Irregular Retinal and RPE Damage After Pressure-Induced Ischemia in the Rabbit

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Purpose. Pressure-induced ocular ischemia is a frequent model for the investigation of the mechanisms and therapy of retinal ischemic damage. It is important to know whether the tissue damage in such experiments is uniform or irregular.

Methods. We reviewed histologic features of Dutch rabbit eyes after 60–80 min of pressure-induced ischemia. The eyes were enucleated 4 hr, 1 day, or 1 wk after circulation was restored, at which times the electroretinogram b-wave was moderately reduced.

Results. Light microscopy showed an irregular distribution of damage involving all retinal layers and retinal pigment epithelium. Some regions of damage (or preservation) were several millimeters wide; others were as small as a few cell widths. Correlation with electroretinogram reduction in individual eyes was difficult.

Conclusions. These results show that pressure-induced ischemic damage in the rabbit, sufficient to reduce the electroretinogram, has a patchy and irregular effect on retina and retinal pigment epithelium. Erroneous judgments may be made about ischemic damage, or therapeutic intervention, if only small or selected regions of retina are examined histologically.

Neuronal cell death after ischemia and reperfusion is mediated in part by secondary events such as the formation of oxidative free radicals and the release of excitatory amino acids that damage cell membranes. We have shown previously that retinal function in intact rabbits can be preserved after 60–75 min of ocular ischemia by pretreatment with an excitatory amino acid receptor antagonist, dextromethorphan, and subsequent reports on antioxidants and other agents that ameliorate retinal ischemic damage have appeared.

In many of these experiments ischemia has been induced by elevating intraocular pressure above systolic levels. This is presumed to produce total ischemia throughout the choroid and retina, and we have observed the rabbit fundus to be blanched and free of blood flow when the pressure is elevated. However, histologic analysis of specimens from our experiments has yielded surprising spatial variation in the degree of cellular damage, as if patches of retina and retinal pigment epithelium (RPE) were damaged or spared selectively. This finding raises concern that examination of small samples could bias the interpretation of experiments on retinal ischemia.

MATERIALS AND METHODS

Pigmented Dutch rabbits weighing 1.2–1.8 kg were used in these experiments. All procedures were performed in accordance with the Association for Research in Vision and Ophthalmology resolution on the
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The animals were pretreated with acepromazine (2 mg/kg) and xylazine hydrochloride (2 mg/kg) administered intramuscularly and anesthetized with an intraperitoneal injection of urethane (1 g/kg). The pupils were maximally dilated with several drops of 1% atropine sulfate and 10% phenylephrine hydrochloride (Neo-synephrine hydrochloride, Winthrop, New York, NY). Animals were then dark-adapted for 40 min and control electroretinogram (ERG) responses were obtained. The anterior chamber was cannulated with a needle or fine catheter and the intraocular pressure was regulated by the height of an attached saline reservoir. After another period of dark adaptation and ERG recording, the intraocular pressure was elevated to 150 mmHg for 60, 70, 75, or 80 min, after which the reservoir was lowered and the circulation was restored. Electroretinograms were recorded at intervals during and after induction of ischemia. Some of the animals were treated with potential therapeutic agents (dextromethorphan, catalase, superoxide dismutase, or mannitol) before or immediately after the period of ischemia.

Enucleation was performed 4 hr, 24 hr, or 1 wk after induction of ischemia. Slits were made behind the limbus and the eyeballs were placed in 1.25% glutaraldehyde-1% paraformaldehyde fixative for 30 min at room temperature. Eyes were then bisected in the equatorial plane and the vitreous humor was removed. A minimum of two tissue blocks of 3-4 mm each were taken from the posterior eyecup of eyes removed 4 and 24 hr after ischemia. Larger sections (about 10 mm) were made from the eyes enucleated 1 wk after ischemia; these were oriented vertically, and extended all the way from the central retina (just below the visual streak) to the inferior edge of the ciliary body. The samples were rinsed in sodium cacodylate buffer, post-fixed in 2% osmium tetroxide, dehydrated in graded ethanol and propylene oxide, and embedded in epoxy. Thick (1 μm) sections for light microscopy were stained with toluidine blue (Fig. 1); thin (70 nm) sections for transmission electron microscopy were stained with uranyl acetate and lead citrate. Samples taken from the same eye were processed together. Sections from more than 25 eyes were examined.

RESULTS

Ischemia was produced by elevating intraocular pressure for 60-80 min. The experiments had been planned to induce partial ischemic damage, and all of the eyes showed a reduction (but not absence) of the ERG b-wave at 4 hr to 1 wk after reperfusion. It was anticipated that histologic damage would be uniform throughout each eye, and would correlate with the amount of ERG loss or recovery. However, in every eye examined that damage was to some degree irregular and patchy across the fundus, and was difficult to correlate with ERG changes or drug treatment status in individual eyes.

As an example, Figure 2 shows variations in the degree of outer retinal damage. Figures 2A and B show different regions from an eye removed 4 hr after 70 min of ischemia, when the ERG b-wave was 35% of the preischemic value. Although one area is relatively well preserved, another shows gross edema of the photoreceptor inner and outer segments. Figures 2C and D show similar regional differences (but more prominent histologic damage) in an eye removed 24 hr after 80 min of ischemia and catalase treatment, in which the b-wave was 74% of preischemic value. One area appears relatively undamaged, whereas the other shows disruption of outer segments and photoreceptor nuclei, and disorganization of the RPE. Regional differences in inner retinal damage were also observed.

By 1 wk after 60 min of ischemia, cellular atrophy and multilayered degeneration was grossly discernible. Figure 3A shows an overview of a long strip of retina, from an eye with a b-wave 53% of control value, that has multifocal areas of ischemic damage. Even at this low magnification one can discern discrete foci of edema and outer nuclear layer cell loss. Higher power views of two regions (Figures 3B and C) show that damage involves all cell layers including the RPE and inner retina (although not necessarily in the same locations). Figure 4A shows an overview of another retina treated in the same manner, with a b-wave 37% of...
Figure 2. Outer retinal structure in different areas of the same eyes (1150X). (A) and (B) Eye enucleated 4 hr after 70 min of ischemia. At that time the ERG showed b-waves 35% of preischemic values. One area (A) shows well-preserved retinal and RPE structure, whereas the other (B) shows edema of the photoreceptor and outer nuclear layer. (C) and (D) Eye enucleated 24 hr after 80 min of ischemia and catalase treatment (b-wave 74% of preischemic value). One area (C) shows relatively undamaged retina and RPE whereas the other (D) shows cellular damage. There is disruption of outer segments, and pyknosis in the outer nuclear layer; the RPE is fragmented and disorganized, with cellular debris filling part of the subretinal space.

control value. The nuclear layers and RPE are relatively preserved at the left end of the section, but show disorganization and degeneration toward the right end. Figure 4B shows another region of this same retina, where there is a discrete patch of photoreceptor loss, although RPE and inner retina seem unchanged. Because this patchy damage was not expected, only limited samples from each retina were prepared and it was impossible to reliably correlate histologic damage in individual eyes with the level of ERG preservation. It also could not be judged whether inner or outer retina was more severely or consistently damaged. Every eye with a relatively good ERG showed some areas of severe histologic damage, and every eye with a relatively poor ERG showed areas of retinal preservation. Morphometry was attempted with one group of our samples, and it was found that by pooling the damage scores from different eyes as well as different areas a tentative judgment could be made that a set of eyes with moderate b-wave preservation (dextromethorphan-treated) showed less cellular destruction than a set of eyes with poor b-wave preservation (untreated). However, the finding was not conclusive without prospective sampling that surveyed every region of the retina.

Transmission electron microscopy generally confirmed the cellular damage seen on light microscopy. Some regions that appeared normal by light microscopy showed subcellular damage (mitochondrial swelling, vacuolization, and loss of basal infoldings in the RPE; mild disruption of outer segment discs). Figure 5 shows that transition between normal and damaged
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FIGURE 3. Areas of focal damage 1 wk after 60 min of ischemia, at which time the ERG b-wave was 53% of the value in the fellow eye. (A) Overview of the retina (45X) showing multiple focal areas of edema and cellular loss in the outer nuclear layer. (B) and (C) Higher magnification views (240X) of the sites indicated. In (B), the upward arrow shows an area of RPE damage, and the downward arrows show regions of cell loss in the outer nuclear area. The asterisks show edema and vacuolization of the retina. In (C), the arrows point to regions of RPE and outer segment disruption, with some overlying edema of the inner retina.

Areas of RPE could be observed at the level of single cells.

DISCUSSION

In different histologic sections within individual eyes subjected to pressure-induced ischemia, patchy areas of tissue preservation and injury were regularly observed. The irregularity involved all layers of retina as well as RPE, and the size of discrete regions ranged from millimeters down to a few cell-widths. Markedly different degrees of damage could even be seen in adjacent cells. The irregularity was seen in drug-treated as well as control eyes, making it difficult to judge drug effects histologically. It could not be determined whether the ganglion cell layer, which has been called more sensitive to ischemia, was more damaged than outer retina.

At 4 hr after ischemia (70 and 75 min) the primary changes were cellular edema, as noted by Johnson and Foulds. By 24 hr after ischemia, cellular disruption was evident. By 1 wk after ischemia, there was prominent cellular drop-out involving the RPE and all layers of retina, and the spatial extent of damage was easier to assess. Presumably this reflects the time required for degenerative changes to develop. Patchiness was evident at all three times, however, so that it appears to involve a variable response to injury, rather than (or as well as) variability in the long-term recovery process.

We can only speculate as to why total ischemia has led to such irregular damage in our experiments. Similar irregularity, including the finding that adjacent
cells may show different degrees of damage, has been observed after light-induced damage in the rabbit. It was not described in one previous study on pressure-induced ischemia in the rabbit, but other studies on rabbit, rat, and monkey have noted regional differences or irregularity in fundus damage without clearly documenting the effect. The normal rabbit does show regional variations in retinal thickness and cellularity, but we observed irregular degrees of ischemic damage within both central and peripheral re-

FIGURE 4. Areas of focal damage 1 wk after 60 min of ischemia, at which time the b-wave was 37% of control value. (A) Transition zone between an area of moderate degeneration on the left, and outer retinal edema with gross disruption of the RPE on the right (150x). (B) Area further to the right in the same section showing focal loss of photoreceptors, but little change in the inner retina (250x).

FIGURE 5. Transmission electron micrograph (5250X) of the junction between areas of normal and damaged RPE. The cell on the left shows normal structure, whereas the one on the right shows cytoplasmic vacuolization and disorganization, swollen and damaged mitochondria, and an absence of basal infoldings.
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regions (see Figures 3 and 4). This may occur because of variations in local cellular metabolism, in local vascular anatomy that governs reperfusion, or in local cellular recovery potential. Some of the variability could relate to pressure as a method of inducing ischemia, either because the pressure is distributed unevenly because of local variations in vascular or scleral rigidity in the rabbit, or because the pressure itself is in some way injuring or modifying cells (although this seems doubtful, as others have observed 12). It remains to be determined whether histologic variability is the same after other methods of inducing ischemia.

We did not anticipate the degree of anatomic heterogeneity that we encountered in these experiments, and did not prepare sufficient samples from each eye to allow reliable quantification of retinal damage. This article represents a cautionary statement to help others avoid the same error. We presume that ERG and histologic results will correlate with each other if properly performed, but investigators should survey many different areas of the retina to reliably assess the effects of retinal ischemia and its therapy. Examination of only a few random or selected samples of histologic material could give a biased impression of normality or abnormality because of the patchy nature of the damage.

Key Words

histology, ischemia, morphometry, retina, retinal pigment epithelium

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References


