Destruction of the Indoleamine-Accumulating Amacrine Cells Alters the ERG of Rabbits

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The indoleamine-accumulating amacrine cells in the rabbit's retina were destroyed by intravitreal injections of 5,7-DHT according to the technique of Ehinger and Florén. One week after the injections, histofluorescence microscopy failed to show the IA-cells in the retina. At this time, the b-wave of the treated eyes was significantly smaller, the OPs were more prominent, and the duration of the b-wave was longer. The a- and c-waves and the off-response were not affected. The loss of the IA-cells resulted in a reduction in the range of dark-adaptation but did not affect the rate of recovery of the threshold. The loss of the IA-cells also had no effect on the response of the retina to flickering light. In a double-flash experiment, the suppression of the response to the second flash was significantly weaker in the treated eyes. These results can be explained by the loss of a negative feedback circuit that has been proposed for the IA-cells from morphologic studies. Invest Ophthalmol Vis Sci 26:1109-1116, 1985

Ehinger and Florén1 reported the presence of one type of amacrine cells in the retina of rabbits, cats, and goldfish which rapidly took up exogenously applied 5,6-dihydroxytryptamine. These IA-cells (indoleamine-accumulating cells) have been found in the retinas of many species of animals.2 Later, Ehinger and Florén3 showed that the injection of 5,7-DHT (5,7-dihydroxytryptamine) into the vitreous of the eye will also be taken up by the IA-cells, and will lead to alterations in the fine structure of the cells. One week after the injections, fluorescence microscopy failed to demonstrate the presence of the terminals of the IA-cells in the retina. Electron microscopy demonstrated that the terminals of these cells form one member of the dyad of bipolar cells in sublamina b of the IPL (inner plexiform layer).4 One week after the destruction of the IA-cells, one member of some of the dyads was replaced by a glial element.4 In the carp, intravitreal injection of 5,7-DHT led to the destruction of the cell bodies as well as the terminals of the IA-cells.5

The question then arose as to what changes would occur in the physiology of the retina following the removal of the IA-cells. We used the technique of Ehinger and Florén3 to destroy the IA-cells, and used the ERG to examine the alterations of the physiologic state of the retina. We shall show that the destruction of the IA-cells results in specific changes in the b-wave of the ERG, and when the b-wave is used to examine the properties of the retina, changes were found in the temporal properties. These results can be explained by the loss of a negative feedback circuit which has been proposed for the IA-cells from morphologic studies.4

Materials and Methods

Animals

The procedures used in this study conformed to the ARVO Resolution on the Use of Animals in Research. The results were obtained from 13 pigmented rabbits weighing 2.2–3.8 kg. The IA-cells were destroyed by the technique developed by Ehinger and Florén.3 To do this, the animal was anesthetized by an intramuscular injection of ketamine (25 mg/kg) and xylazine (25 mg/kg), and injected intraperitoneally with Pargyline (15 mg/kg). About 30 min later, 50 μg/50 μl of 5,7-DHT in saline was injected through the pars plana into the midvitreous of the left eye. The same quantity of saline was injected into the right eye. The procedure was repeated on the next day. Experiments were conducted 1, 2, 3, 4, and 8 wk after the second injection of the 5,7-DHT. Prior to the recordings, the fundus of the eyes was
examined by indirect ophthalmoscopy. None of the eyes revealed any apparent changes.

On the day of the experiment, the rabbit was anesthetized by an intramuscular injection of ketamine and xylazine and the femoral vein canulated. Anesthesia was maintained during surgery by intravenous urethane (250 mg/ml).

The animal was placed in a stereotaxic headholder and paralyzed by flaxedil. During the course of the experiment, anesthesia and paralysis was maintained by a continuous infusion of urethane (25 mg/kg/hr) and flaxedil (10 mg/kg/hr) in a lactated ringer solution. The animal was artificially respired. The pupil was dilated with topical atropine and the nictitating membrane retracted with neosynephrine. A contact lens with an 8-mm artificial pupil was placed on the eye to prevent drying of the cornea.

Recording

A 30-gauge hypodermic needle was placed in the anterior chamber of the eye to pick-up the ERG. The indifferent electrode was placed subcutaneously near the recorded eye, and the animal was grounded by another electrode on the head.

The ERGs were amplified and displayed on an oscilloscope. The low frequency cutoff was set at 10 Hz and the high frequency cutoff at 10 kHz. The responses were recorded on an analogue tape recorder, and signal averaging was done off-line with a Texas Instrument 960A minicomputer. The bin size was set at 2 msec and 20 responses were summed.

Stimulus

The stimulus was obtained from a 150 watt Xenon lamp bulb. The luminance of the full intensity stimulus was $8.6 \times 10^6$ cd/m$^2$, and neutral density filters were used to attenuate the intensity. The stimulus was presented in Maxwellian view with the last lens subtending 26.5 deg at the cornea. The stimulus duration was 300 msec and the interstimulus interval was 3.2 sec.

For the study of the temporal properties of the retina, an array of 7 green LEDs was used for the stimulus. The LEDs were driven by square waves from a Grass stimulator, and placed approximately 2 cm in front of the cornea.

Procedure

The animal was dark-adapted for 1 hr after stimulus alignment before the recordings were started. Recordings were begun with a stimulus intensity which was known to be close to threshold (I = 5.0) for the control eyes, and the stimulus intensity was increased in 0.5 log unit steps. After the entire series of stimulus intensities was recorded from the treated eye, recordings were made from the control eye.

Histology

At the conclusion of the ERG recordings, 50 $\mu$g/50 $\mu$l of 5,7-DHT was injected into the vitreous of both eyes. After 1 hr, the eyes were enucleated, and the ventral half of the retina was separated from the pigment epithelium. The retina was spread on a microscope slide with the vitreous side up. The retina was fixed overnight in a solution of paraformaldehyde, glutaraldehyde and sucrose at 4°C. The slides were air dried and examined with a fluorescent microscope.

Other retinas were fixed in glutaraldehyde and embedded in plastic for light and electron microscopy.

Data Analysis

The averaged data were stored on digital cassette tapes, and the responses were measured from the digital print out. The amplitude of the b-wave was measured from the trough of the a-wave to the peak of the b-wave. The duration of the b-wave was measured at the half-amplitude point, ie, the number of 2 msec bins between the point where the b-wave exceeded the half amplitude level until it fell lower than the half-amplitude point.

Results

Histologic Findings

Two types of fluorescent cells were observed in the flat mounts of the retinas from the control eyes. The more numerous were the yellowish-white and were identified as IA-cells, while the other type of cell was greenish-white and were identified as dopaminergic cells. In the 5,7-DHT treated eyes, only the dopaminergic cells were found. Light and electron microscopic examinations of the treated retinas demonstrated that there were no gross morphologic changes in the retina. The findings were very similar to those reported by Ehinger and Floren, and demonstrated that our techniques led to similar changes in the retina.

Alterations in the Shape of the ERG

The ERGs recorded 1 wk after the injections from the treated and control eyes are shown in Figure 1. The numbers between the ERGs represent the value of the neutral density filters used to reduce the full intensity stimulus. At I = 0, the luminance was $8.6 \times 10^6$ cd/m$^2$.

Several differences in the ERGs can be seen: first, the amplitude of the b-wave is markedly smaller in the treated eye; second, the OPs are much more
prominent in the treated eye; and third, the duration of the b-wave appears longer in the treated eye.

To quantify the difference in the duration of the b-wave, the b-waves of both eyes were normalized, i.e., the maximum amplitude was set to 100% and the other values expressed relative to this value. The results from a 1-wk rabbit are shown in Figure 2. The half-amplitude level (50%) is shown by the horizontal line. The half-amplitude duration for the treated eye was 48 msec while that for the control eye was 28 msec. For the five animals tested at 1 wk, the mean half-amplitude duration of the b-wave was 44.4 msec with a range from 36 to 53 msec for the treated eye. For the control eyes, the mean duration was 31.0 with a range from 26 to 38 msec.

The amplitude of the a-waves of the treated eyes were equal to or even larger than those of the control eyes (Figs. 1, 4). The c-waves recorded with a longer time constant did not differ for the two eyes (not shown). The off-response was also not different for the two eyes.

Intensity-Response Relationship

From ERGs such as shown in Figure 1, the amplitudes of the b-waves were measured for the five rabbits tested 1 wk after the injection of 5,7-DHT. The mean ± SEM of the b-wave amplitudes are plotted in Figure 3 for the treated and control eyes. As was seen in Figure 1, the mean amplitude of the b-wave for the treated eyes was smaller than that of the control eyes at all intensities. An analysis of variance showed that the differences were significant.

We calculated the percentage depression of the b-waves for the different intensities. At $I = 4.0$ and 3.0, the percentage depression of the mean amplitude of the b-wave of the treated eye was 72.7 and 65.5%, respectively.

![Figure 1. ERGs recorded from the 5,7-DHT treated eye (left) and the saline-injected eye. The recordings were made 1 wk after the second injection. The numbers between the ERGs represent the value of the neutral density filter used to reduce the full intensity stimulus. At $I = 0$, luminance = $8.6 \times 10^6 \text{cd/m}^2$. Calibration: 25 $\mu\text{V}$ and 20 msec.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933124/)

![Figure 2. Normalized ERGs recorded 1 wk after the injection of 5,7-DHT. The responses were elicited by the full intensity stimulus. The half-amplitude duration of the b-wave is 28 msec for the control eye and 48 msec for the treated eye.](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933124/)
The question then arose regarding what changes in the properties of the retina would result from the loss of these amacrine cells. Because of the limitations of the ERG, only the nonspatial properties of the retina could be examined.

Course of Dark-Adaptation

To examine whether the loss of the IA-cells altered the course of dark-adaptation, the eye was light-adapted with the full intensity stimulus for 5 min. The course of dark-adaptation was then followed by determining the stimulus intensity necessary to elicit a b-wave of 50 μV. The results from two rabbits are shown in Figure 5. The stimulus intensity required to elicit the criterion b-wave (expressed by the value of the neutral density filter) is plotted on the ordinate and the time in the dark is plotted on the abscissa.

For the control eyes, immediately after the animals were placed in the dark, a criterion b-wave was elicited by the full intensity stimulus reduced by 0.65 log units for rabbit 18 and 0.60 for rabbit 20. There was a rapid decrease in the threshold for the first 8 to 10 min which was then followed by a slower decrease for up to 60 min. At 60 min, the stimulus intensity threshold was 4.65 log units for rabbit 18 respectively. At I = 2.0, 1.0 and 0, on the other hand, the depression was 60.5, 50.5, and 31.5%, respectively. These findings demonstrate that the loss of the IA-cells had greater effect at the lower intensities. The greater depression of the b-wave at the lower intensities may be related to the finding in the cat that the input to the IA-cells is from the rod bipolar cells.

Duration of the Changes

To determine whether the changes induced by 5,7-DHT were long-lasting, we examined rabbits at different periods after the injections. Two rabbits were tested at 2 wk, one at 3 wk, two at 4 wk, and one at 8 wk.

The ERGs recorded from the treated and control eyes of the 8-wk animal are shown in Figure 4. The differences noted in the amplitude and shape of the ERGs in the 1-wk animal (Fig. 1) were found in this animal. The changes, however, were more marked, i.e., the depression of the b-wave was greater and the increase in the duration of the b-wave was greater.

Examination of the Properties of the Retina

These findings demonstrated that the loss of the IA-cells had resulted in specific changes in the ERGs.
and 3.80 log units for rabbit 20. Thus, the range of
dark-adaptation was 4.00 log units for rabbit 18 and
3.20 log units for rabbit 20.

For the treated eyes, the threshold immediately
after the animals were placed in the dark was 0.10
log units for both rabbits. The course of dark-adap-
tation was similar to that of the normal eyes, but the
final threshold was higher in the treated eye of both
rabbits. The range of dark-adaptation was 2.80 log
units for rabbit 18, and 2.85 log units for rabbit 20.

Does the loss of the IA-cells affect the rate of
recovery of the threshold? To determine this, we
calculated the recovery of the threshold as a percentage
of the maximum range of dark-adaptation for the
different periods in the dark for the two rabbits.
When the data were expressed in this way, the course
of dark-adaptation was not different for the treated
and control eyes of the two rabbits (not shown).

These findings demonstrate that the loss of the IA-
cells reduces the range of dark-adaptation but does
not affect the rate of recovery of the threshold. The
higher thresholds in the treated eye were probably
due to the smaller amplitude b-waves in these eyes.

Flicker Responses of the Retina

To determine whether the loss of the IA-cells
altered the flicker response properties of the retina,
we recorded the responses elicited by different fre-
quencies of square wave pulses of light obtained from
an array of LEDs. The luminance of the stimulus
was $3.8 \times 10^3$ cd/m². For this study, 100 responses
were summed. The results from a 1-wk animal are
shown in the top half of Figure 6. The largest
amplitude response was elicited by the 1-Hz stimulus
for the control eye and by the 0.5-Hz stimulus for
the treated eye. With increasing frequencies, there
was a fall in the response amplitude in both eyes. At
all frequencies, the response was considerably smaller
for the treated eye.

To determine whether the difference between the
two eyes could be accounted for by the difference in
the sizes of the b-waves, the responses were normalized
for each of the eyes. The mean data for five rabbits
are shown in the bottom half of Figure 6. There was
very little difference between the two eyes when the
data were expressed in this way. This would then
Fig. 6. Effect of stimulus frequencies on the amplitude of the ERG. The values for one rabbit tested 1 wk after the treatment with 5,7-DHT are shown in the upper graph. The mean amplitudes for the five rabbits are shown in the lower graph. The amplitudes for each eye have been normalized.

indicate that the loss of the IA-cells does not alter the flicker response properties of the retina.

**Double Flash Experiments**

To examine the temporal properties in more detail, a double-flash experiment was conducted on the last four rabbits. The LED stimulus was used for this study with the stimulus duration set at 100 msec. The ISI was set at 4.0 sec, and the time between the end of one flash and the beginning of the next flash varied from 50 to 400 msec. The results from four rabbits are shown in Figure 7. The depression of the b-wave to a second stimulus has been termed suppression and unexpectedly, the suppression was greater for the control eye than for the treated eyes. A second interesting finding was that the degree of suppression in the treated eyes was strongly dependent on the time between the injection and the recordings, i.e., the suppression was weakest when the period was longer. There thus appeared to be a progressive alteration in the degree of suppression.

**Discussion**

The results of this study have shown that destruction of the IA-cells by two intravitreal injections of 5,7-DHT decreases the amplitude of the b-wave, makes the OPs more prominent and increases the duration of the b-wave. When the b-wave was used to examine the course of dark-adaptation, there was a reduction in the range but not in the rate of dark-adaptation in the treated eyes. The response of the treated retinas to flickering light was not altered by the loss of the IA-cells. In the double-flash experiments, the suppression induced by the first flash was found to be greater for the control eyes than for the treated eyes.

The normality of the rate of recovery of threshold and the flicker response function demonstrated that the 5,7-DHT was not having a generalized toxic effect on the photoreceptors and other cells in the retina. The fact that the a- and c-waves were not different in the two eyes also demonstrated the localized effect of the 5,7-DHT.

The IA-cells in the rabbit and cat receive their main input from bipolar cells in sublamina b of the IPL. In cat, Holmgren-Taylor reported that the bipolar inputs were from the rod bipolar cells. The main output of the IA-cells was back onto the bipolar cells by reciprocal synapses in rabbits and cats. The IA-cells also receive a minor input from other amacrine cells. Thus, the IA-cells appear to be involved mainly in a negative feedback type of circuit.

Can this type of neural circuitry account for the changes found? The destruction of the IA-cells will result in the loss of the negative feedback circuit. This loss of the feedback circuit should lead to an increase in the duration of the b-waves as was found. The loss should also make any oscillations present in the pathway more prominent as was observed. The difficulty with this explanation is that it requires the oscillations to be present in the bipolar cells prior to the IA-cells. Intracellular recordings from bipolar cells have not shown any signs of oscillations, although these recordings are made with the feedback circuit active. If this interpretation is correct, then it would predict that intracellular recordings from bipolar cells of retinas treated with 5,7-DHT should show oscillations superimposed on the response.

The greater prominence of the OPs in the treated eyes could also have resulted from an "unmasking"
Fig. 7. The results obtained from four rabbits to two flashes presented at different periods between the two flashes (abscissa). The suppression of the second flash in terms of R2/R1 is shown on the ordinate. The period between the injection and the experiment is shown for each rabbit.

by the smaller b-waves. The present results do not allow us to differentiate between these two choices.

The loss of the feedback circuit can also account for the differences found in the double-flash experiments. As shown, with very short periods between the flashes, the suppression of the second response was greater in the control than in the treated eye. With a bright stimulus, the duration of the response outlasts the duration of the stimulus, the so-called rod after-effect. This would mean that signals from the bipolar cells will continue to flow to the IA-cells well after the first flash is over. This will then result in a continued inhibitory feedback which should then reduce the response to the next flash. In the treated eye, the loss of the feedback circuit will result in the absence of the inhibition, and thus a greater response will be evoked.

Can the proposed neural circuit explain the depression of the b-wave in the treated eye? According to the K⁺ hypothesis of b-wave generation, the b-wave currents arise from localized Mueller cell depolarization generated by light-evoked increases in extracellular K⁺ concentration. There are two sources for the extracellular K⁺, one in the OPL and the other in the IPL. We suggest that the destruction of the IA-cells will reduce the K⁺ source in the IPL and thus lead to smaller light-evoked b-waves.

Key words: retina, electroretinogram, indoleamine-accumulating amacrine cells, oscillatory potentials, rabbit

Acknowledgments

The authors thank Mr. Orlando Navarro for his excellent assistance with the experiments, Dr. Gail S. Tucker for help
with the morphological observations, and Drs. Robert Knighton and Gregory Maguire for critical reading of this paper.

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