Decreased Rhodopsin Regeneration in Diabetic Mouse Eyes

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Purpose. To evaluate the effect of diabetes on rhodopsin regeneration in the excised mouse eye.

Methods. A superfused excised mouse eye preparation that exhibits rhodopsin regeneration after moderate bleaches and that is responsive to the composition of the perfusate was used. Diabetes was induced in albino mice (BALB/c) with the diabetogenic agent streptozotocin. Absorption spectrophotometry of the excised eye was used to monitor rhodopsin concentrations.

Results. Significant reductions in rhodopsin regeneration were observed in diabetic mice. Severely diabetic mice exhibited only 64% and 55% regeneration (at perfusate glucose levels of 5.1 mM and 10 mM, respectively), and moderately diabetic mice exhibited 74% and 73% regeneration, compared to the greater than 100% regeneration observed in nondiabetic mice. Glucose perfusate concentration has a major effect on rhodopsin regeneration. Lower concentrations of perfusate glucose (3 mM) reduced the amount of rhodopsin regeneration in both nondiabetic mice and diabetic mice. The diabetic mice seemed to tolerate higher concentrations of perfusate glucose (20 mM) better than the nondiabetic mice. Neither correction for osmolarity nor substitution with a nonglycolytic substrate increased the amount of rhodopsin regeneration in the diabetic mice.

Conclusions. Diabetes reduced the amount of rhodopsin regeneration that followed moderate bleaches in excised mouse eyes. The data suggest that some process or processes associated with rhodopsin regeneration have been affected in the diabetic. Invest Ophthalmol Vis Sci. 1994;35:3905-3909.

Glucose metabolism is critical to the maintenance and functioning of all animal cells. Therefore, disorders that affect glucose concentration, such as diabetes mellitus, can cause a variety of abnormalities. For cells that do not require insulin for glucose transport, such as photoreceptors, increased extracellular glucose concentrations lead to increases in intracellular glucose concentrations and in metabolic rate. This may initiate a variety of abnormalities, such as increased oxygen consumption and sorbitol concentrations, and decreased myo-inositol concentrations.1-4 Illumination of the rod visual pigment, rhodopsin, transforms the retinal chromophore from the 11-cis configuration to all-trans.5 Because rod cells neither contain the enzyme necessary to isomerize the chromophore back to the 11-cis form6,7 nor store a major supply of 11-cis chromophore,8 a number of reactions and cellular transfers are required to provide adequate chromophore for rhodopsin regeneration.9-11 Recent studies in whole excised mouse eyes have shown that rhodopsin regeneration is sensitive to the concentration of extracellular glucose12 and is inhibited by a lack of oxygen.13 Because both of these factors may be affected in diabetes, it was of interest to evaluate rhodopsin regeneration in diabetic mice. Some aspects of this study have been published in abstract form.14,15

MATERIALS AND METHODS

The superfused excised albino mouse eye preparation developed by Ostroy et al16,18 was used. The prepara-
tion has the advantage of retaining the structural integrity of the eye and remaining viable during multiple rhodopsin bleach–regeneration cycles. Moreover, the preparation can reliably measure small changes in (wavelength-dependent) absorbance, permitting one to follow rhodopsin changes after moderate bleaches, levels of bleaching that may be encountered physiologically. In the present study, only bleaches of 15% to 25% were used. Also, the preparation has been shown to be sensitive to the composition of the perfusate. Previous studies evaluated the effect of perfusate glucose concentration and hypoxia on rhodopsin regeneration. In the present study, we have examined the effect of various levels of perfusate glucose on rhodopsin regeneration in diabetic mice.

Albino mice (BALB/cAnNHsd BR) were bred from animals obtained from Jackson Laboratories (Bar Harbor, ME). Treatment of the animals adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All mice were allowed free access to food and water and were maintained on a 12-hour light/12-hour dark cycle. The male animals used for these experiments were dark adapted for a period of 18 to 22 hours before the experiments. To induce diabetes, the procedure of Le et al was followed with minimal modifications. Intraperitoneal injections of streptozotocin (50 mg/kg dissolved in sterile saline; Sigma Chemical, St. Louis, MO) were administered for 5 consecutive days to male animals 6 to 8 weeks of age (the age specified by Le et al). The animals were generally used 3 to 5 weeks after the final injection. Certain animals (designated as “older diabetic mice”) were studied 9 months after the final injections. It was initially observed that streptozotocin-injected mice, although maintaining high levels of extracellular glucose, had survived without apparent changes in health or weight over a 6- to 10-month period and had not required insulin injections. Because this is unusual for many mammals, some of the diabetic mice were maintained for 9 months and studied at that time.

In all of the experiments, after the eye had been removed, the mice were tested for blood glucose levels with the Accu-Chek II Blood Glucose Monitor (Boehringer Mannheim, Indianapolis, IN) sampled from the heart. Blood glucose levels above the range of the measuring device (that is, greater than 500 mg/dl) were assigned a value of 525 mg/dl. Fifty-six diabetic mice were assigned a value of 525 mg/dl. Fifty-six diabetic mice and 3 nondiabetic mice) were followed for 9 months and studied at that time.

The spectrophotometric procedures used were generally those described by Ostroy et al. Briefly, the rhodopsin of an excised albino eye was monitored by placing the whole eye into a superfused boat-type spectrophotometer cell within the sample chamber of a double-beam spectrophotometer. Both a Cary model 14 spectrophotometer modified for computer use (OLIS, Bogart, GA) and a newly adapted Perkin–Elmer model 6 spectrophotometer (Norwalk, CT) were used. Bleaches were achieved by illuminating the eye for 30 seconds with diffuse fluorescent lights, and only those experiments which exhibited bleaches of 15% to 25% were used. Each spectrum was normalized to 680 nm to correct for nonspecific absorbance changes. A final (baseline) spectrum was obtained at the end of the experiment by illuminating the eye for 1 hour to eliminate all of the rhodopsin and its photoproducts. The absorbance values at 500 nm were used as a measure of the rhodopsin concentration (after zeroing at 680 nm, subtraction of the baseline, and verifying that the absorbance changes were those of rhodopsin by observing that the difference spectrum peaked near 500 nm). The perfusion rate was approximately 2 ml/min. The composition of the perfusate was modified from albino mouse serum values and was as follows: 103.6 mM NaCl, 5.25 mM KCl, 26.2 mM NaHCO3, 1.215 mM Na2HPO4, 0.785 mM NaH2PO4, 1.3 mM MgCl2, 1.4 mM CaCl2, 3 mM HEPES buffer, and varying concentrations of glucose. The pH was 7.4, and the solutions were equilibrated with a 95% O2–5% CO2 gas mixture that was then maintained above the perfusate supply throughout the experiment. Mannitol was used in some of the

![Figure 1](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933401/ on 06/24/2017)

**FIGURE 1.** Ultrafiltrate glucose concentrations. Time axis for the diabetic mice is days after last injection of streptozotocin; for the nondiabetic mice, days after 7 weeks of age. Samples were collected over an 8-hour period. See Materials and Methods for additional details. Error bars represent SEM. Number of mice followed using this procedure: severely diabetic mice, n = 11; moderately diabetic mice, n = 3; nondiabetic mice, n = 3.
experiments to increase the osmolarity of the solution without changing the extracellular glucose concentration. All of the experiments were performed at 23°C, controlled by a temperature-regulated water bath (Lauda; Brinkmann Instruments, Westbury, CT) that circulated water through a closed system positioned within the sample chamber.

RESULTS

Figure 1 presents the effect of the sequence of streptozotocin injections on daily levels of ultrafiltrate glucose. Most of the animals became severely diabetic within 1 week of the injection sequence, remaining at consistently high diabetes levels after approximately 3 weeks. During the 3rd to the 5th week after the injection period, when the experiments were conducted, the glucose concentrations exhibited means from 455 to 570 mg/dl. Twenty-one percent of the injected animals became moderately diabetic, with means from 137 to 255 mg/dl. Both of these groups exhibited higher glucose concentrations than did the nondiabetic mice (at 3 to 5 weeks, means from 63 to 137 mg/dl). Blood glucose measurements, conducted at the time of the experiments, exhibited similar percentages of moderately diabetic mice. Of the 44 animals used for the main regeneration data, 13 (29.5%) exhibited blood glucose concentrations less than 400 mg/dl (the value used to define the severely diabetic mice), with a mean glucose concentration of 287 ± 23 mg/dl (mean ± SEM). These levels were still well above the 94 ± 1 mg/dl measured in nondiabetic mice. The severely diabetic mice exhibited average blood glucose concentrations of 457 ± 7 mg/dl. For those animals in which both ultrafiltrate glucose and blood glucose were measured, there were no instances of inconsistent classifications.

The results illustrating the ability of the eyes to regenerate rhodopsin in diabetic mice and nondiabetic mice at various glucose perfusate concentrations are presented in Figure 2. Compared to the nondiabetic mice, both the moderately and severely diabetic mice exhibited significant reductions in rhodopsin regeneration (P < 0.0001; see caption of Figure 2 for additional details). At 5.1 mM and 10 mM glucose, the severely diabetic mice exhibited only 64% and 55% regeneration, respectively, and the moderately diabetic mice exhibited 74% and 73% regeneration, in contrast to the greater than 100% regeneration observed in nondiabetic mice.

As shown in Figure 2, the extracellular glucose concentration of perfusate glucose on levels of regeneration

**FIGURE 2. Amount of rhodopsin regeneration at various concentrations of perfusate glucose.** For each experiment, the amount of rhodopsin regeneration that followed the first bleach was determined. The figure presents the averages derived from the data of the individual experiments. Error bars represent SEM. Nondiabetic mice data from 0 mM to 10 mM were taken from Ostroy et al. Using analysis of variance (General Linear Models Procedure, or PROC GLM), the effect of diabetes was determined to be highly significant (F (9,67) = 15.4, P < 0.0001). Using Duncan’s multiple range test (using PROC GLM), the nondiabetic mice were determined to be significantly different from both moderately and severely diabetic mice, and the moderately and severely diabetic mice were not significantly different from each other (alpha = 0.05). Also significant was the effect of glucose on levels of regeneration (F (5,67) = 11.7, P < 0.0001). However, the interaction term was not significant, evaluating whether diabetes altered the glucose dependence of the regeneration (using conservative statistical assumptions that did not give extra weight to the data at 20 mM; F (5,67) = 0.72, P > 0.61). Experimental numbers for these data as follows: Severely diabetic mice: 3 mM, n = 10; 5.1 mM, n = 13; 10 mM, n = 4; 20 mM, n = 4. Moderately diabetic mice: 3 mM, n = 6; 5.1 mM, n = 4; 10 mM, n = 3. Nondiabetic mice: 0 mM, n = 4; 1 mM, n = 3; 2 mM, n = 7; 3 mM, n = 4; 4 mM, n = 4; 5.1 mM, n = 6; 7 mM, n = 4; 10 mM, n = 3; 20 mM, n = 4.

maximum or near maximum levels of rhodopsin regeneration in both nondiabetic mice and diabetic mice. Lower concentrations of perfusate glucose (3 mM) reduced the amount of rhodopsin regeneration in all of the groups. However, the diabetic mice seem to be more tolerant of high glucose concentrations. At a perfusate glucose concentration of 20 mM, the severely diabetic mice exhibited regeneration levels that were slightly higher than those observed at 5.1 mM and 10 mM, but the nondiabetic mice exhibited decreases in regeneration level.

To try to find conditions that might modify the levels of regeneration in the diabetic mice, two chemical approaches were used, and some older diabetic mice were tested. None had statistically significant ef-
fects on the levels of regeneration. To increase the osmolarity of the perfusate, 4.9 mM and 14.9 mM mannitol were added to the 5.1 mM glucose. These animals exhibited regenerations of 56% ± 3% (n = 4) and 67% ± 16% (n = 3), respectively. To bypass glycolysis, 10 mM pyruvate was substituted for glucose. These animals exhibited an average regeneration of 59% ± 16% (n = 4). A group of severely diabetic animals (blood glucose = 460 ± 38 mg/dl) that had been diabetic for more than 9 months showed some improvements in regeneration (87% ± 6%, n = 5), but not enough to be considered statistically significantly compared to the diabetic mice (at 5.1 mM, using paired Student’s t-test).

The potential physiological consequences of the observed reductions in the degree of rhodopsin regeneration were evaluated by measuring the total rhodopsin absorbance. To reduce the variability associated with these measurements, the data were selected for experiments conducted within a limited time period using the same spectrophotometer. Total rhodopsin absorbance at 500 nm was as follows: nondiabetic mice, 0.23 ± 0.02 A (n = 6); severely diabetic mice, 0.18 ± 0.02 A (n = 4); moderately diabetic mice, 0.19 ± 0.02 A (n = 4); and older diabetic mice, 0.18 ± 0.02 A (n = 3). Although the data exhibit a consistency in the expected direction (in the diabetic mice, reduced rhodopsin concentrations), the restricted data that could be used for this analysis and the variability of the complete data set require caution in possible interpretations.

DISCUSSION

The clinical manifestation of a reduced rhodopsin concentration is an increase in visual threshold. There are few published reports of such effects in diabetic mice. However, Henson and North21 reported that diabetic patients both exhibited increases in absolute threshold and had longer adaptation times.

The mechanism or mechanisms leading to reductions in the amount of rhodopsin regeneration in the diabetic mice could not be determined with the current data. Previous studies with this experimental system have shown that rhodopsin regeneration was reduced by conditions that affected either glycolysis or aerobic metabolism. Because diabetes can affect these metabolic processes, such a mechanism is likely. In particular, an effect on the cofactor, NADPH, is an obvious suggestion, because it is used in the reduction of retinal to retinol and in the reduction of sorbitol, and it is controlled, in part, by the pentose shunt and is altered in concentration by illumination. The inability of pyruvate to improve regeneration in the diabetic mice might be related to their need for this cofactor. Further information can be derived from the glucose dependence of the regeneration. The similar reductions in levels of regeneration when either diabetic mice or nondiabetic mice are provided with limited substrate (at 3 mM glucose) would suggest that the glycolytic and aerobic pathways have not been inhibited in the diabetic mice. The reduced rhodopsin regeneration at 20 mM glucose in nondiabetic mice, compared to the slight improvement in diabetic mice, may be indicative of a defect related to high circulating concentrations of glucose.

Acknowledgments

The authors thank Dr. Robert N. Frank for detailed comments on the manuscript, Dr. Jeffrey R. Lucas for help with the statistical analyses, Phyllis Crick for animal care, and Anita Robinson for help with the manuscript.

Key Words

diabetes, eye, mouse, rhodopsin, vision

References

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