Electroretinogram Recovery in the Rabbit After Repetitive Short-Term Ischemia in Light and Dark

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Purpose. To determine the time course and reproducibility of electroretinogram recovery after short-term (5- to 20-minute) retinal ischemia in the light and in the dark.

Methods. Electroretinogram recovery was measured in Dutch rabbits after 5-, 10-, or 20-minute episodes of ocular ischemia, repeated three times at 1-hour intervals. Results were compared under light- and dark-adapted conditions.

Results. The rate of b-wave recovery was highly reproducible after repetitive ischemic insults in the same eye. The rate of b-wave recovery varied in proportion to the duration of ischemia but generally reached 100% of preischemic levels within 45 to 60 minutes. Recovery was slower under dark-adapted conditions than under light-adapted conditions, and 10 minutes of ischemia appeared to be a critical duration to maximize light- and dark-adapted metabolic differences.

Conclusions. Because of the speed and reproducibility of electroretinogram recovery, the use of short-term ischemic episodes may facilitate studies on the pharmacologic therapy of retinal ischemia. It remains to be determined, however, whether the retinal effects of short-term ischemia are mediated by the same mechanisms as the effects of longer-term ischemia. Invest Ophthalmol Vis Sci. 1994;35:664-668.

Rabbit retina is irreversibly damaged by periods of ischemia longer than 60 minutes.1-3 However, little permanent injury occurs after ischemic durations of less than 30 minutes.3-5 Studies on pharmacologic protection from ischemia have generally used durations of ischemia (typically 60 minutes or longer) that induce some degree of permanent injury in order to evaluate the potential for rescue. These studies can be laborious because of the length of each ischemic trial and the large numbers of animals necessary to overcome individual variation. We have studied b-wave recovery profiles after brief periods of pressure-induced ischemia (5, 10, and 20 minutes), under both light- and dark-adapted conditions, in order to document the time course of recovery and determine whether these shorter durations of ischemia can be induced repetitively during a single experimental session with a reproducible pattern of recovery. If the mechanism of injury with this technique proves to be similar to that with longer durations of ischemia, then this technique might provide a more efficient and reproducible methodology for the study of ischemia–reperfusion injury.

Our results show that the time course of recovery from short-term ischemia is quite predictable and that ischemia can be repeated sequentially during a single experiment with excellent reproducibility in either the light or the dark. However, it remains to be shown whether the mechanisms of short-term ischemia and recovery are the same as for longer and more irreversible injury.

METHODS

All of the animal experiments conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Pigmented Dutch rabbits (1.5 to 2.0 kg) were sedated with intramuscular ace-
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promazine (1 mg/kg) and xylazine hydrochloride (2 mg/kg) and anesthetized with intraperitoneal urethane (1 g/kg). Pupils were dilated with 1% atropine sulfate and 10% phenylephrine hydrochloride. Animals were not allowed to recover from anesthesia, and were euthanized at the end of each experiment by an overdose of pentobarbital sodium.

Electroretinograms (ERGs) were obtained simultaneously from both eyes, using as the stimulus the brightest flash from a Grass PS-22 (Grass Instrument Co., Quincy, MA) stimulator, positioned 10 cm in front of each eye. Flash duration was about 10μs, with an intensity approximately 75 cd·m⁻² per flash. Monopolar Jet electrodes (Universe SA, La Chausse-Fonds, Switzerland) were placed on the cornea; a silver wire in the conjunctival cul de sac served as the ground. The signals were AC-amplified (0.3 to 1000 Hz) and displayed on a storage oscilloscope. For “light-adapted” experiments, the rabbits were dark-adapted for 40 minutes before a pre- and postcannulation ERG and kept in darkness during all recovery periods. For “light-adapted” experiments, the rabbits were kept under ambient fluorescent room light throughout the experiment (approximately 25 foot-candles).

To control intraocular pressure, the anterior chamber of each eye was cannulated with a heparinized polyethylene tube. A saline reservoir was lowered or raised to change the intraocular pressure. Complete ischemia was induced at a pressure of 150 mm Hg, as confirmed by absent ERGs and a pale fundus on ophthalmoscopic examination. Ischemia of one duration (5, 10, or 20 minutes) was induced three times in each eye with a 1-hour interval between each trial. ERG recordings were made at 2, 5, 10, 15, 30, 45, and 60 minutes after reperfusion. Recovery was quantified by comparing the b-wave amplitude at each time point of recovery to the preischemic (postcannulation) value.

RESULTS

A total of 48 eyes were studied, with 8 eyes used for each of the six experimental conditions studied: ischemia of 5, 10, or 20 minutes under either light- or dark-adapted conditions. The preocclusion b-wave amplitude averaged 116.6 ± 3.0 μV standard error when light-adapted and 309.4 ± 2.31 μV when dark-adapted.

To judge the reproducibility of the time course of recovery of the b-wave after repetitive episodes of short-term ischemia, we induced the same duration of ischemic insults, we combined (averaged) the data from all three runs for each condition to compare the effects of ischemic duration and of light versus dark adaptation. Figure 3 compares the combined b-wave recovery curves after different lengths of ischemia (5, 10, and 20 minutes). Under light-adapted conditions, there was very little difference between the 5- and 10-minute curves. After 20 minutes of ischemia, however, the rate of recovery was markedly slower and the recovery percentages were significantly lower than the 5- or 10-minute curve at all time points up to 45 minutes (when recovery was essentially complete). In contrast, under dark-adapted conditions, the rate of recovery became slower progressively between 5-, 10-, and 20-minute episodes of ischemia. The 20-minute dark-adapted curve was the only experimental condition in our study where complete recovery was not achieved (only 88%).

Figure 4 compares the rates of b-wave recovery in light and dark adaptation. After 5 minutes of ischemia, the light- and dark-adapted recovery curves were statistically indistinguishable. However, after 10 minutes of ischemia, b-wave recovery was significantly slower (P < 0.05) under dark-adapted conditions than light-adapted conditions at all time points up to complete recovery at 45 minutes. This difference became less
FIGURE 2. Reproducibility of ERG recovery after repetitive short-term ischemia. Curves represent data averaged from eight eyes after first (open circles), second (filled circles), and third (triangles) episodes of ischemia under each experimental condition. The curves were virtually the same after each repetitive insult for all ischemic durations under both light conditions. This was confirmed by statistical analysis (see Results). Standard errors of the mean are shown to illustrate the magnitude of variation (for one curve only in each graph, for clarity); the variation was similar in all curves.

prominent after 20 minutes of ischemia (statistically significant \( P < 0.05 \)) only at 5, 10, 15, and 60 minutes of recovery.

DISCUSSION

We found the time course of ERG recovery from short-term ocular ischemia (5 to 20 minutes) to be similar to that reported by others. However, we also showed that ischemic episodes can be repeated at hourly intervals in rabbits, under either light- or dark-adapted conditions, with essentially full recovery of the ERG b-wave after each insult (the possible exception being 20 minutes of ischemia under dark-adapted conditions). Furthermore, the rate of b-wave recovery is unchanged between a first, second, or third episode of ischemia. Because of this reproducibility, the induction of repetitive episodes of short-term ischemia may allow more efficient and accurate studies on the mechanisms or therapy of ischemia than are possible when each ischemic episode requires many hours of recovery and a separate animal.

However, the value of this technique for studies on pharmacologic therapy for retinal ischemia depends on whether the mechanisms that mediate short-term and completely reversible damage are the same as those that mediate irreversible damage from longer periods of ischemia. Histologic studies have demonstrated irreversible changes from ischemia of only 30 minutes' duration, though they are not as prominent as the damage after insults of 60 minutes or longer. Irreversible retinal damage after ischemia results from a cascade of reperfusion injury that involves free radical formation and excitatory amino acid release. Already NMDA receptor antagonists, antioxidants, and other agents have been shown to be partially protective against retinal ischemia lasting 60 minutes or
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FIGURE 4. Effects of light adaptation on b-wave recovery after 5, 10, and 20 minutes of ischemia (same data as in Fig. 3). Dark-adapted recovery was not delayed relative to light-adapted recovery after the briefest (5-minute) period of ischemia but showed a prominent delay after 10 minutes of ischemia and a lesser difference after 20 minutes of ischemia (when the light-adapted recovery began to slow). The error bars show standard error of the mean.

longer. It remains to be determined if the injury and recovery process after our briefer durations (5 to 20 minutes) of ischemia depends upon this same cascade of free-radical and excitotoxic damage.

Our results suggest that the recovery from ischemia is affected by the state of light adaptation. Although no difference between light- and dark-adapted conditions was detected after 5 minutes of ischemia, recovery was considerably slower under our stimulus conditions in the dark than in the light after both 10 and 20 minutes of ischemia. Because we studied only one stimulus intensity, it is theoretically possible that some of this effect could result from shifts in the position of our stimulus on the stimulus-response curve with adaptation rather than differences in recovery rate. However, this seems unlikely because our flashes were relatively bright and would be closer to the maximal response in the dark than in the light; thus, if anything, recovery from ischemia-induced loss of sensitivity should seem more rapid in the dark than the light. Of course, we cannot judge from our single-stimulus data whether the transient effects of ischemia on the ERG b-wave represent a loss of sensitivity, a reduction in the maximal response, or a combination of the two. Stimulus-response curves must be recorded to resolve this question, and to form judgments about the relative degree to which photoreceptors and postreceptor cells contribute to the results.

Another possible confounding factor could be hyper-responsiveness of the ERG, a phenomenon that has been observed during recovery from intermediate durations of ischemia. This could cause recovery to seem falsely rapid, but insofar as we eventually produce abnormally slow recovery in both light and dark adaptation, the relative differences should still be valid. Rods have been reported to show hyper-responsiveness more than cones in the rabbit so that this effect would tend to minimize light–dark differences in the direction we observed rather than cause them. Transient hyper-responsiveness might account for the slight fall-off in b-wave amplitude between 45 and 60 minutes in the 20-minute dark-adapted eyes.

Although our experiments do not distinguish photoreceptor from inner retinal (such as bipolar cell or Müller cell) contributions to the b-wave, we think it most likely that the apparent delay in recovery during dark adaptation represents (at least in part) an effect of metabolic activity in the photoreceptors. Retinal oxygen consumption has been found to be much higher in the dark than in the light, primarily because light reduces the high sodium permeability of the photoreceptors and thus reduces demand for metabolically driven sodium transport to maintain cellular homeostasis. This phenomenon could explain the differences we observed between light- and dark-adapted recovery. Our observation that the greatest difference between recovery rates in light and dark occurred after 10 minutes of ischemia suggests that this duration may be a critical one that just begins to challenge the metabolic capacity of the photoreceptors under dark-adapted but not light-adapted conditions. By this argument, 5 minutes of ischemia would produce little metabolic demand under either condition, whereas 20 minutes of ischemia might begin to compromise light-adapted as well as dark-adapted photoreceptors so that the difference between light and dark would not be as pronounced.

Key Words
dark adaptation, electroretinogram, ischemia, light adaptation, neural recovery, reperfusion, retina
References


