Analysis of ERG a-Wave Amplification and Kinetics in Terms of the G-Protein Cascade of Phototransduction

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Purpose. To test rigorously the hypothesis that the a-wave of the electroretinogram (ERG) is proportional to the rod photocurrent by examining the applicability to a-waves of a recent model of the activation steps in the G-protein cascade of phototransduction.

Methods. ERGs were recorded in response to flashes of graded intensity, from six dark-adapted normal subjects and from two patients, one with retinitis pigmentosa (RP) and one with cone retinal dystrophy with rod involvement (CRD). The a-wave portions of the responses were analyzed with a model of the activation steps of the G-protein cascade. The model is characterized by a parameter, $A$, the amplification constant, with units of $s^{-2}$ (per photoisomerization), which may be expressed as the product of physical and biochemical parameters of the transduction cascade.

Results. Each a-wave family was well described by the model. For the six normal subjects, we obtained $A \approx 7 \text{ s}^{-2}$, about 100-fold greater than in isolated amphibian rods at 22°C, but close to the value for isolated primate rods. For the patient with RP, the maximum a-wave amplitude ($a_{max}$) was considerably reduced, but the amplification constant was normal ($A = 7.5 \text{ s}^{-2}$). In contrast, the patient with CRD had a nearly normal $a_{max}$ but had an amplification constant about sixfold lower than normal ($A = 1.1 \text{ s}^{-2}$).

Conclusions. The authors conclude that the a-wave is a direct reflection of the rod photocurrent and that the rising phase kinetics are accurately described by a simple model of the G-protein cascade. They show that the small volume of the human rod outer segment is crucial to the achievement of high amplification, and they show how their observations constrain the possible pathologies of phototransduction in patients with retinal disease.

The a-wave of the electroretinogram (ERG) recorded in response to a brief flash has long been known to reflect the collective responses of the rod photoreceptors. Features supporting this identification include the continued presence of the a-wave when synaptic transmission from photoreceptors to subsequent retinal neurons is blocked by pharmacologic agents as well as the kinetics, light-sensitivity, and source-sink distribution of the a-wave. Despite the knowledge that the a-wave originates in the receptor photocurrents, clinical application has been hampered by a number of problems, including intrusion by the b-wave, which effectively obscures the a-wave in a complex time- and light-dependent manner (shown later in Figure 3A).

Recent advances in analysis of the a-wave and in the understanding of the biochemistry and electrophysiology of phototransduction now point the way to a quantitative assessment of rod photoreceptor function through analysis of the a-wave. The investigations of Hood and Birch and Breton and Montzka have shown that, with suitably intense flashes, the maximal amplitude of the a-wave can be determined, and that normalization of a family of a-wave responses to this maximum allows direct comparison (up to the point of b-wave intrusion) with theoretically predicted rod photocurrents. Recently, two of us have analyzed the activation steps of the G-protein cascade theory of...
phototransduction and have formulated a model of the cascade that accounts quantitatively for the kinetics and amplification of the rising phase of the rod photocurrent.⁹

In this paper, we apply that analytic formulation to families of clinically recorded a-wave responses, and we demonstrate that the a-waves exhibit the same kinetics and amplification as do the photocurrents of single primate rods. In particular, a gain parameter that we call the "amplification constant" is found to be similar for the human a-wave and the primate rod photocurrent but is about 100-fold higher than in amphibian rods. We describe how our analysis can shed light on the molecular locus of defects underlying clinical conditions.

MODEL OF THE G-PROTEIN CASCADE

The G-protein cascade is now established as the mechanism of transduction in vertebrate rods and cones, and a simplified biochemical model of the activation steps is shown in Figure 1. Upon photoisomerization, rhodopsin becomes an active enzyme, Rh*, which catalyzes the binding of GTP to the α-subunit of the rod G-protein (alias transducin), converting it to an active form, G*. The subsequent binding of G* to the rod phosphodiesterase (PDE) in turn converts this enzyme to an active form, PDE*, which catalyzes the hydrolysis of cGMP. In darkness, the resting concentration of cGMP holds open cation channels in the plasma membrane, allowing a steady influx of Na⁺ and Ca²⁺ ions (the "dark current"). Upon activation of PDE*, the free concentration of cGMP drops, closing the cGMP-gated channels and decreasing the influx of positive current. This hyperpolarizes the cell and decreases the release of transmitter from the synaptic terminal.

Equations for Activation

There are thus five major activation steps in the G-protein cascade: Rh* production; G* production; PDE* production; cGMP hydrolysis; and cGMP-channel closure, with its attendant reduction of the circulating current. The five corresponding variables, Rh*(t), G*(t), PDE*(t), cG(t), and F(t), are defined in Table 1. The analysis of Lamb and Pugh⁹ has shown that, during the activation phase of the photoresponse to flashes of moderate intensity, these variables should be predicted by the following equations:

\[
Rh^*(t) = \Phi \left[1 - \exp\left(-t/t_{Rh}\right)\right] \tag{1}
\]

\[
G^*(t) = \Phi v_{RG} \left(t - t_{RG}\right), \quad \text{for } t > t_{RG} \tag{2}
\]

\[
PDE^*(t) = \Phi v_{RP} \left(t - t_{RGP}\right), \quad \text{for } t > t_{RGP} \tag{3}
\]

\[
cG(t) = \frac{cG(t)}{cG_{dark}} = \exp\left[-\frac{1}{2} \Phi v_{RP} \beta_{sub} \left(t - t_{RGP}\right)^2\right], \quad \text{for } t > t_{RGP} \tag{4}
\]

\[
F(t) = \frac{cG(t)}{cG_{dark}} = \exp\left[-\frac{1}{2} \Phi A \left(t - t_{eff}\right)^2\right], \quad \text{for } t > t_{eff} \tag{5}
\]

The form of these predictions is illustrated in Figure 2, and the symbols are defined in Table 1.

Equation (5) is the overall description of the electrical response. It gives the fraction of circulating current as a function of time in terms of the number of photoisomerizations, Φ, and two composite parameters of transduction: the amplification constant, A (see below), and an effective delay time, t_{eff}.
TABLE 1. Variables and Parameters of the Activation Model of Phototransduction

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Value or Range Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_{\text{enc}} )</td>
<td>Diffusional encounter rate between G-protein and a single Rh*</td>
<td>( s^{-1} ) 17000</td>
</tr>
<tr>
<td>( v_{CG} )</td>
<td>Rate of production of G* by a single Rh*</td>
<td>( s^{-1} ) 10000</td>
</tr>
<tr>
<td>( \beta_{CG} )</td>
<td>Coupling gain for the activation of PDE* by G*</td>
<td>( \approx 0.8 )</td>
</tr>
<tr>
<td>( \beta_{PDE} )</td>
<td>Rate of production of PDE* due to a single Rh*</td>
<td>( s^{-1} ) 8000</td>
</tr>
<tr>
<td>( \beta_{\text{sub}} )</td>
<td>Rate constant of a single catalytic subunit of PDE in a well-stirred volume</td>
<td>( s^{-1} ) 3 ( \times ) 10^{-4}</td>
</tr>
<tr>
<td>( 2k_{\text{eff}} )</td>
<td>Turnover number of PDE with two fully activated catalytic subunits</td>
<td>( s^{-1} ) 4000</td>
</tr>
<tr>
<td>( K_{\text{m}} )</td>
<td>Michaelis constant of fully activated PDE</td>
<td>( \mu M ) 100</td>
</tr>
<tr>
<td>( V_{\text{cyt}} )</td>
<td>Volume of the outer segment cytoplasm; ( \approx \frac{1}{2} V_{\text{envelope}} )</td>
<td>pl 0.04</td>
</tr>
<tr>
<td>( BP )</td>
<td>Buffering power of the cytoplasm for cGMP</td>
<td>( \approx 2 )</td>
</tr>
<tr>
<td>( N_{A} )</td>
<td>Avogadro's number</td>
<td></td>
</tr>
<tr>
<td>( n )</td>
<td>Hill coefficient of opening of the cGMP-activated channel</td>
<td></td>
</tr>
<tr>
<td>( A )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( t_{\text{eff}} )</td>
<td>Effective delay in phototransduction</td>
<td></td>
</tr>
<tr>
<td>( t_{p} )</td>
<td>Radial diffusion of cGMP in the outer segment, and closure time of the cGMP-activated channels.</td>
<td></td>
</tr>
<tr>
<td>( t_{\text{chan}} )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( a_{\text{max}} )</td>
<td>Maximum amplitude of the a-wave</td>
<td>( \mu V ) -500</td>
</tr>
</tbody>
</table>

† For the variables, the entry in this column is the range of the variable; in particular, for the proteins, the upper limit of the range is the total number of such proteins in the outer segment. For the parameters, the column entry is an estimate based upon published literature. For review and discussion of the particular values in the table, see references 9 and 11.

Effective Delay Time. In equation (5), the effective delay in phototransduction, \( t_{\text{eff}} \), represents the sum of the several brief delays:

\[
t_{\text{eff}} = t_{R} + t_{G} + t_{P} + t_{r} + t_{\text{chan}}.
\]

Here \( t_{R} \), \( t_{G} \), and \( t_{P} \) are delays in the formation of the active species Rh*, G*, and PDE*. Of these, \( t_{R} \) in equation (1) is the time constant of formation of metarhodopsin II (the initial form of which corresponds to the active enzyme Rh*); in mammalian rods at body temperature, \( t_{R} \) is about 0.25–0.5 ms. In equations (2) to (4), the cumulative delays are given by \( t_{CG} = t_{R} + t_{G} \) and \( t_{PDE} = t_{R} + t_{G} + t_{P} \). The two additional short delays in equation (6) are: \( t_{r} \), accounting for radial diffusion of cGMP in the outer segment, and \( t_{\text{chan}} \), the closure time of the cGMP-activated channels.

The effective delay expressed in equation (6) is applicable under voltage-clamped conditions, but in the normal "unclamped" cell an additional delay will be contributed by the cell's electrical time constant, \( t_{m} \). Further delays may also be contributed by circuit elements subsequent to the photoreceptors: in particular, by the electrical recording equipment. Later, in equation (11), we use the symbol \( t_{\text{eff}} \) to represent the total effective delay in the complete path up to and including the recording equipment, i.e., it equals \( t_{\text{eff}} \), defined above for the transduction process, plus these possible additional delays. Thus, the experimentally observed \( t_{\text{eff}} \) represents an upper bound for \( t_{\text{eff}} \), the delay in the transduction process. Our a-wave results yield values of \( t_{\text{eff}} \) in the range 1–4 ms (see Results), consistent with the expectation that each of the individual delays in equation (6) should be very short.

Protein Activation Rates. The parameter \( v_{CG} \) in equation (2) represents the rate at which a single Rh* activates G-proteins. This rate is predicted to be approximately constant during the rising phase of the
photocurrent response, and experimental results have suggested that \( \nu_{RG} \) may exceed 10,000 G* s\(^{-1}\) per Rh* in the mammalian rod at body temperature. The parameter \( \nu_{RG} \) in equations (3) to (5) represents the rate at which activated catalytic subunits of PDE are produced per Rh*; this rate depends upon the intermediate step in which a G* binds to one of the two \( \gamma \)-subunits associated with the two catalytic (\( \alpha \)-, \( \beta \)-) subunits of the PDE (see Figure 1). The binding of the G* relieves an inhibitory constraint imposed by the \( \gamma \)-subunit, activating the corresponding catalytic subunit. The theory predicts the rate \( \nu_{RP} \) also to be approximately constant and expresses it in terms of the rate of G-protein activation as

\[
\nu_{RP} = \nu_{RG} c_{GP}.
\]

where \( c_{GP} \) is a “coupling coefficient” expected to be near unity in magnitude.

**Cyclic GMP Concentration.** Equation (4) is derived by solving the differential equation governing the synthesis and hydrolysis of cGMP in the rod. It is expected to provide an accurate description of the rising phase of a-waves for flashes of moderately high intensity. The parameter \( \beta_{sub} \) in equation (4) represents the rate constant of cGMP hydrolysis, produced by a single catalytic subunit of PDE in a well-stirred volume equal to that of the outer segment, that is

\[
\beta_{sub} = \frac{k_{on}/K_m}{N_{Av} V_{cyc} BP}.
\]

The parameters in this expression are described in Table 1. \( \beta_{sub} \) captures the gain contributed by a single catalytic subunit of the PDE and has units of s\(^{-1}\) per PDE*. Based on biochemical data from mammalian rods, it is predicted to have a value of \( \beta_{sub} \approx 3 \times 10^{-4} \) s\(^{-1}\) (Table 1).

**Circulating Current.** The first line of equation (5) follows from the Hill equation, which governs the relation between cG and the fraction of channels open; \( n \) is the Hill coefficient, taken as 3. The second line of equation (5) follows by substitution of equation (4), whereas the final line simply collects the three amplification parameters into a single composite parameter, with units of s\(^{-2}\),

\[
A = \nu_{RP} \beta_{sub} n
\]

which we call the amplification constant. This parameter completely characterizes the amplification of transduction for a given photoreceptor. Estimation of \( A \) for human rods in vivo is a principal goal of our investigation.

**The a-Wave.** The fundamental hypothesis underlying our investigation (and the investigations of others...
who have used a-waves to assess rod function) is that the normalized a-wave, \( \frac{a(t)}{a_{\text{max}}} \), is equal to the normalized rod photocurrent, i.e.

\[
a(t) = R(t),
\]

(10)

where \( a_{\text{max}} \) is the maximal a-wave amplitude, and the normalized photocurrent \( R(t) \) is the complement of \( F(t) \) in equation (5). Equations (5) and (10) can be combined in a convenient form that allows comparison between theory and experiment:

\[
1 - \left[ \frac{a(t)}{a_{\text{max}}} \right] = F(t)
\]

\[
= \exp \left[ -\frac{1}{2} \Phi \left( t - t_{\text{eff}} \right)^2 \right].
\]

(11)

Equation (11) predicts that each member of the family of responses \( 1 - \frac{a(t)}{a_{\text{max}}} \) should trace out a delayed gaussian function of time, whose width from \( t_{\text{eff}} \) (at any criterion response amplitude) should be inversely proportional to \( \Phi \). A-wave families are thus characterized by two composite parameters, \( A \) and \( t_{\text{eff}} \), that can be interpreted in terms of the biochemistry of the G-protein cascade.

**Maximum Rate of Rise and Rate Saturation of the a-Wave.** An important measure of each a-wave response is its maximum rate of rise (Figures 5B and 7). From equation (11) it is possible to derive the maximum rate of rise, \( \frac{dR}{dt}_{\text{max}}(\Phi) \) at intensity \( \Phi \), and this is given by equation (6.13) of reference 9 as

\[
\frac{dR}{dt}_{\text{max}}(\Phi) = \sqrt{A\Phi/e}.
\]

(12)

For \( A \) constant, the maximum rate of rise is thus proportional to \( \sqrt{\Phi} \). Experimentally, it is found that the maximum rate of rise of the a-wave does not increase indefinitely but saturates at high intensity, although such saturation is not predicted by equation (12). In the Results section, we show that the measured decline in amplification constant \( A \) at high intensity is consistent with a simple model of rate saturation (p. 305).

**Altered Outer Segment Length.** In presenting our results, we calculate the amplification constant \( A \) from the ocular parameters and receptor dimensions applicable for an “average” normal observer (see p. 301 and Table 1). This is equivalent to referring \( A \) to the incident illumination at the cornea, rather than to the number of photoisomerizations per rod. We now consider the effect of altered outer segment length (in the absence of any other differences) on the measured amplification constant. For a subject with outer segments of half the normal length, the number of photoisomerizations per outer segment produced by a flash of given retinal illuminance will be roughly halved from normal, assuming the effects of self-screening to be relatively small. In addition, though, the cytoplasmic volume \( V_{\text{cyt}} \) will be halved, thereby doubling the magnitude of \( \beta_{\text{sub}} \) in equation (8). These two effects will have opposite, but approximately equal, effects on the values of \( A \) measured in this paper. Hence, for a subject with altered outer segment length but no other pathology, the amplification constant \( A \) (calculated using corneal intensity and the normal intensity conversion factor) should appear unaltered from normal.

**METHODS**

**Subjects**

a-Waves were recorded from six normal adults (NL1 to NL6), one patient with cone retinal dystrophy with rod involvement (CRD) and one patient with retinitis pigmentosa (RP) (see Table 2). The protocol conformed to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the Presbyterian Medical Center of Philadelphia. Informed consent was obtained from all subjects after an explanation of the procedure and its risks and possible benefits.

**Patient CRD.** Patient CRD was a 53-year-old woman, color blind from childhood, who reported low visual acuity (OD 6/15, OS 6/7.5 at the time of testing). She had clear ocular media. A retinal examination was performed with pupils dilated; this revealed retinal pigment epithelial changes (“beaten metal” appearance) and visible changes in the macular region. No flecks or pigmentary changes typical of retinitis pigmentosa were present. The patient correctly identified only 1 in 10 color plates. The ERG showed a rod a-wave response with maximum amplitude of \(-339 \mu V\) (Figure 6A; Table 2) toward the lower end of the range found for the six normal observers (320–545 \( \mu V \)). However, she had no recordable response to a 30 Hz white stimulus (100–125 \( \mu V \) peak-to-peak response for normal subjects), indicative of cone dysfunction.

**Patient RP.** Patient RP is a 33-year-old woman who had mild problems with night vision. She reported that her mother underwent a progressive loss of vision, with a total loss of vision by age 59. Visual acuities were 6/6 OU at the time of testing. Ophthalmoscopic examination showed clear ocular media with no vitreous debris. The optic nerve head exhibited no waxy pallor. There were symmetric inferonasal sectors of pigmentary changes with bone spicules and attenuated retinal vasculature OU. The remainder of the retina appeared entirely normal. The ERG response evoked with a single white flash showed considerably reduced a-wave and b-wave maximum amplitudes (Figure 6C; Table 2),
### TABLE 2. Estimation of the Amplification Constant of Phototransduction

<table>
<thead>
<tr>
<th>Subject/Status</th>
<th>Age (yrs)</th>
<th>Figures (traces)</th>
<th>Symbol (Fig. 5)</th>
<th>$a_{text}$ (µV)</th>
<th>$dR/dt$</th>
<th>$t_{A}$ *</th>
<th>Range $log_{10}$</th>
<th>A</th>
<th>$t_{A}$ *</th>
<th>Range $log_{10}$</th>
<th>A</th>
<th>$t_{A}$ *</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1.1/W</td>
<td>27</td>
<td>3A, 3B</td>
<td>○</td>
<td>520</td>
<td>750</td>
<td>2.6-4.2</td>
<td>8.7</td>
<td>2.7</td>
<td>2.6-4.2</td>
<td>8.2</td>
<td>2.3</td>
<td>2.6-4.2</td>
</tr>
<tr>
<td>N1.1/B</td>
<td>27</td>
<td>4D</td>
<td>○</td>
<td>490</td>
<td>—</td>
<td>2.9-4.4</td>
<td>7.0</td>
<td>2.6</td>
<td>2.3-4.2</td>
<td>6.5</td>
<td>2.7</td>
<td>2.3-4.2</td>
</tr>
<tr>
<td>N1.2</td>
<td>18</td>
<td>4A, 7A</td>
<td>□</td>
<td>545</td>
<td>579</td>
<td>3.4-5.1</td>
<td>5.8</td>
<td>2.7</td>
<td>2.5-4.2</td>
<td>6.1</td>
<td>2.7</td>
<td>2.5-4.2</td>
</tr>
<tr>
<td>N1.3</td>
<td>29</td>
<td>4B, 7B</td>
<td>△</td>
<td>488</td>
<td>514</td>
<td>3.0-4.8</td>
<td>5.5</td>
<td>3.4</td>
<td>2.4-4.5</td>
<td>6.2</td>
<td>3.4</td>
<td>2.4-4.5</td>
</tr>
<tr>
<td>N1.4</td>
<td>42</td>
<td>4C</td>
<td>▼</td>
<td>392</td>
<td>654</td>
<td>2.5-4.2</td>
<td>8.2</td>
<td>3.6</td>
<td>2.5-4.2</td>
<td>7.3</td>
<td>3.3</td>
<td>2.5-4.2</td>
</tr>
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<td>22</td>
<td>—</td>
<td>*</td>
<td>500</td>
<td>568</td>
<td>3.2-4.5</td>
<td>6.5</td>
<td>2.5</td>
<td>3.2-4.5</td>
<td>5.3</td>
<td>1.3</td>
<td>3.2-4.5</td>
</tr>
<tr>
<td>N1.6</td>
<td>26</td>
<td>—</td>
<td>□</td>
<td>420</td>
<td>580</td>
<td>2.4-4.8</td>
<td>7.3</td>
<td>1.5</td>
<td>2.4-4.5</td>
<td>7.1</td>
<td>1.3</td>
<td>2.4-4.5</td>
</tr>
</tbody>
</table>

Mean ± SD*  
463 ± 81  | 608 ± 82 | 6.9 ± 1.1  | 2.6 ± 0.7  | 6.6 ± 0.8  | 2.4 ± 0.9

**CRD**  
53        | 6A, 6B, 7D | ▼ | 399 | 512 | 3.0-5.2 | 1.1 | 3.7 | 3.0-5.2 | 1.2 | 4.0

**RP**  
33        | 6C, 6D | ▲ | 177 | — | 3.0-4.7 | 7.5 | 3.5 | 2.5-3.9 | 7.2 | 2.4

*In computing the mean ± SD, the two estimates of each parameter obtained from the two sets of responses of subject N1.1 (N1.1/W and N1.1/B) were averaged and treated as a single value.

Column 1 shows the identification of the subjects and their status: N1.1–N1.6, normal observers; CRD, a patient with cone retinal dystrophy; RP, a patient with retinitis pigmentosa (see Methods for further description). For observer N1.1, a complete set of responses was obtained with both a white (W) and a blue (B) stimulus (see Methods and Figures 3 and 4). Column 3 shows the figures in the text in which ERG data from the subject appears; column 4 shows the symbols used in Figures 5A and 5B for each subject. Column 5 shows the maximum amplitude of the a-wave response, obtained from the responses to the most intense flashes. Column 6 shows the saturated rate of rise of the a-wave, determined from the responses to very intense flashes (see Figures 7 and 5B); a dash in this column indicates that the subject was not stimulated with a flash sufficiently intense to produce a saturated rate of rise. Column 7 shows the flash intensity range over which the individual traces were fitted to obtain the mean parameters of best fit given in columns 11 and 12 (see Methods).

* In computing the mean ± SD, the two estimates of each parameter obtained from the two sets of responses of subject N1.1 (N1.1/W and N1.1/B) were averaged and treated as a single value.

indicating reduced rod function. Response to 30 Hz flicker showed reduced amplitude (57.5 µV peak-to-peak, compared to a norm of 100–125 µV) but normal implicit times (27 ms as compared to a norm of 28 ms), indicating decreased cone function as well. Visual fields tested on the Humphrey automated perimeter with the 30/60 peripheral and central 30-2 threshold tests showed localized defects in the peripheral supero-temporal visual field of each eye; the location of these functional defects corresponded to the retinal locations showing pigmentary changes ophthalmoscopically. Goldman-Weekers dark adaptation tests showed rod and cone thresholds in the normal range, with a normal cone-rod break, when the target did not fall on the affected retinal areas. The clinical diagnosis was sector retinitis pigmentosa.

**ERG Recording**

**System.** The ERG-recording system was an LKC Systems EPIC-1000 instrument (Gaithersburg, MD) consisting of a ganzfeld for full-field stimulus presentation, two xenon flash units, an interface for computer and stimulators, and recording amplifiers. One flash unit (Grass Photostimulator, Grass Instruments, Quincy, MA) was used for low-to-medium intensities and repetitive stimulation; the second (Vivitar Photoflash, Vivitar Corp., Santa Monica, CA) was used only for high-intensity stimulation with single flashes separated by intervals. A PC/AT-class computer with Tecmar LabTender (Tecmar Inc. Solon, OH) board was used to control visual stimulation and for analog-to-digital conversion.

**Procedure.** ERGs were recorded in a room completely darkened except for a dim red light illuminating the keyboard. Each subject was dark adapted for at least 30 minutes, and recordings were made from one eye only. Neosynephrine (10%) and tropicamide (1%) drops were used to dilate the pupil. Topical anesthetic was instilled on the cornea, and 2.5% hydroxypropyl methylcellulose gel was used on the inner contact lens surface to protect the cornea. The recording lens was a Jet unipolar disposable type (Universo SA, Switzerland), with an annular gold laminate on the inner surface for electrical contact. The reference electrode was placed on the temple and a ground electrode on the forehead.

**Electrical Recording.** The recording amplifiers were AC-coupled (0.3 Hz) and low-pass filtered, typically at 500 Hz (2-pole Butterworth). To minimize distortion of responses to intense flashes, the high-frequency cutoff was sometimes extended to 1500 or 3000 Hz (Figure 7). Signals were digitized at 0.25- to 0.5-ms intervals by an 8-bit A/D converter and were stored on disc for processing. A baseline was recorded.
with each response, and the zero level was taken as the mean over the 15 ms preceding the flash.

Optical Stimulation

Ganzfeld. The ganzfeld was a plastic sphere 40 cm in diameter, whose interior surface was coated with a highly reflective matte white paint. Two circular apertures were cut out: one, 28 cm in diameter at the equator, was a viewing port; the second, 5 cm in diameter at the north pole, was for illumination. Head position in the ganzfeld was maintained with a chin rest that placed the eyes at about the equator, slightly inside the sphere. A fixation target was provided by a small, red light-emitting diode placed at the equator opposite the viewing port.

Stimulus Control. Flash intensity was varied over 6 log units by means of two different flash units, together with neutral density filters available in steps of 0.2 log units. Filters were calibrated with an IL-700 photometer (International Light, Newburyport, MA), or with an EG&G DR2550 photometer (Gamma Scientific, San Diego, CA). Flashes of low intensity (below about 300 scot td s) were produced by the Grass Photostimulator having a flash duration of 20 μs. Flashes of higher intensity were produced with the Vivitar Photoflash unit, which had a longer duration (1.5 ms at 90th percentile); the most intense Vivitar flash corresponded to 141,000 scot td s. The useful intensity ranges covered by the two flash units overlapped by about 1 log unit. The interval between flashes was closely controlled for the upper two log units of intensity to ensure complete recovery of response amplitude.

Experiments were performed in two locations, at the Scheie Eye Institute and at the Children’s Hospital of Philadelphia; a complete ganzfeld and dual stimulator (Grass and Vivitar) were available at each location. The unattenuated flashes of each instrument were independently calibrated. For three observers (NL1/W, NL4, and CRD), the attenuated Vivitar flashes that produced a-wave responses equivalent to that produced by the unattenuated Grass flash were slightly different from the scotopic equivalence expected from the standard calibrations. For analyzing the responses of these observers to the Grass flashes, corrections (0.15 log units for NL1/W, 0.14 for NL4, and 0.22 for CRD) were made to the scotopic intensity assigned to the unattenuated Grass flash, so that the Grass and Vivitar flashes producing equivalent responses were considered scotopically equal in intensity. Such differences between subjects in the equivalence of the flash units might have arisen from differences in the color of the ocular media, or perhaps from an experimental error that occurred on the day of testing.

For one set of experiments, a short-wavelength “blue” bandpass filter (Wratten 47B, Kodak, Rochester, NY) was used, which gave a scotopic effective λ of 450 nm. The blue filter served to minimize the contribution of cones to the ERG.8,15

Intensity Calibration

The intensity of the flash viewed in the ganzfeld was calibrated in scotopic photometric units using the EG&G DR2500 photometer.8 By aiming the photometer at different angles, the illumination was found to be uniform to within 15%. No intensity corrections were made for the clear contact lens; its aperture, 7 mm in diameter, served as an artificial pupil whose area (38.5 mm²) was used in computing the troland s values.

The conversion from flash intensity at the cornea to units of photoisomerizations rod⁻¹ per flash (i.e., Φ) followed that in Wyszecki and Stiles16 (equation (20)2.4.4) and Schnapf et al17 (equations 1 to 3), and used the equation

$$\Phi = Q k(\lambda) \tau(\lambda) f \frac{\pi d^2}{4} \left[1 - 10^{\frac{-D(\lambda)}{200}}\right] = QK$$  \hspace{1cm} (13)

where Q is the retinal illumination in scot td s. Because the overall conversion factor K in equation (13) is critical in determining the amplification constant, we present our choice of the underlying parameters.

At an effective wavelength of λ = 493 nm, and for an eye with posterior nodal distance 16.7 mm, k(λ) = 5.64 and the preretinal media transmissivity is $\tau(\lambda) = 0.67$, so that k(λ) $\tau(\lambda) = 3.78$ photons s⁻¹ μm⁻² per scotopic troland.16,18 The remaining product of factors, $f(\pi d^2/4) \left[1 - 10^{-D(\lambda)}\right] \gamma$ in equation (13) represents the “end-on” collecting area of a single rod in μm². f is the increase in light collection due to funneling from the inner segment, which we take as 1.8, d is the diameter of the rod outer segment,19 taken as 2 μm, and D(λ) is the end-on optical density of the outer segment, taken as 0.40 log₁₀ units at 493 nm. γ is the quantum efficiency of isomerization, taken as 0.67. Substituting these values, we obtain the end-on collecting area as 2.3 μm².

The conversion factor in equation (13) is then obtained as K = 8.6 photoisomerizations rod⁻¹ per scotopic td s, for a “normal” eye exposed to white light; this is virtually identical to the value of 8.5 obtained in references 17 and 20. Our maximum unattenuated retinal illumination of 141,000 scotopic td s therefor corresponded to 1.21 × 10⁶ photoisomerizations per rod per flash. Taking the total rhodopsin content of a human rod as 7 × 10⁷ molecules (Table 1), our maximum flash would have bleached approximately 1.7% of the rhodopsin.

We emphasize that there is imprecision in the estimates of each of the factors in equation (13), so that we cannot place absolute reliance on the value of the...
conversion factor $K$. Uncertainty in this value will be a particular problem in the case of eye disease, where light absorption may differ as a result of altered photoreceptor dimensions, and so on. Rather than attempt to determine the relevant conversion factor $K$ in individual eyes, we have simply employed a value for the "normal" eye. The amplification constants we obtained must be interpreted with this in mind.

**Curve Fitting**

Equation (11) was fit to the a-wave responses with a least-squares minimization procedure based on the simplex algorithm in the Matlab (Mathworks, Natick, MA) package. We employed two general strategies for curve fitting, individual fitting and ensemble fitting, for each observer. In the first case, equation (11) was fit to each individual response, with both $A$ and $\zeta_{eff}$ varied to obtain the best fit. The individual values of $A$ are plotted in Figure 5, and the averages (and the average values of $\zeta_{eff}$) are presented in Table 2. In the second case, the fitting of equation (11) was performed for the ensemble of responses at different intensities, i.e., the pair of values $A$ and $\zeta_{eff}$ was determined, which produced the best fit to the whole family of responses.
for a given observer. In this case, it was necessary to exclude from the fitting the responses to the very brightest flashes, because the individual values of $A$ decreased in a systematic manner (see Figure 5). In addition, in a few cases it was also necessary to exclude two or three traces at the lowest intensities if there also appeared to be a change in $A$ in that region (Fig. 4).

Because the b-wave intrudes on the a-wave in a complex time- and light-dependent manner, it was necessary to restrict the time range of the fitting. For each response, the maximum time of the fitting was set to about 10%–20% before the point of obvious b-wave intrusion (Fig. 3, for example). However, because the b-wave approximates an inverted and low-pass filtered version of the a-wave, this procedure will not be totally effective, and there will always be some degree of b-wave intrusion.

RESULTS

Estimation of the Amplification Constant $A$ From Normal Subjects' a-Waves

Figure 3A shows a family of ERG responses for a series of flashes that produced from $\Phi = 402$ to $\Phi = 128,000$ photoisomerizations per rod. This family of responses illustrates several well-known features of the early ERG, including graded increases in the amplitude and in the rate of rise of the a-wave and a progressively earlier intrusion by the b-wave, with increasing flash intensity. Particularly important for our analysis is the clear demonstration of amplitude saturation of the a-wave (at a value of $a_{\text{max}} \approx -520 \mu V$), as reported previously by Breton and Montzka. This saturation permits normalization of the a-wave recordings, for comparison with the normalized circulating current, $F(t)$, according to equation (11). In addition to amplitude saturation, the a-wave responses also exhibit rate saturation, whereby the rate of rise reaches a maximum value at the highest intensities; this phenomenon will be examined later. Both amplitude saturation and rate saturation are well-known features of the rod photocurrent.

Ensemble Fitting. Figure 3B shows the normalized a-wave responses extracted from Figure 3A. Here, the a-wave traces have each been truncated in time, at the point where they turn upward, i.e., just before the time where the positive-going b-wave was clearly intruding. The responses to all but the brightest flash have been fit with equation (11), as an ensemble, yielding best fit parameters $A = 8.7 \pm 2$, $\tau_{\text{eff}} = 2.7$ ms. The theoretical traces have been plotted as continuous curves over the region where the fitting was performed, and they have been continued as dashed curves thereafter. The response at the highest intensity was not well described with the parameters obtained from the ensemble fitting (theoretical trace not shown). A comparable discrepancy between experiment and the simple theory also occurs in recordings from individual photoreceptors. The discrepancy will be examined in more detail below (Figure 7).

Figure 4 shows four additional families of a-wave responses, plotted and analyzed as in Figure 3B. The overall pattern for each family is similar to that seen in Figure 3B. Again, the theoretical traces provide a good fit to the ensemble of a-wave responses up to high intensity ($\Phi = 30,000$ to 70,000 for different observers). The nature of the fit at even higher intensities will be described in more detail in Figure 7.

Fitting of Individual Traces. To test whether the amplification constant $A$ was independent of intensity, we determined the best fit of equation (11) to the individual a-waves in each response family. The values of amplification constant $A$ obtained in this way are plotted as a function of flash intensity in Figure 5; a single symbol is used for each family of responses, and the points are connected by straight lines. The results for two patients with retinal disease (filled symbols) will be discussed later.

With the exception of the points for the cone dystrophy patient (▲), the results in Figure 5A bear a striking resemblance to those obtained in recordings from single salamander rods (Figure 6 of reference 9). The sets of points for the different observers cluster together, indicating the occurrence of only minor differences in amplification constant between normal observers. Furthermore, the points for all observers are roughly horizontal for intensities up to about 30,000 photoisomerizations per rod. This indicates that for each observer the amplification “constant” is indeed relatively constant over the 100-fold range of intensities from $\Phi = 300$ to 30,000. Hence, we conclude that the theory with a constant value of $A$ provides a good description of a-wave responses for flashes up to about 30,000 photoisomerizations per rod.

Estimates of the amplification constant at intensities up to this level are summarized in Table 2, for the ensemble and individual fitting approaches. For our group of six normal observers, the ensemble fitting method yielded an overall mean of $A = 6.9 \pm 1.1 \pm 0.8$ s$^{-2}$. These mean values fall approximately midway between the low-intensity asymptotes of the continuous curves shown in Figure 5A; these curves will be discussed below.

Results From Patients With Abnormal Retinas

Figure 6A-B shows a family of ERGs obtained from a patient diagnosed as having cone retinal dystrophy with rod involvement (CRD), plotted in the raw and normalized format of Figure 3. The maximum amplitude of the a-wave for this patient was $a_{\text{max}} = -339 \mu V$, only 75% of the average normal value ($-463 \mu V$) but within the range encountered for the six normal subjects (e.g., NL4). The ensemble fitting analysis (Figure
just 38% of the average amax for normal observers, but the reduction in sensitivity is also clear from the individual estimates of A, plotted as filled inverted triangles (T) in Figure 5B. The maximum amplitude of the a-wave (−177 μV) was about sixfold less sensitive to light at the cornea for this patient than for the average normal observer. This reduction in sensitivity is also clear from the individual estimates of A, plotted as filled inverted triangles (▼) in Figure 5A.

Figure 5B yields \( A = 1.1 \) s\(^{-2}\), indicating that the a-wave was about sixfold less sensitive to light at the cornea for this patient than for the average normal observer. This reduction in sensitivity is also clear from the individual estimates of A, plotted as filled inverted triangles (▼) in Figure 5.

The maximum amplitude of the a-wave (−177 μV) was just 38% of the average amax for normal observers, but the amplification constant was normal, at \( A \approx 7.5 \) s\(^{-2}\) (Table 2 and Figure 5, ▲).

**Rate Saturation of the a-Wave**

At intensities above about 30,000 photoisomerizations per rod, the individual estimates of amplification constant plotted in Figure 5A become smaller for each observer, and the form of the decline is strikingly similar to that illustrated (as Figure 6) in Lamb and Pugh.\(^9\) This similarity of form between individual rod photocurrents and a-waves in the intensity-dependence of the amplification constant provides additional support for the hypothesis that the a-wave is directly proportional to the rod photocurrent at all intensities (equation (10)). It is likely that the decline in the estimates of A at high intensity is a direct consequence of rate-saturation of the photocurrent.\(^{15,23}\)

To establish a connection between the decline in estimates of A and rate-saturation of the photocurrent, we now examine a-wave responses to intense flashes. Panels A-C in Figure 7 present the a-waves recorded from three normal observers, whereas panel D presents responses from the cone dystrophy patient of Figure 6A-B. For each observer, the rate of rise of the a-wave approaches a limiting value at the very highest intensities, i.e., the rise approaches rate-saturation, as described previously by Breton and Montzka.\(^8\) Figure 7 also shows that, provided both \( t'_{efr} \) and A are varied, the best-fitting delayed gaussian curve gives a good description to the a-waves for intense flashes. The slight discrepancy at very early times probably arises from the simplification involved in approximating the series of short delay stages with a pure delay, \( t'_{efr} \), in equation (6).

The approach to characterizing rate saturation of the photocurrent taken by Penn and Hagins\(^{13}\) (in rat) and Cobbs and Pugh\(^{23}\) (in salamander) was to plot the maximum rate of rise of each individual response as a function of flash intensity, and in Figure 5B we apply the same approach to a-wave recordings. Rather than taking the time derivative of the a-waves (a noisy operation), we have obtained the maximum rates, \( dR/ dt\)\(_{\text{max}}\) (= \( \Phi \)) from the best-fitting delayed gaussians (see equation (12) and Figure 5 legend); the good fit to the individual traces in Figure 7 shows that this method is appropriate. A consequence of this approach is that there is an exact correspondence between each of the points plotted in Figure 5B and those in Figure 5A.

Figure 5B yields two important results. First, it shows that for flashes producing less than about 30,000 photoisomerizations per rod, the maximum rate of rise of the human a-wave has a slope of approximately 1/2 in double-logarithmic coordinates. Such a square-root relation is predicted by equation (12), from the delayed gaussian in equation (11), provided that A is constant\(^9\), and this prediction has previously been shown to hold in salamander rods.\(^9\)

Secondly, Figure 5B illustrates that the rate of rise of the human a-wave saturates. For the five normal subjects exposed to sufficiently intense lights, the saturated rate of rise of the normalized a-wave was \( dR/dt\)\(_{\text{sat}} = 578 \pm 52 \) s\(^{-1}\) (mean ± SD, see Table 2). In Figure 5B, we have also plotted the results for the two...
patients with retinal abnormalities (filled symbols). For both patients, the maximum rate of rise shows approximately the same slope of 1/2 dependence on intensity as for normal subjects. Although the points for patient CRD are displaced to the right, the saturated rate of rise appears to be at least as high as for normal subjects. Patient RP was not tested at the highest intensities, so it is not known whether the saturated rate of the a-wave would have been as high in her case.

**Form of Saturation**

The curves plotted in both panels of Figure 5 are based on the idea that the amplification constant $A$ is constant at low intensities but that it declines at high intensities as a result of saturation in the rate of rise of the photocurrent. In Figure 5A, the curves are described by the first-order saturation equation (analogous to the Weber Law):

$$A = \frac{A_0}{1 + \Phi/\Phi_{1/2}},$$

where $A_0$ is the constant value that $A$ assumes at low intensity, and $\Phi_{1/2}$ is the intensity at which $A$ declines to $1/2A_0$; see equation (16) below. In Figure 5B, this same relation has been used to predict $dR/dt_{\text{max}}(\Phi)$, by converting $A$ to maximum rate of rise. This is achieved by substituting equation (14) into equation (12), to give

$$\frac{dR}{dt}_{\text{max}}(\Phi) = \frac{dR}{dt}_{\text{sat}} \sqrt{\frac{\Phi}{\Phi + \Phi_{1/2}}}. \quad (15)$$
FIGURE 6. ERG responses from two patients with retinal disease, plotted as in Figure 3. In each case the upper panel is the raw ERG, and the lower panel is the normalized a-wave together with the ensemble fitting of equation (11). (A,B) Patient with cone retinal dystrophy (CRD); $A = 1.1 s^{-2}$, and $t'_{\text{eff}} = 3.8 ms$. Flash intensity $\Phi$ ranged from to 962 to $1.21 \times 10^6$ photoisomerizations; each trace is the average of 6 to 12 responses, except the responses to the three most intense flashes, which were averages of 4, 2, and 1 responses, respectively. (C,D) Patient with retinitis pigmentosa (RP); $A = 7.5 s^{-2}$, and $t'_{\text{eff}} = 3.5 ms$. $\Phi$ = 349 to 192,000 photoisomerizations; each trace is the average of six responses, except for the two most intense flashes, where four responses were averaged.

For both equations (14) and (15), $\Phi_{1/2}$ is given by

$$\Phi_{1/2} = \frac{e(dR/dt)_{\text{sat}}^2}{A_0}$$

With this value for $\Phi_{1/2}$, equation (14) shows that at high intensity the amplification constant declines inversely with $\Phi$, according to $A = (e(dR/dt)_{\text{sat}}^2)/\Phi$.

For all the curves in Figure 5, $dR/dt|_{\text{sat}}$ has been set equal to 576 $s^{-1}$, giving the common upper limit in panel B, and also giving the common high-intensity asymptote in panel A, at $A = (9 \times 10^9)/\Phi$. For the three curves, $A_0$ has been altered, thus altering $\Phi_{1/2}$ according to equation (16). In Figure 5A, this causes the Weber curves to slide diagonally along a line of slope $-1$ in double logarithmic coordinates, whereas in Figure 5B it causes a lateral shift. The pair of solid curves (in each panel) has been chosen to encompass most of the observed range for normal subjects by setting $A_0$ to $5 \text{ and } 10 \ s^{-2}$; the broken curve has been chosen to provide a good fit to the results for patient CRD by setting $A_0$ to $1.2 \ s^{-2}$.

Hence, all the results in Figure 5 are consistent with these ideas: (1) the amplification constant saturates in a first-order manner at high intensities; (2) the saturating slope for all normal observers and both patients was close to 600 $s^{-1}$; (3) the differences between different normal observers (and patient RP) are equivalent simply to an intensity scaling factor (i.e., a change in $\Phi_{1/2}$) of no more than about twofold; (4) and the behavior for patient CRD is equivalent to a reduction in amplification (or a reduction in effective intensity) of about sixfold below that for the average normal observer.

DISCUSSION

Relationship Between Human a-Waves and Rod Photocurrents

The results reported here add to a substantial body of evidence supporting the hypothesis embodied in equation (10) that the a-wave is a direct reflection of the rod photocurrent.\textsuperscript{5,6,7,8,13} Our results show that the rising phase of the a-wave has exactly the same delayed gaussian kinetics as previously found for the photocurrents of a variety of species\textsuperscript{9,11} including primates.\textsuperscript{20} Furthermore, the rate of rise of the response
FIGURE 7. a-Waves from three normal observers and a patient with cone retinal dystrophy, in response to intense flashes. The dashed traces show the best-fitting delayed gaussian curves (equation (11)) when both $A$ and $t'_{cr}$ were allowed to vary. 

$A$ (NL2), $\phi_1 = 5.29 \times 10^4, 1.27 \times 10^5, 3.34 \times 10^5, 9.63 \times 10^5$; $B$ (NL3), $\phi_1 = 1.75 \times 10^5, 4.83 \times 10^5, 1.21 \times 10^6, 9.63 \times 10^5$; $C$ (NL1), $\phi_1 = 1.75 \times 10^5, 5.29 \times 10^5, 1.92 \times 10^6, 1.21 \times 10^6$; $D$ (CRD), $\phi_1 = 1.75 \times 10^5, 4.83 \times 10^5, 1.21 \times 10^6$.

The greater amplification constant of human rods may be explained in terms of three factors: a geometrical difference in outer segment volume, temperature dependence of the PDE hydrolytic rate constant, and temperature dependence of the protein diffusional contact rates.

Firstly, from equation (8), the rate constant $\beta_{inh}$ of PDE hydrolysis is predicted to depend inversely on the volume $V_{cyto}$ of the outer segment cytoplasm; this effect arises because hydrolysis of a given number of cGMPs corresponds to a greater concentration change in a smaller volume. Because a human rod outer segment has only about $1/25$ the volume of its amphibian counterpart ($0.04 \mu l$ cf $1 \mu l$), the volume effect accounts for a 25-fold greater amplification constant. Secondly, biochemical evidence (reviewed in reference 11) indicates that at $37^\circ$C the ratio $k_{cat}/K_m$ for the PDE (Table 1) is about double its value at room temperature. This would increase $\beta_{inh}$ by a further factor of two. Thirdly, the “encounter rate” between a single Rh* and molecules of G-protein is expected on theoretical grounds to have a strong temperature dependence, increasing about twofold between room temperature and body temperature. Provided that the coupling coefficient $c_{p}$ between G* and PDE* activation (equation (7)) is reasonably high, an elevation in $v_{KC}$ would produce a concomitant increase in $v_{RP}$, the rate at which a single Rh* causes activation of PDE catalytic subunits. Hence, it is likely that in human rods $v_{RP}$ is about double its value in amphibian rods (about $8000 s^{-1}$ cf $4000 s^{-1}$).

Thus, it seems that the combination of the smaller outer segment volume and the temperature dependence of $v_{RP}$ and $k_{cat}/K_m$ provide the basis for the higher amplification constant $A$ of phototransduction in human rods in comparison with amphibian rods. The three effects appear to increase the amplification constant by factors of about 25, 2, and 2, respectively.

It is satisfying that our analysis demonstrates a functional advantage for the small size of human rods, in conferring a much higher amplification constant. The elevated amplification constant is likely to be of greatest importance at very low levels of illumination, where individual rods are responding to only one or a few isomerizations per integration time. A small outer segment has the important advantage that the rod’s response to a single photon will rise rapidly, and this is likely to be of great importance in enabling the detection of quanta at very low light levels, as in the “photon-counting” region.

Effective Delay of Transduction, $t_{ef}$

We have used the composite delay parameter $t_{ef}$ to approximate the effects of several cascaded activation steps in phototransduction, simply by taking the sum of the individual delays in equation (6); more exactly, the overall delay should be expressed as a cascade of low-pass filters with very short time constants.9,23 The experimentally measured parameter $t_{ef}$ (including all the delays up to and including the recording amplifiers) had a mean value of 2.5 ms (Table 2), which can...
be used to provide an upper bound to $t_{\text{eff}}$. Because the mammalian rod has an electrical time constant of about 1 ms,\textsuperscript{13} and because the recording delay was also around 1 ms for most of these recordings, we conclude that the transduction delay $t_{\text{eff}}$ in human rods does not exceed approximately 1 ms.

**Rate Saturation of the a-Wave**

At the highest flash intensities, the rate of rise of the a-wave reached a saturated level of $dR/dt|_{\text{sat}} = 600$ s$^{-1}$, more than three times the corresponding value of 168 s$^{-1}$ for voltage-clamped salamander rod photocurrents at 22°C.\textsuperscript{9,23} The higher value obtained for a-waves indicates that the maximum rate constant of PDE hydrolysis, $\beta_{\text{max}}$, is much greater in human rods than in amphibian rods.

The PDE hydrolytic rate constant, $\beta(t) = PDE^{\text{m}}(t) / PDE_{\text{sub}}$, should rise approximately linearly with time, and it will continue rising even after substantial suppression of the circulating current, when it can no longer feasibly be measured. It is possible to show that $\beta(t)$ is bound to rise to at least the measured value of $dR/dt|_{\text{sat}}(\Phi)$ at each intensity. Hence, we can write

$$\beta_{\text{max}} \geq \frac{dR}{dt}|_{\text{sat}}$$

where $dR/dt|_{\text{sat}}$ is the limiting value of the rate of rise at very high intensities. Therefore, we expect that in human rods $\beta_{\text{max}}$ will exceed 600 s$^{-1}$. Substitution in equation (8), with reasonable values for $V_{\text{cyt}}$, $BP$, and the total number of PDE subunits per rod (see Table 1), indicates that for each catalytic subunit of human PDE, the hydrolytic constant $k_{\text{cat}}/K_m$ should exceed about $10^7$ M$^{-1}$ s$^{-1}$.

**Comparison With Previous Theoretical Analyses of a-Waves**

In several recent papers, Hood and Birch\textsuperscript{6,7,22} have analyzed the human a-wave with a formal model previously developed for rod photocurrents.\textsuperscript{18,19,24,25} That model comprises a linear, multistage filter followed by an instantaneous nonlinearity (exponential saturation). It is characterized by four parameters: $a_{\text{max}}$, as here, a gain factor $C$ linking intensity to response amplitude, the time-to-peak $t_p$ of the dim flash response, and the number $N$ of filter stages. As argued by Hood and Birch, the success of that model in fitting a-wave responses, in conjunction with its previous success in fitting rod photocurrents, provides support for the notion that the a-wave does indeed reflect the rod photocurrent; see equation (10). However, as is the case for all previous applications of multistage filter descriptions to photoreponses, we think this model is of limited value in understanding the biochemical mechanisms. In particular, there is no explicit physical basis for the parameters $t_p$, $N$, or $C$ in that model, or for the exponential form of saturation.

Instead, the simple description employed in equation (11) follows directly from a theoretical analysis of the chain of molecular steps underlying phototransduction. This formulation describes the activation stages of transduction in human rod photoreceptors in terms of a fundamental parameter $A$, termed the amplification constant of transduction, which can in turn be related to the physical and biochemical constants of the photoreceptor by equation (9). Furthermore, the exponential form of saturation follows as a direct consequence of the cascade of reactions illustrated in Figure 1. Thus, the present model has the advantage of providing a good description of the rising phase kinetics for the entire family of a-wave responses in terms of known molecular mechanisms.

**Potential and Limitations of the Cascade Model in Characterizing Retinal Disease**

We have presented results from two patients with examples of retinal disease, and we now discuss the way in which our analysis of ERGs can help to characterize the locus of any abnormality in phototransduction.

Our analysis provides a clear measure of the maximum a-wave amplitude, $a_{\text{max}}$, obtained with extremely bright flashes. For a given eye, $a_{\text{max}}$ will be directly proportional to the magnitude of the current circulating in the photoreceptors at the time the flash was delivered. If all other retinal and ocular factors were constant, then $a_{\text{max}}$ would be proportional to the length of the outer segments.\textsuperscript{13,25} However, differences in $a_{\text{max}}$ between observers may alternatively result either from the absence of outer segments (whether in patches or uniformly) or from alteration in the radial resistance of the electrical path in the neighborhood of the receptors, e.g., as a result of altered permeability of the outer limiting membrane. Nevertheless, for patient CRD (with $a_{\text{max}} = -339 \mu V$), it seems clear that the total circulating current of all the photoreceptors in the retina cannot be far short of normal. In contrast, patient RP had a greatly reduced maximal amplitude ($a_{\text{max}} = -177 \mu V$), suggesting either a substantial loss of total outer segment membrane or a leaky pathway in the vicinity of the photoreceptors.

A more important parameter for our characterization of a-wave dysfunction is the amplification constant, $A$. For patient RP, we obtained $A \approx 7.5$ s$^{-2}$, indistinguishable from the the mean normal value of 6.9 s$^{-2}$. Hence, the results from patient RP are consistent with the hypothesis that her rods are either shortened or partly absent (because $a_{\text{max}}$ is small), but that there is relatively little change in the intrinsic biochemical steps of phototransduction (because $A$ is close to normal). For patient CRD, on the other hand, we obtained $A = 1.1$ s$^{-2}$, about 1/6 the mean for normal.
Human ERG a-Wave Amplification and Kinetics

subjects. This suggests that patient CRD exhibits a significant reduction in one or more of the biochemical parameters that multiply together to yield $A$ in equation (9), i.e., that one or more of the activation steps of phototransduction in her rods exhibits a significantly reduced gain.

An additional clue to the nature of the underlying pathology is provided by the rate-saturated a-wave response. Because $dR/dI_{sr}$ for subject CRD reached the normal level, it is plausible that $\beta_{max}$ in her rods is the same as in normal subjects; see equation (17). This would imply that she has a normal complement of PDE with normal kinetic parameters and would, therefore, place the defect in amplification at an earlier stage in the cascade, for example in the catalytic rate $k_{RG}$ of activation of G-protein by $R^*$. In summary, knowledge of the three parameters $\alpha_{max}$, $A$, and $dR/dI_{sr}$ of the a-wave responses should provide considerable insight into the nature and location of defects underlying human rod photoreceptor dysfunction.

Key Words
ERG a-wave, rod photocurrents, phototransduction, G-protein cascade, amplification

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