Recovery of Retinal Pigment Epithelial Function After Ischemia in the Rabbit

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Survival of the rabbit retinal pigment epithelium (RPE) after ischemia was studied. The ischemia was induced by elevating intraocular pressure; retinal and RPE function were monitored by electrophysiologic recordings. The b-wave recovered to control amplitude in 1–4 hr after 30–60 min of ischemia, but it never recovered more than about 50% amplitude after 90 min of ischemia. The c-wave recovered after 30 min of ischemia but was replaced by a negative response after 60–90 min of ischemia. The RPE hyperosmolarity response was normal after 60 min of ischemia, but it was severely depressed after 90 min of ischemia. The RPE response to acetazolamide (cornea positive in the rabbit) was lost after both 60 and 90 min of ischemia. These results suggest that different components of RPE function have different tolerances to ischemia and is consistent with evidence that the RPE electrophysiologic responses differ in mechanism and response to disease. Invest Ophthalmol Vis Sci 32:73–77, 1991

Retinas from rabbits and other mammals can survive total ischemia of up to 90 min, as documented by recovery of the electroretinogram (ERG) and the maintenance of normal histologic appearance.1–6 Little attention has been paid to the survival time of retinal pigment epithelium (RPE).7 Choroidal ischemia occurs in various local or diffuse fundus disorders, and in such circumstances, the ability of the RPE to resist ischemia becomes critical to the ultimate viability of the overlying retina.

We found previously that rabbit retina can be protected partially against ischemic damage by preadministration of dextromethorphan, an excitatory amino acid receptor antagonist.6 Interestingly, this drug provided protection against photoreceptor and inner retinal degeneration. There is some evidence of photoreceptor responsiveness to excitatory neurotransmitters,8 but there is also evidence that the RPE may be responsive to these agents.9 It is important to know whether the RPE is damaged by ischemia comparable to that which damages the retina, as a prelude to determining whether damage to the RPE can be modified by dextromethorphan.

To investigate this question, we needed a measurement of RPE function that was independent of retinal function. The most common electrophysiologic tests of the RPE, the c-wave of the ERG and the light response of the standing potential (electro-oculogram), both depend on light reception by the photoreceptors.10 They may give clues to RPE function, but they cannot be interpreted under conditions where the retina may also be injured. The nonphotic responses of the RPE, which have hyperosmolarity or acetazolamide as the stimulus instead of light, are more specific for the RPE since they are independent of the neurosensory retina.11,12 In this report, we examine the b- and c-waves of the ERG and nonphotic responses of the RPE to document the time course of RPE damage after choroidal ischemia in the rabbit.

Materials and Methods

These experiments adhered to the ARVO Resolution on the Use of Animals in Research. The experiments were done on pigmented Dutch rabbits weighing 1.4–1.8 kg. For electrophysiologic recording, the animals were sedated with acepromazine (1.5 mg/kg) and then deeply anesthetized with intraperitoneal urethane (1 g/kg) and intramuscular xylazine (2 mg/kg). The acepromazine and xylazine were repeated as necessary.

The ERGs were recorded as previously reported,13 between the cornea and a reference on the sclera, using silver–silver chloride amalgam electrodes. The corneal electrode was embedded in a silicone rubber surgical contact lens for stability. The pupils were dilated maximally with 1% atropine and 10% phenylephrine drops. Stimuli of 2000-Lux intensity at the cornea and 1-sec duration were presented through a fiberoptic light guide that terminated 1 cm above the...
cornea. The conventional ERG was recorded by alternating-current amplification (0.1–1000 Hz) and displayed on an oscilloscope; c-waves and nonphotic responses were recorded by direct-current amplification on a pen recorder.

At the start of each experiment, an intravenous catheter was placed, and lactated Ringer's solution was infused at roughly 25–30 ml per hr to keep the line open for later drug administration. The animals were then dark adapted for 40–60 min, and control responses were obtained. The anterior chamber of one eye was then cannulated under red light, using a heparinized silastic tube, and the intraocular pressure (IOP) was regulated by the height of an attached saline reservoir. The rabbits were again dark adapted for 30 min, after which control ERG responses were again obtained. The IOP was then raised rapidly to 147 mmHg for 30, 60, or 90 min, after which the reservoir was lowered and the circulation restored. This IOP elevation reliably produced total choroidal occlusion, evident by fundus examination, so that the ischemic effect was independent of blood pressure.

The ERGs were recorded at frequent intervals before, during, and after ischemia and 24 hr later. The nonphotic responses could not be recorded as frequently, since recovery was slower and the administered agent did not immediately disappear. They were obtained using either 25% mannitol (6 ml/kg) or sodium acetazolamide (25 mg/kg) given intravenously over 1 min.

**Results**

Control ERGs that were recorded before and after cannulation of the anterior chamber were identical. When the IOP was elevated, the amplitude of both b- and c-waves fell rapidly, and within 10 min there was no recordable signal (Fig. 1). The recovery of the b- and c-waves depended on the duration of ischemia (Figs. 2, 3). Both b- and c-waves recovered fully within 90 min after ischemia of 30-min duration and continued to rise to supernormal amplitudes over the next 3 hr. After 60 min of ischemia, the b-wave recovered almost all of its control amplitude in 3–4 hr, but the c-wave was essentially unrecordable during this time. By 24 hr after the ischemic episode, the c-wave had returned to normal. After 90 min of ischemia, the b-wave was reduced permanently to roughly 50% of its control amplitude, and the c-wave was replaced by a large, negative response still present 24 hr later.

To monitor RPE function independently of the retina, we recorded hyperosmolarity (mannitol) and acetazolamide responses. Representative recordings are shown in Figure 4. The responses were typically biphasic. The hyperosmolarity response began with a brief (< 1 min) cornea-positive wave followed by a
negative response that averaged $-0.95 \text{ mV}$ (range, 0.7 - 1.3; $n = 13$) and lasted 20 - 30 min. We found empirically that this response could be repeated about every 2 hr, but more frequent repetitions diminished the amplitude. The acetazolamide response began with a very brief cornea-negative deflection followed by a 10-min positive response that averaged $+0.76 \text{ mV}$ (range, 0.6 - 1.0; $n = 9$). The acetazolamide response could not be repeated more often than every 24 hr without diminishing its effect.

The effects of ischemia were monitored with the hyperosmolarity and acetazolamide responses. Control hyperosmolarity responses were obtained 1 hr before ischemia and then at 1, 3, and 24 hr after the restoration of circulation. Figure 5 shows that the responses were normal at all these times after 60 min of ischemia, but they were markedly reduced at all times (including 24 hr) after 90 min of ischemia. In contrast (Fig. 6), the acetazolamide response was severely diminished by both 60 and 90 min of ischemia. Nevertheless, the acetazolamide response was clearly more sensitive to ischemia than the hyperosmolarity response.

**Discussion**

Our data indicate that the RPE is sensitive to durations of ischemia that cause retinal damage. Previous investigators, and earlier work in our own laboratory, showed that the b-wave is compromised by 60 min of ischemia and seriously damaged by 90 min of ischemia.1-6 We found that the c-wave and nonphotic responses are all abolished by 90 min of ischemia but were affected variably by 60 min of ischemia. Both the b- and c-wave showed supernormal re-
and edema, will diminish the c-wave independently of the subretinal space, as can occur with tissue injury or tissue that has been noted previously. The loss of the c-wave after 60–90 min of ischemia is consistent with RPE damage, but it is important to recognize that a reduction of the c-wave is not necessarily proof of RPE damage. The c-wave reflects changes of potassium concentration in the subretinal space as a result of photoreceptor activity, and as such, it is exquisitely sensitive to any change in the size or composition of the subretinal space. For example, widening of the subretinal space, as can occur with tissue injury and edema, will diminish the c-wave independently of any direct damage to the RPE apical membrane. The fact that the hyperosmolarity response remained normal after 60 min of ischemia, while the c-wave was severely depressed, suggests that the subretinal space was altered as a result of the ischemia or possibly that ischemia has differential effects on the apical and basal membranes of the RPE. Theoretically, the loss of the c-wave could also reflect an alteration in the light-evoked potassium decrease or a relative increase in the cornea-negative (slow PIII) retinal component of the c-wave. These are unlikely alternatives since the a-wave (which represents photoreceptor activity) returned to normal (and was not supernormal).

Both the hyperosmolarity and acetazolamide responses have been shown experimentally to be derived from the basal membrane of the RPE. Choroidal hyperosmolarity hyperpolarizes the basal membrane directly; acetazolamide appears to act by its effects on the apical membrane that lead to a relative basal polarization. These responses can be generated in isolated choroid/RPE preparations and thus do not require photoreception or the presence of a retina. Clinical studies show that the two responses behave differently in many retinal and RPE diseases. The hyperosmolarity response is very sensitive to retinal or RPE pathology and is abnormal in a wide range of disorders including retinitis pigmentosa, Stargardt's disease, retinal detachment, and severe diabetic retinopathy. The acetazolamide response is rather resistant to disease but has been abnormal in selected cases of fundus flavimaculatus or pigment epitheliopathy. These data suggest that the responses have different electrophysiologic generators, and thus it is not surprising to find that these responses have different sensitivities to choroidal ischemia.

It is interesting that the acetazolamide response in the rabbit is cornea positive under our recording conditions; the response in the frog, monkey, and humans has been reported as cornea negative. However, the polarity of RPE electrical responses is the result of a delicate balance between apical and basal membrane behavior, and differences in polarity do not necessarily implicate differences in the mechanism of response. Cornea-positive acetazolamide responses have also been noted in the cat (F. Yamamoto and R. H. Steinberg, personal communication).

Our results have some clinical implications. Whereas brief periods of both choroidal and retinal ischemia may be tolerated, ischemia longer than 60–90 min will be damaging permanently to the RPE and the retina. Thus, even if the tolerance of retinal tissue to ischemia can be enhanced by the use of excitotoxic amino acid inhibitors or similar agents, the RPE must also be protected. This rabbit model can help to investigate the use of dextromethorphan or related agents on RPE function after ischemia. (Indeed, the rabbit is a more appropriate model for the retina. They can monitor RPE function independently of the retina.)

Key words: retinal pigment epithelium, ischemia, c-wave, hyperosmolarity response, acetazolamide

References