Diurnal Rhythm in the Human Rod ERG: Retinitis Pigmentosa

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Rod ERGs were measured at three times of day over an extensive range of retinal illuminances in six light-entrained patients with autosomal recessive or isolate forms of retinitis pigmentosa and at five times of day in six light-entrained normal volunteers. B-wave amplitude versus retinal illuminance functions from each time of day were described by determining the parameters of the best-fit Naka-Rushton function. Results from normal subjects showed that rod ERG threshold (defined as the log retinal illuminance necessary to elicit a 2.0 μV response) was elevated 1 1/2 hr after daily light-onset due to both an increase in log k (semi-saturation constant) and a decrease in log Vmax (maximum rod amplitude). The magnitude of the threshold elevation 1 1/2 hr after light-onset was comparable in patients with retinitis pigmentosa and normal subjects. Whereas thresholds returned to pre-light exposure levels rapidly during the light-phase of the daily cycle in normal subjects, thresholds continued to rise in patients with retinitis pigmentosa due primarily to a further increase in log k. These findings are consistent with abnormal rod photoreceptor disc renewal mechanisms in retinitis pigmentosa. Invest Ophthalmol Vis Sci 28:2042–2048, 1987

Patients with retinitis pigmentosa typically report night blindness at an early age and show elevations in dark-adapted visual thresholds.1–5 Rod-mediated electoretinograms (ERGs) are reduced in amplitude or non-detectable6–10 and rod outer segments (ROS) are shortened, disorganized and reduced in number.11–13 In normal vertebrates, long-term stability of ROS length depends upon a balance between the rates of rod disc shedding and disc synthesis which is regulated, at least in part, by the daily light cycle.14–19 Histological studies have documented a burst of disc shedding from the ROS tip between 1 and 2 hr after daily light onset.14–19 During the course of the day, new discs are synthesized at the base.16 The entire rhesus monkey outer segment is renewed every 10 days,20 suggesting that approximately 10% of the ROS is shed each day. An imbalance in the rates of shedding and synthesis could lead to the progressive loss of rod function found in retinitis pigmentosa.21

Previous studies suggest that electrophysiological techniques can provide functional correlates of rod renewal. In light-entrained normal pigmented rats, there is an inverse correlation between log b-wave sensitivity and log phagosome frequency during the daily cycle.22 In light-entrained human subjects, systematic variations occur in thresholds derived from full-field rod ERGs.23,24 Rod ERG thresholds are 0.13 log unit higher following light-onset than at other times of day.23 This threshold elevation is believed to reflect rod ROS shedding since the time course is similar to that documented in anatomical studies of shedding14–19 and since neither rod responses of fellow eyes23 nor rod responses in unentrained normal subjects24 vary with time of day. The entrainment process in human subjects takes 3 days; that is, by the third day of entrainment, the magnitude of the diurnal variation in rod ERG threshold is similar to that obtained following 7 or more days of entrainment.24 The threshold elevation measured 1 1/2 hr after light onset was significantly greater than predicted from a 10% shortening of ROS length and raised the possibility that factors other than reduced quantal catch influenced the magnitude of the diurnal rhythm. One purpose of the present study was to use an analysis of the rod ERG based on the Naka-Rushton function25 to isolate the component of diurnal variation in the rod ERG that relates to quantal catch and, indirectly, to shortened ROS length. Rod responses were obtained over a range of retinal illuminances from those producing a just-detectable re-
Table 1. Summary of clinical findings

<table>
<thead>
<tr>
<th>I.D. #</th>
<th>Genetic type</th>
<th>Age (yrs)</th>
<th>Acuity</th>
<th>Rod amp* (uV)</th>
<th>Cone amp† (uV)</th>
<th>Rod/cone‡ ratio</th>
<th>30 Hz amp§ (uV)</th>
<th>30 Hz IT§ (msec)</th>
<th>Dark-adapted¶ threshold</th>
<th>Visual** field</th>
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<tr>
<td>945</td>
<td>isolate</td>
<td>7</td>
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<td>57.0</td>
<td>0.32</td>
<td>17.0</td>
<td>32.5</td>
<td>2.01</td>
<td>Full†</td>
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<tr>
<td>1199</td>
<td>isolate</td>
<td>18</td>
<td>20/20</td>
<td>2.1</td>
<td>15.2</td>
<td>0.14</td>
<td>9.1</td>
<td>45.0</td>
<td>3.77</td>
<td>35</td>
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<tr>
<td>1071</td>
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<td>18</td>
<td>20/15</td>
<td>3.2</td>
<td>21.7</td>
<td>0.15</td>
<td>12.6</td>
<td>48.0</td>
<td>2.69</td>
<td>90</td>
</tr>
<tr>
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<td>27</td>
<td>20/125</td>
<td>7.0</td>
<td>12.1</td>
<td>0.58</td>
<td>6.3</td>
<td>43.0</td>
<td>2.83</td>
<td>60</td>
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<tr>
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<td>20/40</td>
<td>11.4</td>
<td>24.0</td>
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<td>16.9</td>
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<tr>
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<td>20/20</td>
<td>19.9</td>
<td>21.5</td>
<td>0.93</td>
<td>21.2</td>
<td>39.0</td>
<td>0.90</td>
<td>NA</td>
</tr>
</tbody>
</table>

* Mean normal ± 1 sd = 151 ± 38 µV; dark-adapted rod (-0.1 log scot td-sec short wavelength flash).
† Mean normal ± 1 sd = 152 ± 39 µV; computer-isolated dark-adapted cone (0.4 log phot td-sec long wavelength flash).
‡ Mean normal ± 1 sd = 1.02 ± 0.24: dark-adapted rod/dark-adapted cone.
§ Mean normal ± 1 sd = 28.9 ± 1.5 msec: cone b-wave implicit time.
¶ Log microamp equivalents (mean normal ± sd = 1.45 ± 0.24) after 45 min dark-adaptation; 11 deg white test; 7 deg above fovea—Goldmann-Weekers adaptometer.
** Equivalent circular field (degrees): Goldmann IV or equivalent (normal ± 120 deg).
NA Not available.
†† By confrontation.

Sensitivity to those producing rod saturation. A second goal of the study was to obtain functions from normal observers at additional times of day around light-onset to better delineate the time course of the diurnal variation. A third goal of the study was to obtain functions at three times of day from six patients with isolate or recessive forms of retinitis pigmentosa to assess the magnitude and/or pattern of diurnal variation and possible differences from the normal rod diurnal rhythm.

Materials and Methods

Eight adults (ages 18–49 yr; mean = 26 yr) with normal ophthalmic examinations and normal rod and cone full-field ERGs were entrained to a 14 hr light: 10 hr dark diurnal cycle for 3 days by patching one eye between the hours of 10:00 PM and 8:00 AM. Beginning on day 4 and continuing for 3 consecutive days, full-field rod ERGs were obtained at four times around daily light-onset in a subset of six subjects. Sessions lasted approximately 15 min and began at 7:45 AM (prior to daily light-onset), 8:45 AM (45 min after light onset), 9:30 AM (1½ hr after light onset) and 10:15 AM (2½ hr after light onset). A subset of six normal subjects was also tested at 4:00 PM (8 hr after light onset). Subjects were exposed for 10 min to a full-field white light (34 cd/m²) at 8:00 AM and repatched at 8:10 AM. Subjects were patched for 1 hr prior to the 4:00 PM test session. In a subset of three normal subjects, full-field rod ERGs to a single retinal illumination (~0.25 log scot td-sec) were monitored continuously in the dark beginning at 9:10 AM.

Diurnal variations in the full-field rod ERG were analyzed in six patients with isolate or recessive forms of retinitis pigmentosa following entrainment to the same 14 hr light: 10 hr dark cycle. A summary of clinical findings is shown in Table 1. Each patient showed a rod-cone form of degeneration as indexed by the ratio of dark-adapted rod amplitude to dark-adapted cone amplitude. Each patient entrained at home to a 14L:10D light cycle for 3 days prior to testing. With younger patients, the parent helped by assessing compliance and providing transportation. Each patient was given a detailed description of the study prior to signing the consent form and the patching requirements were carefully demonstrated. Patients were encouraged to note any accidental light leaks, oversleeping or other problems that could compromise entrainment but none were reported.

Pupils were maximally dilated (10% phenylephrine hydrochloride and 1% cyclopentolate hydrochloride) prior to testing. Responses were recorded from the topical anesthetized cornea with a bipolar Burian-Allen contact lens electrode (Hansen Ophthalmic, Iowa City, IA) which was inserted in dim red illumination from a head-mounted flashlight. Full-field ERGs were obtained over a 4.1 log unit range of retinal illuminances and 0.5 Hz for higher illuminances; by confrontation.

Pupils were maximally dilated (10% phenylephrine hydrochloride and 1% cyclopentolate hydrochloride) prior to testing. Responses were recorded from the topical anesthetized cornea with a bipolar Burian-Allen contact lens electrode (Hansen Ophthalmic, Iowa City, IA) which was inserted in dim red illumination from a head-mounted flashlight. Full-field ERGs were obtained over a 4.1 log unit range of retinal illuminances (approx. 0.2 log unit steps) with short-wavelength flashes (Wratten #47A; λmax = 470 nm; half-bandwidth = 55 nm). Retinal illumination was controlled by a combination of calibrated neutral density filters and intensity settings on the photostimulator. Standard conversion factors were used to convert photometric calibrations (UDT 61 optometer, United Detector Technology, Culver City, CA) to scotopic trolands. Responses were also obtained to long wavelength (Wratten #26; λ50% cut-on = 605 nm) stimuli, photometrically equated to short wavelength stimuli of 1.1 log scot td-secs and higher. Responses were amplified (gain = 10,000; 3 dB down at 2 and 300 Hz) and averaged (n ≥ 20) with a minicomputer using software to reject sweeps containing artifacts more than twice the average amplitude. Stimulus repetition rates were 1 Hz for low retinal illuminances and 0.5 Hz for higher illuminances; pilot studies have shown that there is no diminution of amplitude over time at these rates.
Fig. 1. Isolation of rod responses from mixed rod-cone responses at high retinal illuminances. Responses to short wavelength flashes over 0.75 log scot td-secs contained a small cone component. For a normal subject and patient with retinitis pigmentosa, an example is shown of the computer subtraction procedure used to isolate the rod component. In each example, the large response at the top is to a 1.66 log scot td-sec short wavelength flash. The smaller paired response is to a photometrically-matched (0.4 log phot td-sec) long wavelength flash. The result of the subtraction procedure is shown below. Eliminating the cone component has more practical importance in the patient with retinitis pigmentosa, where cones contribute a relatively larger portion of the response to the short wavelength flash. Vertical calibration bars indicate that responses from the patient are shown at four times the magnification of those from the normal subject.

Responses to short wavelength stimuli between 0.75 and 2.04 log scot td-secs included a small cone component. Over this range, rod-isolated responses were derived by computer-subtracting the response to a photometrically-matched long wavelength stimulus (Fig. 1). Rod amplitudes were measured from the peak of the corneal negative a-wave (or baseline in the absence of an a-wave) to the corneal-positive peak of the b-wave. Rod b-wave amplitude as a function of retinal illuminance was analyzed by finding the parameters of the best-fit Naka-Rushton function:

\[ \frac{V}{V_{\text{max}}} = I^n / (I^n + k^n) \]  

where \( V \) = rod b-wave amplitude, \( V_{\text{max}} \) = maximum rod b-wave amplitude, \( I \) = retinal illuminance, \( k \) = retinal illuminance at half-amplitude and \( n \) is an exponent describing the slope of the function. Parameters of each function were initially determined through non-linear analysis. Previous studies have shown that the exponent (\( n \)) is approximately 1.0 in patients with retinitis pigmentosa and normal subjects. A repeated measures analysis of variance in normal subjects showed that the exponent did not differ significantly with time of day (\( F_{3,15} = 0.09, \text{n.s.} \)). Therefore, the exponent was assumed equal to 1.0 and the parameters \( k \) and \( V_{\text{max}} \) were re-determined through linear regression (correlation coefficients ranged from 0.69 to 0.98; mean = 0.91). Rod ERG threshold (defined as the retinal illuminance necessary for a 2.0 \( \mu \text{V} \) response) was determined using the parameters of the Naka-Rushton equation. With the exponent equal to 1.0, the elevation in ERG threshold at any time of day relative to baseline before light onset is equal to the sum of the increase in \( \log k \) and the decrease in \( \log V_{\text{max}} \). A repeated measures analysis of variance of rod threshold was used to identify possible differences in the rod diurnal rhythm between patients with retinitis pigmentosa and normal subjects.

As a direct index of photoreceptor activity, rod a-wave slope was analyzed at four times of day surrounding diurnal light-onset. A-wave slope was analyzed at each time of day from the best-fit linear function between slope and retinal illuminance by finding the retinal illuminance necessary for a 1.0 \( \mu \text{V/msec} \) slope.

Results

Variations in parameters of best-fit Naka-Rushton functions from four times of day around light-onset in six entrained normal subjects are shown in Figure 2. All values were normalized relative to the 7:45 AM value for each subject. Rod b-wave threshold (log retinal illuminance necessary to elicit a 2.0 \( \mu \text{V} \) response) was elevated at 8:45 AM [\( 3/4 \) hr after light onset \( (t_5 = 6.25, P < 0.01) \)] and at 9:30 AM [\( 1/2 \) hr after light onset \( (t_5 = 2.7, P < 0.05) \)], but had recovered to near the pre-exposure value by 10:15 AM [\( 2/4 \) hr after light onset \( (t_5 = 1.09, \text{n.s.}) \)]. Because the exponent of the Naka-Rushton function was assumed to be 1.0 (linear) for the purposes of this analysis, the magnitude of the threshold elevation was independent of threshold criterion. The elevation in b-wave threshold at 8:45 AM (0.14 log unit) was associated with an increase in \( \log k \) of 0.10 log unit \( (t_5 = 9.26, P < 0.01) \) and a decrease in \( \log V_{\text{max}} \) of 0.04 log unit \( (t_5 = 2.27, \text{n.s.}) \). The elevation in threshold at 9:30 AM (0.11 log unit) was due to an average increase in \( \log k \) of 0.06 log unit \( (t_5 = 1.41, \text{n.s.}) \) and an average decrease in \( \log V_{\text{max}} \) of 0.05 log unit \( (t_5 = 1.77, \text{n.s.}) \).

Results from three entrained normal subjects who were tested repeatedly with a single retinal illuminance are shown in Figure 3. Following light expo-
sure at 8:00 AM, amplitudes initially increased to approximately the pre-exposure value within 1 hr of daily light onset. A sharp amplitude decline began approximately 80 min after light onset in each subject and reached a minimum at 9:40 AM. The magnitude of the decline averaged approximately 20% of the pre-exposure amplitude. One subject was able to continue wearing the contact lens electrode comfortably and showed a return toward the pre-exposure value beginning at 9:50 AM. Open triangles with standard error bars show the results at the same retinal illuminance from six subjects tested at three discrete time intervals. Also shown is the time course of amplitude increase following light exposure in the absence of light entrainment (dashed curve).

A reduction was also seen in rod a-wave slope at 8:45 AM and 9:30 AM at each retinal illuminance. Five subjects had measurable rod a-waves over a sufficient range of retinal illuminances to determine threshold. The mean retinal illuminance (±1 standard error) necessary for a criterion slope (1.0 \mu V/msec criterion) showed a similar diurnal pattern of variation to that necessary for a criterion rod b-wave amplitude (Fig. 4), with thresholds elevated at 45 min \((t_4 = 2.98, P < 0.05)\) and 1½ hr \((t_4 = 2.98, P < 0.05)\) after light onset and returning to near pre-exposure levels within 2½ hr of light onset \((t_4 = 1.82, \text{n.s.})\).

Rod responses obtained over 1 day of testing are shown in Figure 5 for a representative normal subject (top) and a patient with retinitis pigmentosa (bottom). Both the normal subject and the patient with retinitis pigmentosa show a drop in b-wave amplitude at 9:30 AM. Amplitudes returned to pre-light onset
Fig. 6. Naka-Rushton functions based on responses shown in Figure 6. Top: Threshold elevation at 9:30 AM in the normal subject is due to both an increase in log k (horizontal shift) and a decrease in log Vmax (vertical shift). Bottom: Threshold is highest in the patient with retinitis pigmentosa at 4:00 PM due primarily to a large increase in log k (horizontal shift).

levels by 4:00 PM in the normal subject, but were even lower at 4:00 PM than at 9:30 AM in the patient with retinitis pigmentosa. Naka-Rushton functions (solid lines) describing each response series are shown for the normal subject and patient with retinitis pigmentosa in Figure 6. The decrease in amplitudes at 9:30 AM in both the patient and the normal subject was due to both an increase in log k and a decrease in log Vmax. The additional decrease in amplitude at 4:00 PM in the patient was due primarily to an additional increase in log k. In five patients with responses across a sufficient range for the Naka-Rushton analysis, the threshold elevation at 9:30 AM was associated with an increase in log k of 0.08 log unit ($t_4 = 1.43$, n.s.) and a decrease in log Vmax of 0.04 log unit ($t_4 = 2.87$, $P < 0.05$). The additional increase in threshold at 4:00 PM was associated with an increase in log k of 0.23 log unit ($t_4 = 2.3$, $P < 0.05$) and a decrease in log Vmax of 0.03 log unit ($t_4 = 0.48$, n.s.). Normalized mean thresholds (±1 standard error) are shown in Figure 7 for all three days of testing in six normal subjects and six patients with retinitis pigmentosa. A repeated measures analysis of variance (Table 2) of the actual thresholds showed a significant main effect of diagnosis (retinitis pigmentosa versus normal), with patients having significantly higher thresholds than normals at all times of day. The main effect of time of day was also significant, with both patients and normal subjects showing diurnal variation. The analysis also revealed a significant interaction effect, with patients exhibiting a different diurnal pattern than normal subjects.

Table 2. Analysis of variance

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<th>df</th>
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<th>$F$</th>
<th>$p$</th>
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<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Between subjects</td>
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<td>11</td>
<td>—</td>
<td>—</td>
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<td>Diagnosis (normal vs r.p.)</td>
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<td>31.489</td>
<td>23.377</td>
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<tr>
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<td>Time of day</td>
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<tr>
<td>Time of day X diagnosis</td>
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<td>Error$_w$</td>
<td>0.288</td>
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<td>0.014</td>
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Discussion

Light-entrained normal subjects show a significant elevation in rod ERG threshold following daily light onset. This finding was replicated in the present study with eight additional normal subjects. The present results further delineate the time course. Amplitudes initially recovered to pre-exposure levels following daily light onset, ruling out an explanation of the diurnal decline in terms of incomplete dark adaptation. In these well-entrained subjects, the reduction in amplitude occurred over a brief period of time and in all subjects, thresholds had recovered to near pre-light exposure values within 2½ hr of light onset. A similar diurnal decrease in b-wave amplitude has been reported recently in the light-entrained rat and rabbit.

The Naka-Rushton analysis of rod b-wave amplitude versus retinal illuminance functions at each time of day revealed at least two factors contributing to the threshold elevation at 9:30 AM. Normal subjects showed an average increase in log k of 0.06 log unit. Since increases in log k result from factors that decrease quantal catch, this finding is consistent with a 10% reduction in ROS length which has been suggested by previous studies in monkey. The average decrease in log Vmax 1½ hr after daily light onset was 0.05 log unit, which represents a 10% decrease in maximum rod amplitude during the period of shedding. Previous studies have shown that reduced log Vmax values are found in delimited retinal diseases that cause regional loss of rods and in normal subjects when the extent of the retinal stimulus is decreased. One possible explanation for the diurnal decrease in log Vmax might be a temporary inability for rods to generate responses during the active shedding phase. Consistent with this suggestion is the rapid recovery of log Vmax by 10:15 AM.

Previous studies showed that the percent reduction in rod a-wave amplitude 1½ hr after light onset was approximately equal to the percent reduction in rod b-wave amplitude. In the present study, a-wave slope was analyzed to further determine whether the diurnal variation in the rod b-wave originates at the photoreceptor level. A-wave slope, unlike a-wave amplitude, is independent from variations in b-wave amplitude and timing and is, therefore, a “pure” index of photoreceptor activity. The present results showed that the threshold variation in a-wave slope (retinal illuminance necessary for 1.0 μV/msec slope) paralleled the diurnal variation in rod b-wave amplitude.

The magnitude of the threshold elevation at 9:30 AM was comparable in patients with retinitis pigmentosa to that seen in normal subjects (Fig. 7). In both patients and normal subjects, the threshold elevation was due to both an increase in log k and a decrease in log Vmax. The increase in log k was not significantly different between patients and normals, suggesting an equivalent fractional decrease in quantal catch in each group. In the context of ROS shedding, these results suggest that normal subjects and patients with retinitis pigmentosa lose approximately an equal proportion of ROS length within 1½ hr of light onset. Pre-light-exposure values of log k in patients ranged from 0.23–1.06 log units higher than mean normal, suggesting shorter than normal ROS length. Thus the absolute number of ROS discs shed by patients within 1½ hr of light onset must be lower than in normal subjects.

Whereas thresholds returned to near pre-exposure levels by 10:15 AM in normal subjects, thresholds were higher at 4:00 PM than at 9:30 AM in patients with retinitis pigmentosa. The additional threshold increase at 4:00 PM was associated with an increase in log k of 0.23 log unit over pre-exposure values. At least part of this increase in log k during the day in patients with retinitis pigmentosa could be due to a prolonged period of shedding following light onset. Previous studies suggest a slowed or reduced rate of ROS disc synthesis during daylight hours in at least one genetic type of retinitis pigmentosa. It has also been suggested that shedding could result in rod de-sensitization analogous to that which occurs in response to light adaptation. Regardless of the origin of this prolonged decrease in sensitivity, the results of this study demonstrate abnormalities in the rod diurnal rhythm in patients with recessive and isolate forms of retinitis pigmentosa.

Note added in proof: Rod ERG diurnal rhythms were measured in three affected siblings from a family with dominant retinitis pigmentosa (Sandberg MA, Baruzzi CA, Hanson AH, and Berson EL. Invest Ophthalmol Vis Sci 25(Suppl):196, 1984). Functions relating b-wave amplitude to log retinal illuminance were shallower than normal immediately before light onset of 13 hr after light onset.

Key words: retinitis pigmentosa, rod, electroretinogram, diurnal rhythm, entrainment

References


