following formulas:

Positive IOP Integral = \[ \sum_{i=1}^{n} (t_i - t_{i-1}) \times \frac{1}{2} \times (IOP_R - IOP_L)_{t_i} \]

IOP Integral = \[ \sum_{i=1}^{n} (t_i - t_{i-1}) \times \frac{1}{2} \times (IOP_R + IOP_L)_{t_i} \]

where \( t_i \) indicates the time point \( i \) (in days), IOP_R, IOP in the right eye; and IOP_L, IOP in the left eye.

Serum glucose levels were measured in all rats at day 14 before the animals were humanely killed by means of cardiac perfusion with physiologic saline solution under terminal anesthesia.

**TISSUE PROCESSING AND HISTOLOGY**

Each eye with the ON and chiasm attached was carefully dissected. A short piece of proximal ON, 1.5 mm behind the globe, was removed for resin processing. The globe, middle ON, and chiasm were fixed in 10% buffered formalin for 24 hours and processed for routine paraffin-embedded sections. Globes were embedded sagittally, whereas ON and chiasmata were embedded longitudinally. In all cases, 5-µm serial sections were cut. Toluidine blue staining of resin sections of the proximal ON was performed as previously reported.

**IMMUNOHISTOCHEMISTRY**

Immunohistochemistry was performed as previously described.25 In brief, tissue sections were deparaffinized and treated with 0.3% hydrogen peroxide to block endogenous peroxidase activity. Antigen retrieval was achieved by microwaving the sections in 10mM citrate buffer (pH, 6.0) for 10 minutes at 95°C to 100°C. Tissue sections were then blocked in phosphate-buffered saline containing 3% normal horse serum, incubated overnight at room temperature in primary antibody (Table 1), followed by consecutive incubations with biotinylated secondary antibody and streptavidin-peroxidase conjugate. Color development was achieved with 3',3'-diaminobenzidine. Sections were typically counterstained with hematoxylin, then dehydrated and mounted. Specificity of antibody labeling was confirmed by incubating adjacent sections with mouse IgG1 isotype control (50878; BD Pharmingen, North Ryde, Australia) or normal rabbit serum.

**EVALUATION OF IMMUNOHISTOCHEMISTRY**

Immunolabeling for each antigen was performed in a single batch, and all analyses were conducted in a masked fashion. Left retinas and left ONs served as control specimens.
Sections were evaluated by 3 independent observers (including A.E. and G.C.). To minimize sampling errors, all analyses were performed on sections taken at the level of the ON head. Retinal ganglion cells immunostained for NeuN, Brn-3, and heat shock protein 27 (HSP27) were counted across the entire retina. Two sections per animal were analyzed for NeuN and the counts were averaged. Loss of RGCs was calculated by subtracting the NeuN count of the glaucomatous right eye from the normotensive left eye. Immunoreactivities for macroglia (labeled with glial fibrillary acidic protein) and microglia (labeled with Iba1 and ED1) were scored using a 4-point grading system, as described previously,21 after appraisal of the entire retina.

Optic Nerve

For each antigen, immunohistochemistry was performed using 1 section of the middle ON and 1 section of the distal ON (adjacent to the optic decussation). One photomicrograph from each location was taken using the 20 × microscope objective. Evaluations were performed using a software package platform (ImageJ 1.42q; http://rsb.info.nih.gov/ij/). For counterstained sections, color deconvolution was applied to extract the 3’-,3’-diaminobenzidine staining. After thresholding, the area of staining was measured.

EVALUATION OF SEMITHIN TOLUIDINE BLUE STAINED ON CROSS SECTIONS

Axon numbers were estimated using a semiautomated, quantitative method with targeted sampling, similar to that used by others20 (eMethods section and eFigure; http://www.archophthalmol.com).

TABLE 1. Antibodies Used for Immunohistochemistry

<table>
<thead>
<tr>
<th>Target</th>
<th>Source</th>
<th>Clone or Catalog No.</th>
<th>Host</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>αB-crystallin</td>
<td>Novocastra Laboratories Ltd, Newcastle upon Tyne, England</td>
<td>NCL-ABCrys-512</td>
<td>Mouse</td>
<td>1:1000</td>
</tr>
<tr>
<td>Brn-3</td>
<td>Santa Cruz Biotechnology Inc, Santa Cruz, California</td>
<td>Sc-6026</td>
<td>Goat</td>
<td>1:500</td>
</tr>
<tr>
<td>ED1</td>
<td>AbD Serotec, Oxford, England</td>
<td>MCA431</td>
<td>Mouse</td>
<td>1:500</td>
</tr>
<tr>
<td>GFAP</td>
<td>Dako, Glostrup, Denmark</td>
<td>Z0334</td>
<td>Rabbit</td>
<td>1:20 000</td>
</tr>
<tr>
<td>HSP27</td>
<td>Stressgen, Ann Arbor, Michigan</td>
<td>SPA-801</td>
<td>Rabbit</td>
<td>1:2500</td>
</tr>
<tr>
<td>Iba1</td>
<td>Wako Pure Chemical Industries Ltd, Osaka, Japan</td>
<td>019-19741</td>
<td>Rabbit</td>
<td>1:50 000</td>
</tr>
<tr>
<td>Nestin</td>
<td>Millipore, Temecula, California</td>
<td>MAB353</td>
<td>Mouse</td>
<td>1:1000</td>
</tr>
<tr>
<td>NeuN</td>
<td>Chemicon, Temecula, California</td>
<td>MAB377</td>
<td>Mouse</td>
<td>1:1000</td>
</tr>
<tr>
<td>Smi32</td>
<td>Sternberger, Princeton, New Jersey</td>
<td>SMI-32R</td>
<td>Mouse</td>
<td>1:20 000</td>
</tr>
</tbody>
</table>

TABLE 2. IOP Exposure and Serum Glucose Levels in NG-OHT and HG-OHT Rats

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Peak IOP, mm Hg</th>
<th>IOP Exposure, mm Hg–Days</th>
<th>IOP Integral, mm Hg–Days</th>
<th>Day 14 Serum Glucose Level, mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG-OHT (n=26)</td>
<td>41.9 (9.3)</td>
<td>179.5 (103.5)</td>
<td>348.7 (104.7)</td>
<td>123 (23)</td>
</tr>
<tr>
<td>HG-OHT (n=26)</td>
<td>38.7 (7.7)</td>
<td>183.1 (91.6)</td>
<td>318.7 (95.0)</td>
<td>497 (92)</td>
</tr>
<tr>
<td>P valueb</td>
<td>.18</td>
<td>.90</td>
<td>.29</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

| Abbreviations: HG-OHT, hyperglycemic group; IOP, intraocular pressure; NG-OHT, normoglycemic group. |
| SI conversion factor: To convert glucose to millimoles per liter, multiply by 0.0555. |
| a Calculated as the comparison between the NG-OHT and the HG-OHT experimental groups (unpaired, 2-tailed t test). |

Retina

Sections were evaluated by 3 independent observers (including A.E. and G.C.). To minimize sampling errors, all analyses were performed on sections taken at the level of the ON head. Retinal ganglion cells immunostained for NeuN, Brn-3, and heat shock protein 27 (HSP27) were counted across the entire retina. Two sections per animal were analyzed for NeuN and the counts were averaged. Loss of RGCs was calculated by subtracting the NeuN count of the glaucomatous right eye from the normotensive left eye. Immunoreactivities for macroglia (labeled with glial fibrillary acidic protein) and microglia (labeled with Iba1 and ED1) were scored using a 4-point grading system, as described previously,21 after appraisal of the entire retina.

STATISTICAL ANALYSIS

Our hypothesis was that short-term hyperglycemia would protect RGC axons and their somata. This was a unified hypothesis, and no correction for multiple hypothesis testing was made. Other analyses of the ON and retina were considered as complementary outcomes. An unpaired t test was used if parametric assumptions were met; a Mann-Whitney test was used otherwise. Regarding the complementary outcomes, we were not interested in the notion of finding at least 1 statistically significant result; hence, adjustments for multiple statistical tests would have unnecessarily inflated the type II error rate and were not performed. No post hoc comparisons were made, and P values were interpreted in the context of the experiment, relevant biological factors, and scientific knowledge. Statistical analyses were performed using commercially available software (GraphPad Prism 5.0b; GraphPad Software Inc, La Jolla, California).

RESULTS

Streptozocin caused a sustained elevation of the serum glucose level. Peak pressure and IOP exposure were comparable in both experimental groups (Table 2) and were similar to values reported by other groups using the same model.20

OPTIC NERVE

Estimated axon counts of ON cross sections, the primary outcome, revealed protection of the ON by hyperglycemia (Figure 1). In NG-OHT and HG-OHT rats, the mean (SEM) counts were 66 500 (4000) and 77 600 (2800), re-
relate well with axonal injury during experimental chemically using antibodies against Iba1 and ED1, correlation normalities. Compared with control ONs, increased heavy, is a sensitive marker of axonal neurofilament abnormalities were evident in the treated ONs of HG-OHT rats, although the difference did not reach statistical significance (Table 3; $P = .04$ and $P = .02$, respectively, for Iba1), a result that is consistent with the control ONs (D and lower part of A and B). Likewise, increased expression of HSP27 (C) and $\alpha$B-crystallin (G-I, not counterstained) by astrocytes and oligodendrocytes is apparent in the treated optic nerves (H, I, and upper part of C). Controls consist of contralateral optic nerves. The immunostains used are identified in each image. Scale bars indicate 100 $\mu$m. HG-OHT indicates hyperglycemic group; NG-OHT, normoglycemic group.

Figure 2. Quantification of axonal damage on longitudinal optic nerve sections. Representative images of optic nerves 14 days after induction of ocular hypertension (OHT) show immunohistochemical markers indicative of axonal cytoskeletal damage, microglial activation, and expression of heat shock proteins (HSPs) by astroglia and oligodendrocytes. Sections were obtained at the level of the optic decussation (A-C) and from the middle part of the optic nerve (D-I). Axonal cytoskeletal abnormalities (A) with increased microglial activity and density (B and D-F) are evident in the treated optic nerves (E, F, and upper part of A and B) compared with the control nerves (D and lower part of A and B). Likewise, increased expression of HSP27 (C) and $\alpha$B-crystallin (G-I, not counterstained) by astrocytes and oligodendrocytes is apparent in the treated optic nerves (H, I, and upper part of C). Controls consist of contralateral optic nerves. The immunostains used are identified in each image. Scale bars indicate 100 $\mu$m. HG-OHT indicates hyperglycemic group; NG-OHT, normoglycemic group.

Quantitative analyses of distal and middle ON sections immunolabeled for markers of axonal cytoskeletal damage, microglial activation, and astroglial and oligodendroglial stress also indicated white matter protection by hyperglycemia. Representative immunohistochemical images are shown in Figure 2, whereas the results are documented in Table 3. In detail, Smi32, an antibody that labels nonphosphorylated neurofilament heavy, is a sensitive marker of axonal neurofilament abnormalities.

Compared with control ONs, increased Smi32 abnormalities were evident in the treated ONs of NG-OHT rats. The Smi32 abnormalities tended to be less prevalent in the HG-OHT rats, although the difference did not reach statistical significance (Table 3; $P = .39$ and $P = .40$ for distal and middle ONs, respectively).

We have previously demonstrated that microglial number and phagocytic activity, as detected immunohistochemically using antibodies against Iba1 and ED1, correlate well with axonal injury during experimental glaucoma. In the present study, both markers were significantly less abundant in the distal and middle ONs of HG-OHT rats compared with NG-OHT rats (Table 3; $P = .04$ and $P = .02$, respectively, for ED1; $P = .007$ and $P = .01$, respectively, for Iba1), a result that is consistent with greater axonal preservation in the HG-OHT group.

To assess the macroglial stress response, we quantified immunolabeling for 2 members of the small HSP family, HSP27 and $\alpha$B-crystallin, which are differentially expressed in astrocytes and oligodendrocytes, respectively, after axonal injury. Marked upregulation of both small HSPs were detected in the treated ONs of NG-OHT rats. These changes were significantly attenuated in the distal and middle ON of HG-OHT animals (Table 3; $P = .01$ and $P < .001$, respectively, for HSP27; $P < .001$ and $P = .02$, respectively, for $\alpha$B-crystallin).

The combined results demonstrated decreased axonal loss, attenuated microglial proliferation and phagocytic activity, and a less elevated macroglial stress response in the glaucomatous ONs of HG-OHT rats.

RETINA

The primary retinal outcome of the study was survival of RGCs. This was assessed by counting NeuN-positive cells in the ganglion cell layer (Table 4 and eTable). NeuN has been proved to be a reliable marker for quantification of RGC survival in models of RGC degeneration. In the treated retinas of NG-OHT rats, the mean (SEM) loss was 64.3 (9.9) vs 31.1 (9.3) RGCs in HG-OHT rats ($P = .02$).

In contrast to the primary outcome, the secondary retinal outcomes did not point toward greater protection in the HG-OHT group. Representative immunohistochemical images are shown in Figure 3, and the results are provided in Table 4. In detail, grading of sections immunolabeled with markers of microglial and macroglial activation revealed similar magnitude glial injury responses ($P = .54$ and $P = .46$, respectively). Similarly, quantification of the number of HSP27-positive RGCs in the retinas of NG-OHT and HG-OHT rats demonstrated a small number of RGCs in all laser-treated eyes but no difference between the 2 experimental groups ($P = .75$). Heat shock protein

Figure 1. Quantification of axonal damage on optic nerve cross sections. Representative images of control optic nerves (A and D) and representative examples for the normoglycemic (NG-OHT; B and E) and hyperglycemic (HG-OHT; C and F) experimental groups at different magnifications (A-C, original magnification ×10; D-F, original magnification ×40) are shown. Scale bars indicate 100 $\mu$m (A-C) and 25 $\mu$m (D-F). The graph (G) illustrates the mean (SEM) loss of axons based on the estimated axon counts (Mann-Whitney test).
27 is not constitutively expressed by RGCs; however, within an ongoing pathological setting, such as experimental glaucoma35 or chronic ischemia,21 it is persistently upregulated in some RGCs, indicating cellular stress.

COMMENT

Hyperglycemia exacerbates ischemic brain injury.17 The situation in the retina, however, which exhibits unique features of glucose metabolism, is different. Previous studies have shown that providing glucose to retinal neurons in vivo during acute20 and prolonged21 periods of ischemia affords a robust neuroprotective effect. Epidemiological data investigating the association between diabetes mellitus and glaucoma is controversial and published results are inconsistent. The aim of this study was to evaluate the short-term effect of hyperglycemia on axonal damage and RGC survival in a rat model of experimental glaucoma, dissecting the potentially beneficial effect of additional energy supply (ie, elevated serum glucose levels) from the noxious chronic changes that occur in diabetic retinopathy.

As for our primary outcome, we found convincing evidence that hyperglycemia attenuated axonal and somal RGC degeneration after experimentally induced OHT. The secondary outcomes of the study, which provided information on the status of the axonal cytoskeleton and the response of microglial and macroglial cells in the ON and retina, yielded mixed results.

OPTIC NERVE

Immunolabeling with Smi32 highlights any axon fibers in the process of degeneration and provides information about ongoing, rather than cumulative, injury. Although there was a trend for decreased Smi32 labeling in the HG-OHT rats, statistical significance was not attained. The short period during which axons show abnormalities in Smi32 immunolabeling might explain this result. Alternatively, it may simply be the case that axonal degeneration was proceeding at a similar rate in NG-OHT and HG-OHT animals by 14 days after elevation of IOP. In contrast to Smi32, analysis of microglial markers in the glaucomatous ONs supported the finding of increased axonal survival in HG-

Table 3. Effect of Hyperglycemia on Chronic OHT–Induced Neurofilament Degeneration and Glial Activation in Distal and Middle ON

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Distal ON</th>
<th>Mean (SEM) a</th>
<th>Microglial Activation</th>
<th>Macrogial Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Neurofilament Degeneration, Smi32</td>
<td>Microglial Response, ED1</td>
<td>Iba1</td>
</tr>
<tr>
<td>Controls (n=51)</td>
<td>Absent</td>
<td>35.2 (2.4)</td>
<td>285.0 (36.1)</td>
<td>285.6 (21.3)</td>
</tr>
<tr>
<td>HG-OHT (n=26)</td>
<td>10.6 (3.3)</td>
<td>3.1 (1.1)</td>
<td>54.9 (4.6)</td>
<td>388.1 (43.9)</td>
</tr>
<tr>
<td>P value b</td>
<td>.39 c</td>
<td>.04 c</td>
<td>.007 c</td>
<td>.001 c</td>
</tr>
<tr>
<td>Middle ON</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HG-OHT (n=23)</td>
<td>16.0 (5.9)</td>
<td>5.4 (2.0)</td>
<td>76.4 (7.2)</td>
<td>682.7 (66.6)</td>
</tr>
<tr>
<td>HG-OHT (n=25)</td>
<td>9.9 (4.5)</td>
<td>1.1 (0.3)</td>
<td>53.9 (5.0)</td>
<td>458.3 (44.3)</td>
</tr>
<tr>
<td>P value b</td>
<td>.40 c</td>
<td>.02 c</td>
<td>.01 c</td>
<td>.02 c</td>
</tr>
</tbody>
</table>

Abbreviations: HG-OHT, hyperglycemic group; HSP27, heat shock protein 27; NG-OHT, normoglycemic group; OHT, ocular hypertension; ON, optic nerve.

aNumbers given represent the area of immunostaining expressed in pixels (× 103). The total area of a microphotograph is 1920 × 103 pixels. Controls are contralateral ONs.

bCalculated as a comparison between the NG-OHT and HG-OHT ONs.
cCalculated using the Mann-Whitney test.
dCalculated using the unpaired t test.

Table 4. Effect of Hyperglycemia on Chronic OHT-Induced RGC Loss and Glial Activation in the Retina

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Control (n=44)</th>
<th>Mean (SEM) a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute Loss, NeuN</td>
<td>RGC Stress, HSP27</td>
</tr>
<tr>
<td>Controls (n=44)</td>
<td>Nil</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>NG-OHT (n=24)</td>
<td>64.3 (10.9)</td>
<td>4.3 (1.5)</td>
</tr>
<tr>
<td>HG-OHT (n=20)</td>
<td>31.1 (9.3)</td>
<td>3.2 (1.2)</td>
</tr>
<tr>
<td>P value b</td>
<td>.02 c</td>
<td>.75</td>
</tr>
</tbody>
</table>

Abbreviations: HG-OHT, hyperglycemic group; NG-OHT, normoglycemic group; OHT, ocular hypertension; RGC, retinal ganglion cells.

aThe NeuN-measured loss and heat shock protein 27-positive RGCs were counted along the entire retina at the level of the optic nerve head. Glial cell–marker immunostaining was scored on a 4-point grading system ranging from 0 to 3. The mean (SEM) NeuN count for control eyes was 346 (6) neurons. Loss of RGCs was calculated by subtracting the NeuN count of the glaucomatous right eye from that of the normotensive left eye.

bCalculated as the comparison between NG-OHT vs HG-OHT groups using the Mann-Whitney test. All treatment groups are significantly different from the controls (P<.01).
They are also markedly upregulated in many neurode-
tosis inhibitors, antioxidants, and cytoskeleton stabilizers.
multiple functions acting as molecular chaperones, apop-
tosis. Heat shock protein 27 (HSP27), is absent from healthy RGCs (C), but induction of HSP27 expression occurs in a small proportion of the RGC population (arrows) during ocular hypertension (D); the arrowhead indicates an astrocyte, whereas the short arrow points to a putative RGC that has been cut peripherally. Iba1 labels quiescent and activated microglia. In control retinas (E), Iba1-positive microglia are mainly located in the nerve fiber and inner plexiform layers, are few in number, and display slim cell bodies and ramified processes. After 14 days of experimental glaucoma (F), Iba1-positive microglia are more numerous and often exhibit an amoeboid morphology. GFAP labels astrocytes and Müller cell end-feet in control retinas (G). After 2 weeks of OHT (H), a striking upregulation of GFAP is observable within astrocytes and Müller cells.

OHT animals. We have previously demonstrated that microglial number and phagocytic activity, as detected by Iba1 and ED1, correlate well with axonal injury during experimental glaucoma. Both Iba1 and ED1 were less abundant in HG-OHT nerves, denoting reduced axonal damage and myelin breakdown. Heat shock protein 27 and αB-crystallin belong to the family of small HSPs, which have multiple functions acting as molecular chaperones, apoptosis inhibitors, antioxidants, and cytoskeleton stabilizers. They are also markedly upregulated in many neurodegenerative diseases. That HSP27 and αB-crystallin are differentially expressed in astrocytes and oligodendro-
cytes during ON degeneration has been previously dem-
strated. The more limited induction of these proteins in HG-OHT rats points toward a reduced pathological pro-
file in these ONs compared with those of NG-OHT rats.

## RETINA

A recent study demonstrated that hyperglycemia is neu-
roprotective against chronic retinal ischemia caused by permanent ligation of the common carotid arteries. In ad-
tion to enhancing RGC survival, retinas from hyper-
glycemic rats had fewer RGCs that were metabolically stressed (ie, HSP27-positive), a reduced microglial re-

Figure 3. Quantification of retinal ganglion cell (RGC) loss and glial activation in the retina. Representative images of control (A, C, E, and G) and treated (B, D, F, and H) retinas 14 days after induction of ocular hypertension (OHT) show immunohistochemical markers for RGCs, microglia, and macroglia. NeuN labels RGCs and amacrine cells. Fewer NeuN-positive cells were evident in the ganglion cell layer in the retinas of glaucomatous eyes (B) compared with controls (A). Heat shock protein 27 (HSP27) is absent from healthy RGCs (C), but induction of HSP27 expression occurs in a small proportion of the RGC population (arrows) during ocular hypertension (D); the arrowhead indicates an astrocyte, whereas the short arrow points to a putative RGC that has been cut peripherally. Iba1 labels quiescent and activated microglia. In control retinas (E), Iba1-positive microglia are mainly located in the nerve fiber and inner plexiform layers, are few in number, and display slim cell bodies and ramified processes. After 14 days of experimental glaucoma (F), Iba1-positive microglia are more numerous and often exhibit an amoeboid morphology. GFAP labels astrocytes and Müller cell end-feet in control retinas (G). After 2 weeks of OHT (H), a striking upregulation of GFAP is observable within astrocytes and Müller cells. Controls (A, C, E, and G) are retinas of the normotensive contralateral eyes. NeuN sections (A and B) are not counterstained. Scale bars indicate 25 µm (C and D) and 50 µm (A, B, and E-H). HG-OHT indicates hyperglycemic group; NG-OHT, normoglycemic group.
CONCLUSIONS

We found a neuroprotective effect of hyperglycemia on the ON and RGCs in this model of experimental glaucoma. In light of this experiment, the results from the Ocular Hypertension Treatment Study,13,14 which suggested delayed glaucoma progression in patients with diabetes, seem plausible. Quigley16 has proposed several mechanisms by which underlying diabetes may protect the retina from glaucoma-related insults. These include the intracellular invasion of the trophic factors from leaky vasculature, localized episodes of hypoperfusion leading to localized ischemic preconditioning, or increased glycation of structural tissue elements such as collagen, culminating in increased connective tissue rigidity and prevention of pressure-related structural damage. Finally, we do not stipulate changes in clinical practice patterns in patients with glaucoma and diabetes mellitus. However, a bioenergetic approach to delay neurodegeneration is worth further pursuit.

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Additional Contributions: Mark Daymon provided expert technical assistance.

REFERENCES


**Ophthalmic Images**

Giant Conjunctival Mucosa-Associated Lymphoid Tissue Lymphoma

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Slitlamp examination of a 70-year-old man who had blurred vision and eyelid swelling for 2 years in both eyes (A [left eye] and B [right eye]). Giant salmon-pink conjunctival tumors obstructed the pupil and affected the patient’s vision. Histologically, the conjunctival tumors consisted of a monomorphic population of atypical B lymphocytes (C), positive for CD20 immunohistochemical stain (D) (original magnification ×1100). DNA analysis obtained from tumor tissue by Southern blot demonstrated immunoglobulin heavy-chain gene rearrangement in the JH region (E, arrows). kb Indicates kilobase; OD, right eye; and OS, left eye.