Retinomotor activity and the c-wave of the hypoxic trout retina. J. Russell Hoffert and John L. Ubels.

The teleost retina exhibits retinomotor activity in response to changing light intensity. We have shown that hypoxia interferes with normal retinomotor activity in the dark-adapted rainbow trout, so that the retina assumes an essentially light-adapted configuration with the response to changing light intensity. We have shown that cones contracted, the rods extended toward the pigmented epithelium, and epithelial pigment expanded. The teleost retina exhibits retinomotor activity in response to decreasing light intensity via movements of the photoreceptors and retinal epithelial pigment (REP) granules. The c-wave of the ERG is due to a rod-dependent hyperpolarization of the apical membrane of the pigmented epithelium in response to decreasing extracellular potassium ion ([K+]o) increase in c-wave amplitude may be related to the movement of the rods toward the pigmented epithelium, which would cause a greater than normal change in extracellular [K+]o near the apical membrane in response to a light stimulus, leading to an increase in c-wave amplitude.

Previous reports from our laboratory concerning the electroretinogram (ERG) of the dark-adapted rainbow trout (Salmo gairdneri) have shown that retinal hypoxia caused by acetazolamide, ischemia, or decreased ventilatory flow results in a transient increase in c-wave amplitude of as much as 400%. In our study of the cause of this increase in c-wave amplitude we have considered the retinomotor activity of the teleost retina and the physiological origin of the c-wave. Retinomotor activity has been described in detail by Altschuler and is the mechanism by which the teleost, which has a fixed pupil, can adjust to changes in light intensity via movements of the photoreceptors and retinal epithelial pigment (REP) granules.

The a-wave is not immediately affected by hypoxia, this increase in c-wave amplitude may be related to the movement of the rods toward the pigmented epithelium. Since the amplitude of the a-wave (receptor potential) is not immediately affected by hypoxia, the increase in c-wave amplitude may be caused by the close proximity of the rods to the pigmented epithelium which would result in a greater [K+]o change near the apical membrane upon photostimulation, thus increasing the degree of hyperpolarization of the apical membrane.

Experiments which combine both electroretinographic and histologic techniques were designed to determine whether the rod movements suggested above actually occur during hypoxia. In this paper we report the changes in the positions of the rods, cones, and REP granules which were observed during retinal hypoxia in light- and dark-adapted rainbow trout. These movements are correlated with simultaneously observed changes in the a- and c-waves of the ERG.

Materials and methods

Experimental protocol. Rainbow trout (S. gairdneri), weighing 200 to 300 gm, were obtained from Midwest Fish Farming Enterprises, Inc. (Harrison, Mich.) and maintained in aerated filtered tap water at 12° C. The photoperiod was 16 hr light and 8 hr dark. To facilitate handling during preparation for the experiment, the fish were lightly anesthetized with tricaine methane sulfonate (MS-222). ERGs were recorded by a method similar to that of Forman et al. The fish were not anesthetized during the experiment, however, they were paralyzed with 2 to 4 U of tubocurarine chloride, and the local anesthetic procaine hydrochloride was applied to the cornea prior to the insertion of the recording electrode into the vitreous humor. Aerated water (12° C) was pumped over the gills at 535 ml/min, and ERGs were recorded at 5 min intervals during 50 min of dark adaptation, which was a sufficient period of time to allow the ERG to reach a constant ampli-
Fig. 1. A, ERG recorded from a light-adapted rainbow trout under normoxic conditions (ventilatory flow 535 ml/min). Note lack of c-wave. B, Effect of 10 min of hypoxia (ventilatory flow 55 ml/min) on the ERG of a light-adapted trout. C, ERG recorded from dark-adapted trout under normoxic conditions. Note presence of the c-wave. D, Effect of 10 min of hypoxia on the ERG of the dark-adapted trout. Note large c-wave and persistence of a-wave.

At this time a control ERG was recorded. Following this recording, in one group of fish the ventilatory flow was maintained at 535 ml/min for a period of 10 min, after which another ERG was recorded. The fish were then killed by cervical section, and the eyes were quickly enucleated. In the other group of fish, the control ERG was recorded, and hypoxia was induced by reducing the ventilatory flow to 55 ml/min for 10 min. An ERG was then recorded, and the eyes were enucleated. Light-adapted fish were treated similarly except that an incandescent bulb delivering 330 candle power was placed 15 cm from the eyes throughout the procedure.

Histology. An eyecup was immediately prepared from the enucleated eye, fixed in a solution of 9% ethyl alcohol, 3% formaldehyde, and 1% glacial acetic acid, and dehydrated in four changes of tetrahydrofuran for 1 hr each. The tissue was embedded in paraffin and sectioned at 6 μm. One set of slides was stained with Harris hematoxylin and eosin; the second set of slides was bleached to remove the melanin pigment, with a 0.25% aqueous potassium permanganate solution followed by a 5% aqueous oxalic acid solution, before being stained with hematoxylin and eosin.

Cell measurements. Measurements of the relative positions of the photoreceptors and the epithelial pigment were made with a calibrated ocular micrometer. Twenty-four areas of the retina of each fish were examined, and the means of these observations were analyzed by a two-by-two factorial analysis of variance and the Student-Newman-Keuls multiple-range test. Fiducial levels were set at p < 0.05. For the purposes of this study, the thickness of the retina was defined as the distance between external limiting membrane (ELM) and the lamina basalis. To compensate for variations between retinas, all measurements were expressed as a percentage of this distance. Since the melanin granules move within the pigmented epithelial cells during retinomotor activity, we refer to the “retinal epithelial pigment” (REP) and measure this layer as the distance between the lamina basalis and the mean border of the REP. Measurements of the rods and cones were made within the pigmented epithelial cells during retinomotor activity, we refer to the “retinal epithelial pigment” (REP) and measure this layer as the distance between the lamina basalis and the mean border of the REP. Measurements of the rods and cones were made within the pigmented epithelial cells during retinomotor activity, we refer to the “retinal epithelial pigment” (REP) and measure this layer as the distance between the lamina basalis and the mean border of the REP.

Results. Electroretinographic data are shown in Table I and Fig. 1. Although there was much variation in ERG amplitude among animals, several trends are evident. A c-wave was recorded...
Table I. Effect of hypoxia on the ERG of light- and dark-adapted trout

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control (recorded after 50 min adaptation)</th>
<th>Experimental (recorded 10 min after control value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photo condition</td>
<td>a-wave (μV)</td>
<td>b-wave (μV)</td>
</tr>
<tr>
<td>Light, Light</td>
<td>44.2 ± 9.1</td>
<td>191.4 ± 27.2</td>
</tr>
<tr>
<td>Light, Dark</td>
<td>27.0 ± 6.0</td>
<td>179.8 ± 57.4</td>
</tr>
<tr>
<td>Dark, Light</td>
<td>42.8 ± 16.4</td>
<td>209.6 ± 82.5</td>
</tr>
<tr>
<td>Dark, Dark</td>
<td>69.8 ± 7.6</td>
<td>274.6 ± 27.9</td>
</tr>
</tbody>
</table>

X ± S.E.; N = 5.
* Significantly different from unmarked values in same column (p < 0.05).
† Significantly different from control value (p < 0.05).

Table I. cont’d

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ventilatory flow (ml/min)</th>
<th>a-wave (μV)</th>
<th>b-wave (μV)</th>
<th>c-wave (μV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light, Light</td>
<td>535</td>
<td>40.6 ± 8.9</td>
<td>196.2 ± 16.8</td>
<td>6.6 ± 6.6</td>
</tr>
<tr>
<td>Light, Dark</td>
<td>55</td>
<td>8.2 ± 8.2*†.</td>
<td>9.4 ± 9.4*†.</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Dark, Light</td>
<td>535</td>
<td>39.4 ± 19.7</td>
<td>162.4 ± 73.0</td>
<td>29.6 ± 18.3</td>
</tr>
<tr>
<td>Dark, Dark</td>
<td>55</td>
<td>51.2 ± 20.1</td>
<td>29.8 ± 13.7*†</td>
<td>202.6 ± 90.8*†</td>
</tr>
</tbody>
</table>

Table II. Effect of hypoxia on positions of photoreceptors and epithelial pigment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ventilatory flow (ml/min)</th>
<th>Retinal thickness (μm)</th>
<th>Epithelial pigment (%)</th>
<th>Cones (%)</th>
<th>Retinal thickness, bleached (μm)</th>
<th>Rods, bleached (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light, Light</td>
<td>535</td>
<td>137.5 ± 7.2</td>
<td>73.2 ± 2.0</td>
<td>7.8 ± 0.7</td>
<td>135.3 ± 7.7</td>
<td>66.4 ± 4.5</td>
</tr>
<tr>
<td>Light, Light</td>
<td>55</td>
<td>149.3 ± 3.6</td>
<td>68.5 ± 3.0</td>
<td>9.2 ± 1.0</td>
<td>152.7 ± 1.8</td>
<td>67.2 ± 3.2</td>
</tr>
<tr>
<td>Dark, Light</td>
<td>535</td>
<td>132.4 ± 7.5</td>
<td>39.0 ± 3.1*†</td>
<td>23.6 ± 4.7*</td>
<td>138.6 ± 2.6</td>
<td>34.7 ± 4.1*</td>
</tr>
<tr>
<td>Dark, Light</td>
<td>55</td>
<td>143.6 ± 11.4</td>
<td>51.4 ± 5.7*†</td>
<td>13.3 ± 1.8</td>
<td>155.1 ± 13.8</td>
<td>56.6 ± 3.3</td>
</tr>
</tbody>
</table>

X ± S.E.; N = 5.
* Significantly different from unmarked values in same column (p < 0.05).
† Significantly different from other marked values in same column (p < 0.05).

under control conditions from only one light-adapted animal, whereas a low-amplitude c-wave was recorded from most dark-adapted animals. Hypoxia abolished the ERG in all but one light-adapted animal. As expected, b-wave amplitude decreased during hypoxia in all animals tested. Of greatest importance to this study is the dramatic increase in c-wave amplitude recorded from the dark-adapted, hypoxic trout.

Histological data are shown in Table II and Fig. 2. As expected, in the normoxic, dark-adapted retina, the band of REP was narrower, the rods were shorter, and the cones were longer than in the light-adapted retina.

During hypoxia in the dark-adapted state, the REP expanded significantly but did not reach its light-adapted position. The cones contracted to their light-adapted length, and the rods extended to their light-adapted length. Hypoxia had no effect on photoreceptor or melanin position in the light-adapted retina.

Discussion. The possibility that hypoxia could affect retinomotor activity was suggested by the work of Ali, who reported that the enucleated eye of the Atlantic salmon (Salmo salar) is incapable of retinomotor responses. We have now shown that hypoxia does in fact affect retinomotor activity in the dark-adapted trout retina. A relationship appears to exist between retinomotor activity and the ERG during hypoxia. We have shown that the
Fig. 2. A, Light-adapted, normoxic (ventilatory flow 535 ml/min) rainbow trout retina. The cones are contracted, and the REP is expanded. B, Light-adapted, normoxic trout retina. The melanin has been bleached to reveal the position of the rods (arrows) which are extended. C, Dark-adapted, normoxic trout retina. The REP is contracted, the cones are extended, and the rods (arrows) are contracted. D, Dark-adapted trout retina after 10 min of hypoxia (ventilatory flow 55 ml/min). The retina has assumed an essentially light-adapted configuration. E, Dark-adapted, hypoxic trout retina. Melanin has been bleached to reveal rods, which are extended and lie near the apical membrane of the pigmented epithelium. (Calibration bars = 30 μ.)
light-adapted a-wave disappears immediately during hypoxia whereas the dark-adapted a-wave persists for some time. In the teleost the myoids of the rods are very thin, so that in the light-adapted retina the rods are shielded by the pigmented epithelium not only from light approaching the rod from an angle but also from axial light. This means that the photopic a-wave is primarily a cone response. The telost retina is avascular, and therefore all \( O_2 \) must reach the retinal cells by diffusion from the choroid. The cone outer segments lie very near the ELM in the light-adapted retina, so that diffusion distance for oxygen is increased, leading to the rapid effect of hypoxia on the photopic ERG. Since the rods elongate in the dark-adapted, hypoxic retina, they could initially have received adequate oxygen for maintenance of the scotopic a-wave. The rods would not be completely shielded from light, since although the REP expands in the dark-adapted, hypoxic retina, it does not reach its light-adapted position.

Our suggestion that the increase in c-wave amplitude observed in the trout during hypoxia may be correlated with retinomotor activity is supported by the fact that in several mammals, including dog, rabbit, cow, and hooded rat, retinal hypoxia results in a decrease in c-wave amplitude. Retinomotor activity does not occur in mammals. It should be noted, however, that Fujino and Hamasaki observed an initial increase in c-wave amplitude in the monkey during hypoxia, although this response did not occur as consistently or to the extent that it does in the trout.

Oakley and Green called the \( [K^+]_o \) decrease in the retina following photostimulation the "potassium efflux" or KRG. They found that the KRG amplitude varies depending on the position of a \( K^+ \)-sensitive electrode within the retina and that over a distance of 30 \( \mu \)m it decreased as the electrode moved from the rods toward the pigmented epithelium. This distance (30 \( \mu \)m) is the average distance over which the rod outer segment moved in the study. Cavaggioni et al. have shown that illumination of rod outer segments results in a decrease in \( K^+ \) efflux from the outer segments secondary to membrane hyperpolarization. In the retina this would result in a decrease in \( [K^+]_o \). Movement of the rods toward the apical membrane of the pigmented epithelium during hypoxia in the dark-adapted retina would cause a greater change in \( [K^+]_o \) near the membrane upon photostimulation, leading to generation of a larger c-wave, since it is known that c-wave amplitude varies directly with KRG amplitude. If this explanation is valid, the amplitude of the c-wave of animals in which retinomotor activity occurs would be a function not only of KRG amplitude (and therefore receptor potential amplitude) but also of the spatial relationship between the rods and pigmented epithelium. Matsunura et al. have also suggested that close apposition of photoreceptors to pigmented epithelium may favor \( K^+ \)-mediated interaction between these cells.

We have shown that hypoxia can affect retinomotor activity and that changes in photoreceptor position can be correlated with changes in the ERG. Therefore, during electrophysiological studies of the retina of lower vertebrates, oxygen supply to the animal or retina should be carefully controlled. This will prevent any alterations of the electrophysiological recordings due to the effects of hypoxia, not only upon cellular metabolism but also upon the spatial orientation of the photoreceptors.

We thank Esther Brenke and C. Dennis Peale for technical assistance.

From the Physiology Department, Michigan State University. This work was supported in part by National Eye Institute research grant EY00009. Submitted for publication Feb. 6, 1979. Reprint requests: J. Russell Hoffert, Physiology Department, Michigan State University, East Lansing, Mich. 48824.

Key words: retinomotor activity, hypoxia, c-wave, pigmented epithelium, rods, cones, electroretinogram (ERG), teleost

REFERENCES


High-molecular-weight (HMW) protein from human cataractous lenses, isolated by differential centrifugation, was deaggregated in 7M urea and then reaggregated in either the presence or absence of 10 mM CaCl₂. By contrast, only 20% to 25% of the material reaggregated in 7M urea buffer can be converted to HMW species if the deaggregating agent is removed. However, only 5% to 10% of this protein is converted to HMW aggregates if the deaggregation step is eliminated. Experiments with ⁴⁰Ca indicate that whereas calcium is necessary for the formation of the HMW aggregates, only one calcium per approximately 5 x 10⁶ daltons remains bound in the reaggregated material. The data suggest that although calcium may be required to induce aggregation to HMW species, it is not required to stabilize such macromolecules. SDS-polyacrylamide gel electrophoresis of the HMW species formed upon reaggregation of the dissociated HMW species with calcium indicates the presence of all the major polypeptide subunits of the original HMW species present in the lens; however, reaggregation in the absence of calcium yields HMW species lacking in the 9600 dalton component.

The experimental studies of Spector et al.¹ and the theoretical work of Benedek² clearly suggest that the generation of high-molecular-weight (HMW) aggregates in the lens may lead to lenticular opacity (see Harding and Dilley³ for additional references). It was shown by Jedziniak et al.⁴ that calcium can induce the aggregation of bovine lens alpha-crystallin to HMW aggregates. Spector et al.⁵ demonstrated that deaggregated bovine HMW alpha-crystallin reaggregates to HMW species upon removal of the deaggregating agent. However, if calcium is omitted, only low-molecular-weight (LMW) species are observed. Calcium is not effective in reaggregating dissociated LMW alpha-crystallin to HMW aggregates. It is probable that a cooperative reaction involving modified polypeptides and calcium is required to reconstitute the HMW alpha-crystallin.

Unlike the bovine lens, in which alpha-crystallin is the primary HMW species, in the human lens, polypeptides from all major proteins appear to be present in the HMW species.⁶ The deaggregated human HMW species have also been shown to reaggregate in the presence of calcium⁷ to HMW aggregates. In conjunction with these studies of the in vitro effects of calcium on the lens proteins, there also have been reports of the presence of higher levels of calcium in some cataractous lenses than in normal lenses. The results obtained from these studies have been summarized by Jedziniak et al.⁸ In a recent investigation of normal and cataractous human lenses, Jedziniak et al.⁹ concluded that the mean calcium concentration of cataractous lenses is two to 13 times higher than is normally found.

How calcium brings about this transformation of the deaggregated proteins to HMW aggregated forms and whether it is required for the stabilization of these giant macromolecules has never been clear. The present studies strongly suggest that although calcium induces aggregation of the deaggregated human lens proteins to giant aggregates, it is not required to stabilize the HMW aggregate structure.

Materials and methods. Cataractous human lenses ranging in color 1 to 5 and opacification 2 to 4 on the basis of the classification of Anderson and Spector⁴ were homogenized at a concentration of 0.7 to 1.0 gm of lens wet weight to 10 ml of 0.01M Tris, 0.1M KCl at pH 7.6. The HMW and LMW fractions were separated by the method of differential centrifugation.⁶ According to this procedure, the supernatant obtained after the first centrifugation step of the lens homogenate at 12,000 rpm for 15 min at the average centrifugal force of 9900 x g contains a mixture of HMW and LMW proteins. Further centrifugation of this su-