The Effect of Hyperglycemia on Experimental Retinal Ischemia

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Objective: To determine the effect of hyperglycemia and intraocular glucose delivery on ischemic retinal injury.

Methods: Experimental diabetes was induced in age- and sex-matched Wistar rats by an injection of streptozocin. The functional and structural retinal injury in these rats after a period of pressure-induced retinal ischemia was compared with the injury in appropriate controls and with rats made hyperglycemic by an injection of systemic glucose. The effect of high intraocular pressure-induced ischemia with the use of several different isotonic substrates in the elevated reservoir (isotonic sodium chloride solution, glucose, 2-deoxyglucose, and lactate) was also determined. Electoretinography, reverse transcriptase polymerase chain reaction, and histologic examination were used to assess the retinal injury.

Results: Streptozocin-induced diabetes, glucose injection–induced preexisting hyperglycemia, and intraocular glucose delivery during ischemia markedly reduced the functional and structural ischemic retinal injury. Neither posts ischemic hyperglycemia nor the intraocular delivery of lactate significantly affected the ischemic injury; however, the intraocular delivery of 2-deoxyglucose significantly exacerbated the retinal injury.

Conclusion: Preexisting hyperglycemia and the intraocular delivery of glucose markedly attenuate ischemic retinal injury.

Clinical Relevance: These findings highlight fundamental differences in energy metabolism between brain and retina, have important implications for the pathophysiology of diabetic retinopathy, and may lead to novel therapeutic strategies for ischemic retinopathies.


Preexisting hyperglycemia has a well-described deleterious effect on the clinical outcome of cerebral ischemia; however, information on the effect of hyperglycemia on retinal ischemia is lacking. The many functional and structural similarities between retina and brain and the causal association between long-term hyperglycemia and diabetic retinopathy suggest that hyperglycemia would also exacerbate ischemic retinal injury; however, several factors may undermine this assumption. Unlike the brain, the isolated mammalian retina derives a considerable amount of adenosine triphosphate (ATP) from the conversion of glucose to lactate, even in the presence of oxygen (aerobic glycolysis); and, importantly, it has the remarkable ability to maintain most of its ATP requirement in the absence of oxygen by anaerobic glycolysis. Furthermore, an intraocular delivery of glucose before or during retinal ischemia has been reported to attenuate ischemic histologic changes, and experimental diabetes mitigates hypoxia-induced electoretinographic (ERG) changes. We therefore hypothesized that if preexisting hyperglycemia increased the availability of glucose to the ischemic retina, then it may in fact attenuate ischemic retinal injury, rather than exacerbate it. Herein, we present evidence in support of this hypothesis and discuss the implications of the findings.

METHODS

TREATMENT OF ANIMALS

Procedures used in this study conformed to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Home Office in England. Male Wistar rats (250-300 g) housed in a 12-hour light-dark cycle were used for all experiments; food and water were provided ad libitum. Anesthesia was achieved with a combination of intramuscular fentanyl citrate (0.315 mg/mL) and...
fluanisone (10 mg/mL) (Hypnorm; Janssen Pharmaceutica, Beerse, Belgium), 0.4 mL/kg, and diazepam, 0.4 mL/kg.

TREATMENT GROUPS

Baseline ERG recordings were taken 1 to 4 days before further intervention, as previously described. Diabetes was induced by a single intraperitoneal injection of streptozocin (60 mg/kg), and control rats received a single injection of the saline buffer solvent. Four days later, the blood glucose level was measured from tail vein blood by means of a blood glucose sensor (Abbott Laboratories, Medisense Products, Abbott Park, Ill). Diabetes was defined by a blood glucose level greater than 250 mg/dL (13.8 mmol/L) at this measurement and at second measurement just before ischemia. Diabetic rats were divided into 2 groups: group 1 underwent ischemia 5 days after streptozocin injection, and group 2 rats underwent ischemia 6 weeks later; streptozocin nonresponders were not used in further experiments. Rats received unilateral pressure-induced retinal ischemia for either 45 or 60 minutes, as previously described. The ERGs were recorded 3 and 7 days later, after which the retinas were removed for reverse transcriptase polymerase chain reaction (RT-PCR) analysis or histopathological examination.

Hyperglycemia was also induced by an intraperitoneal injection of glucose (2 g/kg) 20 minutes before ischemia, or at the end of the ischemic period. Control animals received isotonic sodium chloride solution (saline) (1 g/kg). The infusate used during the pressure-induced ischemia was routinely 0.9% saline, but for experimental purposes this was replaced with 5% glucose, a glycolysis inhibitor (3% 2-deoxyglucose), and 3.7% lactic acid.

ASSESSMENT OF RETINAL MESSENGER RNA LEVELS

Seven days after ischemia, retinal cyclophilin and Thy-1 messenger RNA (mRNA) levels were determined by means of RT-PCR as previously described. Briefly, total RNA was isolated from whole retinas, and first-strand complementary DNA synthesis was performed on 2 µg of DNase-treated RNA. The individual complementary DNA species were amplified in a 10-µL reaction, containing the 2-µL complementary DNA aliquot, PCR buffer (10mM Tris hydrochloride, pH 8.3, 30mM potassium chloride), 4mM magnesium chloride, 200µM of each deoxynucleotide triphosphate, 4 ng/µL of both the sense and antisense primers, and Taq polymerase (2.5 U). Reactions were initiated by incubating at 94°C for 10 minutes, and PCRs (94°C, 15 seconds; 52°C, 55°C, or 56°C, 30 seconds; 72°C, 30 seconds) were performed for a suitable number of cycles followed by a final extension at 72°C for 3 minutes. Interexperimental variations were avoided by performing all amplifications in a single run. The PCR products of the primer pairs were separated on 1.5% agarose gels with the use of ethidium bromide for visualization and yielded single bands corresponding to the expected molecular weights. The relative abundance of each PCR product was determined by analysis of digital gel photographs software (Labworks; Ultra-Violet Products Ltd, Cambridge, England). For semiquantitative analysis, the ratio of the Thy-1 densitometric readings between the ischemic and control eyes was calculated and was normalized to the internal standard mRNA ratio (cyclophilin), which was assumed to be unaffected by the ischemia.

HISTOPATHOLOGICAL EXAMINATION

Terminally anesthetized rats were transcardially perfused with 50 mL of 10mM phosphate-buffered saline, followed by 4% paraformaldehyde. The eyes were enucleated and immersion fixed in a single run. The PCR products of the primer pairs were separated on a single gel line) in the diabetic group (n=9) was 89%±5%, but in the diabetic rats also displayed remarkable preservation of structural integrity (Figure 3C) compared with Figure 3C). The group 2 rats had no significant change in the ERG over time (data not shown), but after a 60-minute

MEASUREMENT OF GLUCOSE IN THE VITREOUS AND RETINA

The vitreous and retinas were removed from freshly killed rats, placed on ice, and weighed (the vitreous from both eyes was pooled, as were the 2 retinas from each rat). After the retina samples were diluted with 1 mL of deionized water, the samples were sonicated, then centrifuged at 12000g for 10 minutes. The supernatant was removed and the vitreous samples were made up to 100 µL with distilled water. This volume was then added to a glucose hexokinase assay reagent (Sigma-Aldrich Corp, St Louis, Mo) for 15 minutes, and the spectroscopic absorbance was then read at 340 nm. The final glucose concentration (milligrams per milliliter) in the vitreous was determined by allowing for the dilution factor and comparing the absorbance with a previously constructed calibration curve. The weight of glucose per weight of retina was determined in a similar manner.

STATISTICAL ANALYSIS

To compare independent samples and normalize for slight day-to-day variation in the ERG, the ratio of the a- and b-wave amplitudes between paired eyes (one eye treated and the fellow untouched) was used as the unit of statistical analysis. Similarly, the paired-eye ratio of the RT-PCR densitometric readings was used as the unit of statistical analysis. A 1-way analysis of variance was used to compare means between 2 or more independent groups, and a Tukey honestly significantly difference test was used for post hoc comparisons. A Bonferroni correction was applied to the ERG measurements at 2 time points. All statistical determinations were performed with SPSS for Windows, version 10 (SPSS Inc, Chicago, Ill), and all data are expressed as mean±SEM; a P value of less than .05 was considered statistically significant.

RESULTS

In the first set of experiments, we found a remarkable degree of protection in the group 1 diabetic rats 3 and 7 days after a 45-minute period of ischemia: after 7 days, the mean b-wave amplitude (as a percentage of baseline) in the diabetic group (n=9) was 89%±5%, but in the control group (n=9) it was only 24%±4% (P<.001); similarly, the reduction in Thy-1 mRNA (as a percentage of cyclophilin mRNA) was only 5%±3% in the diabetic group (n=6) compared with 22%±4% in the control group (n=6; P=.02). When the duration of ischemia was increased to 60 minutes, the b-wave still exhibited better preservation in the diabetic group than the control group; the mean amplitudes are shown in Figure 1, and representative ERG tracings from an untreated, ischemic control and ischemic group 1 diabetic rat are shown in Figure 2A-C, respectively. The group 1 diabetic rats also displayed remarkable preservation of structural integrity (Figure 3C) compared with Figure 3C).
period of ischemia, they displayed marked preservation of the b-wave amplitude (Figure 1 and 2D). Similarly, the Thy-1 mRNA was remarkably preserved compared with the controls (Figure 4), as was the structural integrity (Figure 3F).

In the second group of experiments, an alternative method was used to induce hyperglycemia: injecting rats with glucose (2 g/kg) 20 minutes before ischemia. After 45 minutes of ischemia and 7 days of reperfusion, the mean b-wave amplitude (as a percentage of baseline) in the glucose-injected group was 95%±5%, but in the control group it was only 28%±4% (P<.001; Figure 5A). Similarly, the reduction in Thy-1 mRNA (as a percentage of baseline) was only 5%±4% in the hyperglycemic group (n=6) compared with 20%±3% in the control (P=.03; n=6). After 60 minutes of ischemia, the b-wave still exhibited better preservation in the hyperglycemic group than in the control group (Figure 5B). The hyperglycemic group also displayed marked preservation of structural integrity (Figure 3G compared with Figure 3C). However, when glucose (2 g/kg) was administered at the end of the period of ischemia, the mean b-wave amplitude (after 3 and 7 days of reperfusion) was not significantly different from the control mean amplitude (Figure 5A).

Although the blood glucose level in the glucose-injected rats, at the onset of ischemia, was significantly lower (274±25.2 mg/dL [15.2±1.4 mmol/L]) than the blood glucose level in the streptozocin-induced diabetic rats (420±46.8 mg/dL [23.3±2.6 mmol/L]; P<.001), there was no significant difference in the degree of protection.

The effect of using different energy substrates in the intraocular infusate during the pressure-induced ischemia was also investigated. After ischemia, the glucose-infused eyes had significantly greater b-wave amplitudes than saline-infused eyes (Figure 6A, and Figure 2F compared with Figure 2B), had significantly greater preservation of Thy-1 mRNA (Figure 6B), and showed better structural preservation than the saline-infused eyes (Figure 3H compared with Figure 3C). However, eyes infused with a glycolysis inhibitor (2-deoxyglucose) had significantly lower b-wave amplitudes than the saline-infused eyes (P=.01; Figure 6A) and had a corresponding exacerbation of structural injury (Figure 3D compared with Figure 3C). When isotonic lactate was used as the intraocular infusate, the mean b-wave amplitude was not significantly different from that of the saline controls (P=.76; Figure 6A).

The Table compares the glucose concentration in the retina and vitreous of normoglycemic and hyperglycemic rats. The concentration of glucose in the vitreous was significantly greater (P<.001) in the hyperglycemic rats than in controls, in both the diabetic rats and the glucose-injected rats; however, the amount of free glucose in the retina was not significantly different between groups.

**COMMENT**

The b-wave amplitude of the ERG is a functional measure that is particularly susceptible to ischemia12,13 and provides a quantitative measurement of middle and in-
ner retinal function, but it excludes information about retinal ganglion cells. Thy-1 mRNA is a retinal ganglion cell marker, and its measurement by semiquantitative RT-PCR provides a useful gauge of ischemic insult, which predominantly affects the inner retina; histopathological examination provides qualitative structural data about all retinal layers. Using these complementary techniques, we found that preexisting hyperglycemia provided a remarkable degree of protection against ischemic injury, a level of protection that, in our experience, is unrivaled by any other method of neuroprotection. The most likely explanation for this phenomenon is that the elevated glucose levels in the vitreous of the hyperglycemic rats caused relative preservation of retinal ATP levels by anaerobic glycolysis during the period of ischemia. There are, however, several alterna-

![Figure 3. Digitally processed images of untreated retina (A), retina of rats after 6 weeks of diabetes (B), and retinal sections from rats that received 60 minutes of ischemia and 7 days of reperfusion (C-H). C, Normoglycemic rat with isotonic sodium chloride solution (saline) infusate. D, Intraocular infusate of 5% 2-deoxyglucose. E, Five-day diabetic rat. F, Six-week diabetic rat. G, Glucose-induced preexisting hyperglycemia. H, Infusate of 5% glucose in a normoglycemic rat (for all parts, hematoxylin-eosin, original magnification x250).](image)

![Figure 4. Thy-1 messenger RNA (mRNA) levels in rats after 6 weeks of diabetes and control rats, 7 days after a 60-minute period of ischemia. A, Representative polymerase chain reaction; top gel is from control eyes, and bottom gel, diabetic eyes (odd lanes, ischemic eyes; even lanes, untouched eyes). B, Ratio of Thy-1 to cyclophilin mRNA. The ratio was significantly greater in diabetic rats than in controls. Asterisk indicates P<.001; limit lines, SEM.](image)

![Figure 5. b-Wave amplitudes after 45 minutes (A) and 60 minutes (B) of ischemia. A, Glucose-induced preexisting hyperglycemia preserved the b-wave amplitude against 45 minutes of ischemia, but postischemia hyperglycemia did not. B, Preexisting hyperglycemia also significantly protected the b-wave amplitude when the duration of the ischemia was increased to 60 minutes. Asterisk indicates P<.001; limit lines, SEM.](image)
possible increase in the activity of the pentose phosphate pathway is the critical protective event. Accumulated lactate in the postischemic period was likely produced during the ischemic period rather than the use of glucose during the ischemic period, indicates that the anaerobic use of glucose and retinal glycogen are used during ischemia. In the present study, diabetic rats and the rats that received a bolus of glucose displayed a similar degree of protection, despite the higher glucose levels in the diabetics, which may reflect a saturation of the enzyme systems at moderate glucose levels. Another protective mechanism using glucose is the diabetic-neuronal lactate shuttle is important for the recovery of neuronal function after hypoxic injury. Hence, it is conceivable that the anaerobic buildup of lactate and its use in the immediate postischemic period, rather than the intracellular use of glucose per se, was the protective factor. However, the finding that intracellular lactate was not protective, coupled with the lack of protection afforded by hyperglycemia in the postischemic period, indicates that the anaerobic use of glucose during the ischemic period rather than the use of accumulated lactate in the postischemic period was likely to be the critical protective event.

Another protective mechanism using glucose is the possible increase in the activity of the pentose phosphate pathway with a corresponding increase in nicotinamide adenine dinucleotide phosphate and cellular reducing power, thereby decreasing free radical injury, particularly in the early reperfusion period. However, Winkler et al showed that the activity of the pentose phosphate pathway is not significantly increased in the isolated retina when the concentration of glucose is raised from 5mM to 30mM. Furthermore, the complete lack of neuronal protection by hyperglycemia in the early reperfusion period goes against the possibility that the pentose phosphate pathway is involved in the neuronal protection.

The present results highlight fundamental differences between retinal and cerebral energy metabolism. Under aerobic conditions, brain tissue converts only 13% of its available glucose to lactate and obtains 94% of its ATP from oxidative metabolism. Conversely, under aerobic conditions, the isolated rat retina converts 90% of its available glucose to lactate, deriving 36% of its ATP from glycolysis. Hence, under anaerobic conditions the retina has “less distance to make up” and, by a 2-fold up-regulation of glycolysis (the Pasteur effect), can maintain ATP levels at 50% to 70% of aerobic levels as long as glucose is abundant. Similarly, under aerobic conditions, human Muller cells convert 99% of available glucose to lactate and require only a 30% increase in glycolysis to maintain ATP production anaerobically. Several in vivo studies have also shown that vitreous glucose and retinal glycogen are used during ischemia. Using a pressure-induced ischemia model in rabbits, Weiss found that in the first 20 minutes of ischemia, retinal glycogen was used, but after this period the vitreous became an important source of glucose for anaerobic glycolysis. Weiss concluded that the vitreous glucose was the most important energy substrate for retinal ischemia lasting more than 20 minutes and that the exhaustion of anaerobic glycolysis was a crucial factor in determining the ischemic tolerance time of the rabbit retina. In the present study, diabetic rats and the rats that received a bolus of glucose displayed a similar degree of protection, despite the higher glucose levels in the diabetics, which may reflect a saturation of the enzyme systems at moderate glucose levels. Given that rats store low amounts of retinal glycogen, and the finding that the free retinal glucose levels were not significantly different in the hyperglycemic and normoglycemic groups.

![Figure 6](image-url)

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood Glucose, mg/dL</th>
<th>Vitreous Glucose, mg/dL</th>
<th>Retinal Glucose, mg of Glucose/µg of Retina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoglycemic (n = 4)</td>
<td>129.7 ± 14.8</td>
<td>68.5 ± 7.7</td>
<td>5.3 ± 0.24</td>
</tr>
<tr>
<td>Glucose injected† (n = 4)</td>
<td>291.9 ± 27.0</td>
<td>133.3 ± 21.6</td>
<td>5.4 ± 0.32</td>
</tr>
<tr>
<td>Diabetic† (n = 4)</td>
<td>457.7 ± 57.7</td>
<td>219.8 ± 34.2</td>
<td>5.3 ± 0.18</td>
</tr>
</tbody>
</table>

SI conversion factor: To convert glucose to millimoles per liter, multiply by 0.0555.

*Values are expressed as mean ± SEM.
†Rats were injected with streptozocin (60 mg/kg) and measurements were taken 5 days later.
‡Rats were injected with glucose (2 g/kg) intraperitoneally and measurements were taken approximately 20 minutes later.

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normoglycemic rats, it seems likely that vitreous glucose is an important energy source for the ischemic rat retina. The finding of elevated vitreous glucose levels in the diabetic rats was consistent with the well-described correlation between blood glucose and vitreous glucose levels. In addition, we found that vitreous glucose levels are rapidly elevated after a systemic bolus of glucose, a finding that almost certainly accounts for the protection against ischemia that was observed in this group of rats. Hence, in summary, these experiments have shown that the retina’s relative lack of reliance on mitochondrial metabolism can be exploited under anaerobic conditions in vivo by increasing glucose availability to the oxygen-starved retina.

The present study has implications for the pathophysiology of diabetes. Long-term hyperglycemia has a clear association with diabetic retinopathy, which commonly includes an ischemic element. Hence, although long-term hyperglycemia can cause retinal microangiopathy and neuronal apoptosis, short-term hyperglycemia can paradoxically preserve neuronal structure and function after acute ischemia.

The finding that preexisting moderate hyperglycemia is protective against retinal ischemia may also have clinical applications. Conceivably, retinal injury caused by temporary ischemia in a variety of conditions, including retinal detachment and venous occlusion, could be ameliorated by short-term moderate hyperglycemia. In addition, the sustained administration of glucose to the vitreous may represent a treatment strategy for conditions involving an element of chronic retinal ischemia.

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