Abnormal Activation and Inactivation Mechanisms of Rod Transduction in Patients With Autosomal Dominant Retinitis Pigmentosa and the Pro-23-His Mutation

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Purpose. The leading edge of the rod a-wave in normal human subjects can be fit with a computational model of the activation phase of transduction to provide parameters analogous to those obtained from individual photoreceptors. The authors extend this work to the kinetics of recovery after saturating flashes.

Methods. Electroretinograms were recorded from three patients with autosomal dominant retinitis pigmentosa and the pro-23-his rhodopsin mutation, two patients with rod monochromatism, and five normal subjects. Rod-only a-waves were obtained for a series of flashes ranging from 4.4 to 10.1 In (1.9 to 4.4 log) scot td-sec. One set of parameters describing the activation process was derived from fits to the a-wave model. A double-flash paradigm was used to study inactivation mechanisms. The first flash was achromatic and varied in intensity (I_f) from 6.1 to 13.9 In (2.6 to 6.0 log) scot td-sec. The second flash was a short-wavelength probe held constant at 9.3 In (4.0 log) scot td-sec. Cone components were elicited with a photopically matched long-wavelength stimulus and were computer subtracted. Recovery at each I_f was followed by measuring the amplitude to the probe flash at various interstimulus intervals (ISI). The critical time (T_c) before the initiation of rod recovery was determined from the function relating relative rod amplitude to ISI.

Results. Recovery from activation was similar in normal subjects and in patients with rod monochromatism. Over a large range of I_f above rod saturation, T_c increased in proportion to ln I_f. The mean slope of the function relating T_c to ln I_f was 2.3 s/ln I_f when I_f varied between 11 and 13.9 ln scot td-sec. Patients with retinitis pigmentosa and the pro-23-his rhodopsin mutation had a decrease in the gain of activation. They also had significantly slower than normal recovery after high test flash intensities, such that the slope of the function relating T_c to ln I_f was 12.1 seconds.

Conclusion. Available data from other species imply that complete, transient activation of transducin (T* saturation) occurs within or below the investigated range of flash intensities. Based on the slope of the delay function (ΔT_c/Δ ln I_f) above 11 ln scot td-sec, the authors hypothesize that the lifetime of activated rhodopsin (R*) in normal human rods is approximately 2.3 seconds. In patients with the pro-23-his mutation, the gain of the activation mechanism is reduced and the reaction determining the ΔT_c/Δ ln I_f slope is markedly slowed. The activated species that exhibits this prolonged lifetime could be the mutant rhodopsin itself.

circular current. Recent studies examined the dependence of this delay period \( T_c \) on flash intensity \( I_f \) in photocurrent recordings obtained from isolated single rods of the salamander. The results showed that \( T_c \) increases in proportion to the natural logarithm (ln) of \( I_f \) over a large range of saturating flash intensities and suggested that the slope of the \( T_c \) versus ln \( I_f \) function, represents the lifetime of \( R^* \).\(^3\)-5

The current study addresses the relation between flash intensity and the delay period \( T_c \) for human rods in vivo. It is well established that the flash-induced electroretinographic (ERG) a-wave expresses, as a voltage signal, the massed photocurrent response of rods.\(^6\)

The leading edge of the human rod a-wave can be fit by a computational model of the activation phase of phototransduction.\(^7\)-9 Based on the Lamb and Pugh\(^10\) model of transduction, the leading edge is defined by:

\[
P^3(i, t) \cong [1 - \exp(-i \cdot S \cdot (t - \omega)^5)] \times R_{mp} \text{ for } t > t_d \quad (1)
\]

where \( P^3 \) is the sum of the responses of individual rods. The amplitude of \( P^3 \) is a function of flash intensity, \( i \), and time, \( t \), after flash onset. \( S \) is a sensitivity parameter that scales \( i \), \( R_{mp} \) is the maximum response, and \( t_d \) is a brief delay. For brief flashes up to approximately 9 ln (4.0 log) scot td-sec, equation 1 provides good fits to the human rod a-wave as it increases in proportion to the natural logarithm (ln) of \( I_f \), as it does to photocurrent data obtained from single isolated rods from human donor eyes.\(^11\)

The agreement between ERG a-wave data and isolated rod data provides support for the validity of a key method used here—that of determining the saturating amplitude of rod photoreceptors from the bright-flash a-wave response. Recovery of the rod photocurrent after a saturating flash cannot be measured directly in the ERG because of intrusion of the b-wave and other postreceptoral components. However, recovery from a bright (test) flash can be inferred from the amplitude of the a-wave response to a subsequent (probe) saturating flash. Repetition of this two-flash paradigm with variations of the interval between two flashes allows determination of the recovery time course and, thus, \( T_c \) at a given test flash intensity \( I_t \). Using these methods, we measured the dependence of \( T_c \) on \( I_t \) for a group of five normal subjects. We also obtained data from three patients with retinitis pigmentosa caused by the pro-23-his rhodopsin mutation to determine whether structural alterations in the opsin could influence recovery.

**METHODS**

**Subjects**

Screening for rhodopsin mutations in patients with autosomal dominant retinitis pigmentosa (adRP)) was conducted through a collaborative agreement with Dr. Stephen Daiger (Molecular Biological Laboratory, University of Texas Health Science Center, Houston). We selected three patients from three different families, each of whom had the pro-23-his mutation and detectable (>3.0 µV) responses to standard protocol stimuli. Two patients with complete achromatopsia (rod monochromatism) and five normal subjects (age range, 40 to 50 years; mean age, 44 years) also participated in the study. Patients and normal subjects consented to the study after the experimental procedures were described to them. Tenets of the Declaration of Helsinki were followed, and approval was obtained from the institutional committee on human experimentation.

**Procedure**

Full-field ERGs were recorded with a bipolar gold electrode (Doran Instruments, Littleton, MA) placed on the anesthetized cornea after full pupil dilation and 45 minutes of dark adaptation. A cup ground electrode was placed on the forehead. Signals were amplified (×10,000; 3 dB down at 2 and 10,000 Hz) and acquired through an A/D converter (National Instruments, Austin, TX) in a personal computer.

All stimuli were derived from a circular xenon gas flash tube (Novatron, Dallas, TX). To study activation stages of transduction, a series of a-waves was obtained to flashes varying from 4.4 to 10.1 ln (1.9 to 4.4 log) scot td-sec. The best fit of equation 1 to the leading edge was determined using techniques previously described.\(^9\) A double-flash technique was used to analyze inactivation stages of transduction. The first (test) flash of each two-flash pair was achromatic and varied in retinal illumination from 6.1 to 13.9 ln scot td-sec (2.6 to 6.0 log scot td-sec). All flashes were well above the rod semisaturation value of 2 ln scot td-sec but below intensities that produce a sizable bleach. Assuming that 1 scot td-sec produces 8.6 isomerizations per rod, our highest intensity test flash produced an approximately 12% bleach.) The second (probe) flash was either a saturating, short-wavelength (W47B; \( \lambda_{\text{max}} = 449 \text{ nm} \) ) “blue” or a photopically matched long-wavelength (W26; \( \lambda_{\text{max}} = 605 \text{ nm} \) ) “red” flash. The interstimulus interval (ISI) was controlled by computer. The flash tube recharged to within 0.1 log unit of the baseline intensity within 1 second and was fully recharged by 2 seconds. The retinal illumination of the probe flash was fixed at 9.3 ln (4.0 log) scot td-sec (2.7 log phot td-sec) for the blue flash and 6.2 ln phot td-sec (2.7 log phot td-sec) for the red flash. In preliminary studies, comparable results were obtained with saturating probe flashes ranging in intensity from 7.1 to 13.9 ln scot td-sec. Test flashes were followed by probe flashes with ISIs from 1 second to 128 seconds. A 2-minute interval after each trial was sufficient.
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TABLE 1. Clinical Findings

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<th>30 Hz Implicit Time (msec)</th>
<th>30 Hz Amplitude (μV)</th>
<th>Dark-Adapted Threshold (log y asb)</th>
<th>Goldmann Field (W4e)</th>
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* t_d was increased by 0.6 msec from that reported previously^3 to account for the slightly longer flash duration used here.

RESULTS

Summary clinical data from three patients with adRP from the pro-23-his rhodopsin mutation are shown in Table 1. In response to standard protocol stimuli, all patients had severely reduced rod responses and cone responses that were reduced in amplitude and delayed in b-wave implicit time. In general, visual thresholds after 45 minutes of dark adaptation and visual field diameters were abnormal in rough correspondence with patient age.

Representative ERGs to high-intensity stimuli are shown in Figure 1 for a subject with rod monochromatism (upper panel), a normal subject (middle panel), and patient 3674, who had the pro-23-his form of adRP (lower panel). The left column shows responses to short-wavelength stimuli, whereas the right column shows the rod responses isolated by computer-subtracting responses to photopically matched long-wavelength stimuli from the responses to short-wavelength stimuli. The dashed curves are the fit of equation 1 obtained by estimating a single set of parameters for each series of responses. Because the subject with rod monochromatism showed no evidence of cone ERG responses to any stimuli, the subtraction procedure had no effect on the responses to short-wavelength stimuli. The parameters (log S, log R_mP3; log [R_mP3 (μV)]; t_d (msec)) for rod responses from the fit of equation 1 were 1.03, 2.21, and 3.32. Removing the cone components in the normal subject had only a small effect, as discussed in detail previously. Parameters for responses to short-wavelength flashes in the left column were 0.9, 2.42, and 2.61. Parameters for the rod-only responses in the right column were 0.95, 2.36, and 2.66.

Removing cone components had a greater effect on the parameters derived from patient 3674, who had adRP. The best-fit parameters (log S; log R_mP3; t_d) from equation 1 for short-wavelength responses in the left column were 0.54, 1.52, and 4.0 compared with the values 0.42, 1.3, and 3.84 for the rod-only responses in the right column. Note, however, that the cone subtraction procedure does not influence the major finding that log S is lower than normal in the patient with retinitis pigmentosa. Summary parameters from the fit of equation 1 to rod-only responses from all three patients and both subjects with rod monochromatism are shown in Table 1, along with previously published average values from 15 normal subjects. Patients with the pro-23-his form of adRP had significant reductions in log S and log R_mP3.

Recovery of the rod a-wave in a subject with rod monochromatism after high-intensity test flashes is shown in Figure 2. Because this patient showed no cone ERG response to any stimulus, the responses to the short-wavelength probe flashes of 9.5 In scot td-sec were entirely rod mediated. The left column indicates recovery after test flashes of 10.9 In scot td-sec. Because each trace is a single response (i.e., no computer averaging), there was more noise in the recordings than in those shown in Figure 1. Nevertheless, the responses showed reasonable agreement with the dashed curves, which were the best fits of equation 1 to the data with S and t_d held constant. (Values of S and t_d were obtained from fits of the model to a range of dark-adapted responses; see Fig. 1). The responses were barely detectable at ISI of 2 and 3 seconds. After 5 seconds, there was rapid growth in amplitude, and by 8 seconds, the response was back to approximately 50% of the baseline amplitude to a single 9.5 In scot td-sec flash. The right column shows recovery after a 13.1 In scot td-sec test flash. Here, the initiation of recovery was considerably delayed relative to that after the 10.9 In scot td-sec flash. Furthermore, once initiated, the recovery after this test flash was more gradual. It took a full minute before the response recovered to more than half its baseline amplitude. The
FIGURE 1. (left column) Responses (solid curves) to short-wavelength flashes ranging up to 10.1 ln scot td-sec (4.4 log scot td-sec) in approximately 0.7 ln (0.3 log) unit steps. (right column) Rod-only responses derived by computer-subtracting cone responses to photopically matched long wavelength stimuli from the responses in the left column. The dashed curves are the fit of equation 1. (top) Subject with rod monochromatism. (middle) Normal subject. (bottom) Patient with autosomal dominant retinitis pigmentosa. See Results and Table 1 for parameters of best fit.

dashed curves again represent the best fit of equation 1 with S and t held constant. Slightly better fits could be obtained for some ISI by decreasing log S by 0.2 log unit (solid curves), suggesting that this extremely intense test flash leads to a slight decrease in gain during recovery.

The two-flash paradigm illustrated in Figure 2 was used to determine the relationship between test flash intensity (I) and the delay that precedes recovery of the response to a saturating probe flash. Shown in Figure 3 are the results obtained from a subject with rod monochromatism when a probe flash of 9.3 ln scot td-sec was presented at varying times after a test flash of 10.9 ln scot td-sec (circles) or 13.1 ln scot td-sec (squares). In each trial, the response to the probe flash was analyzed through equation 1 to yield R_{m3}. This value of R_{m3} was normalized to the baseline level of R_{m3} (obtained from the response to the probe flash alone) to obtain R/R_{max}, the relative amplitude of the response. As illustrated by the curves in Figure 3, R/R_{max} versus time at each test flash intensity can be described in terms of the exponential relation:

\[ R/R_{max} = 1 - \exp\left[-(t - T_0)/\tau_r\right] \]
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10.9 In scot td-sec

2 sec ISI
3 sec ISI
4 sec ISI
5 sec ISI
7 sec ISI
8 sec ISI

13.1 In scot td-sec

5 sec ISI
8 sec ISI
16 sec ISI
32 sec ISI
48 sec ISI
64 sec ISI

FIGURE 2. Responses to second (probe) flash (9.3 In scot td-sec) of two-flash paradigm in subject with rod monochromatism. Dashed curves represent best fit of equation 1 with $S$ and $t_i$ held constant at values derived from fits to a full set of dark-adapted a-waves (see Fig. 1). Slightly better fits were obtained for some interstimulus intervals (ISI) by decreasing log $S$ by 0.2 log unit (solid curves). (left column) Responses at various ISI after test flash of 10.9 In scot td-sec. (right column) Responses at various ISI after test flash of 13.1 In scot td-sec.

where $T_c$ was the critical delay before the initiation of recovery, and $\tau_r$ was the recovery time constant. Analysis of the data through equation 2 (free variation of $T_c$ and $\tau_r$) indicated that, with an increase in test flash intensity from 10.8 to 13.1 In scot td-sec, there was an increase in critical time ($T_c$) from 2.5 to 7.2 seconds and an increase in recovery time constant ($\tau_r$) from 9 to 60.4 seconds.

In normal subjects, the analysis of recovery was complicated by the presence of cone-mediated activity in the responses to short-wavelength flashes of 9.3 In scot td-sec. Figure 4 shows recovery of the a-wave in a normal subject to short-wavelength (left column) and long-wavelength (right column) flashes after test flashes of 10 In scot td-sec. The responses in the right column were pure cone and were identical at all ISI—i.e., cone photoreceptor responses showed full recovery within 1 second. The rapid completion of cone recovery was indicated by the similarity of the cone response at 1-second ISI (dashed curve in the right column) with that obtained at 5-second ISI. The responses in the left column vary qualitatively as a function of ISI. Responses to the short-wavelength flash were comparable to responses to long-wavelength flashes at 2-second ISI; only cones were responding shortly after the test flash. A small rod component was present at 5 seconds and continued to grow in amplitude through at least 16 seconds. To isolate the rod photoreceptor response, the photopically matched cone responses were computer-subtracted for each ISI-test flash intensity combination. Rod-only responses obtained in this fashion in a normal subject for two test flash intensities are shown in Figure 5. The absolute amplitudes of the a-waves are larger in the normal subject than in the subject with monochromatism shown in Figure 2, but the relative growth in
FIGURE 3. Amplitude of second (probe) flash relative to baseline amplitude at various interstimulus intervals for a subject with rod monochromatism. The test flashes are those shown in Figure 2. Solid curves are fits of equation 2 to the data.

FIGURE 4. Responses to a short wavelength flash (left column) and a photopically matched long-wavelength flash (right column) at various interstimulus intervals after a test flash in a normal subject. The dashed curve in the right column is the cone response at 1 second superimposed on the response at 5 seconds to show that cones have fully recovered at the shortest ISI measured. The left column shows that cone responses to the blue flash are present at the shortest interstimulus interval (ISI) but that rod responses are not present before an ISI of 3 seconds.

time after each flash was virtually identical. Again, the dashed curves are the best fits of equation 1, with $S$ and $t_d$ (from full fits to dark-adapted a-waves) held constant; the solid curves during recovery from 13.1 ln scot td-sec test flashes are slightly better fits, with log $S$ decreased by 0.2 log units. Figure 6 shows a family of recovery functions obtained from one normal subject over three recording sessions. The results demonstrate clearly that, with increasing $I_f$, there was an increase in critical time, $T_c$. As for the subject with monochromatism (Fig. 3), the recovery curves leading to the determination of $T_c$ also appeared to have an intensity-dependent time constant ($\tau_r$).

Critical times for this normal subject are plotted as a function of ln $I_f$ in Figure 7 (solid circles). For test flash intensities of up to 10.5 ln scot td-sec, $T_c$ exhibits a relatively weak, approximately linear dependence on ln $I_f$ (slope = 0.2 seconds). Beginning at approximately 11 ln scot td-sec and continuing up to the highest $I_f$ examined (13.9 ln scot td-sec), $T_c$ grows much more rapidly with ln $I_f$. This higher branch of the $T_c$ versus ln $I_f$ function is well described by a straight line of slope ($\tau_r$) 2.3 seconds. Four additional normal subjects were tested over a more limited range of test flash intensities. The bold dashed curve repre-
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FIGURE 5. Rod-only responses to second (probe) flash (9.3 In scot td-sec) of two-flash paradigm in a normal subject. Dashed curves represent best fit of equation 1 with S and $t^h$ held constant at values derived from fits to a full set of dark-adapted a-waves (see Fig. 1). Slightly better fits were obtained for some ISI by decreasing log S by 0.2 log unit (solid curves). (left column) Responses at various ISI after test flash of 10.9 In scot td-sec. (right column) Responses at various ISI after test flash of 13.1 In scot td-sec.

These results represent the mean slope of the function relating $T_c$ to $I_f$. The average value of $\tau$ for values of $I_f$ above 11 In scot td-sec was 2.3 seconds; 95% confidence limits for normal are shown as dotted curves. Also shown are the results from two subjects with monochromatism tested with two test flash intensities within this range (open symbols). Results were similar to those from the normal subject, with an average $\tau$ value of 2.1 seconds.

Rod responses in patients with the pro-23-his form of adRP were less than one fourth the magnitude of rod responses in normal subjects. The responses from patient 3540 shown in Figure 8 are representative of the three patients. The short, dashed curves again represent the best fits of equation 1 to each flash-ISI combination, allowing only the maximum response, $RmP3$, to vary. (Values of S and $t^h$ were obtained from fits of the model to a range of dark-adapted responses; see Fig. 1). The left column shows rapid recovery after test flashes of 10.9 In scot td-sec. A response clearly is present at an ISI of 2 seconds and grows to >50% of the baseline response by 4 seconds ISI. In contrast, the recovery shown in the right column to flashes of 13.1 In scot td-sec was much slower than in normal subjects because the response was barely detectable at an ISI of 16 sec. The growth in relative amplitude for each test flash intensity is plotted against ISI in Figure 9. Values of $T_c$ derived from the best fits of equation 2 to each curve are shown for patient 3540 and for two other patients in Figure 10. These patients' values of $T_c$ for test flashes up to 10.9 In scot td-sec were normal. However, all three patients (including one patient tested on two occasions) had $T_c$ values that exceeded the normal confidence limit at high test flash intensities. The solid line indicates the average slope of the relationship between $T_c$ and $I_f$ for the three patients. Although this upper limb was based on only two measures per patient, the slope of the line...
DISCUSSION

We have analyzed the recovery of a-wave amplitude after a bright test flash. The results show that, after correction for the cone contribution to the a-wave, the initiation of recovery exhibits a delay, $T_c$, that increased with test flash intensity. Recovery of the cone-mediated response was complete within 1 second, i.e., within a period substantially shorter than that for rod-mediated responses at high test flash intensities. The appropriateness of the techniques used for isolating the rod photoreceptor component was evident from the similarity to the results from subjects with rod monochromatism. Based on this and on the agreement of ERG-derived rod properties with those observed in single-cell recordings from human rods, we interpret the parameter $T_c$ to represent the period of rod photocurrent saturation produced by the test flash.

The current study was motivated by evidence that the slope of the $T_c$ versus $\ln I_t$ function in salamander rods (20°C to 25°C) represents the exponential lifetime of $R^*$. A proportionality between $T_c$ and $\ln I_t$ is in itself consistent with an exponential decline of any flash-activated transduction intermediate that precedes cGMP hydrolysis, i.e., activated rhodopsin, transducin, or cGMP phosphodiesterase ($R^*$, $T^*$, or PDE*).

In the earlier studies, the association between the slope of the $T_c$ versus $\ln I_t$ function and the lifetime of $R^*$ was based in part on the finding that the proportionality of $T_c$ and $\ln I_t$ extends to flash intensities well above those calculated to produce near-complete, transient activation of transducin. Based on light-scattering data from bovine rods at 20°C to 22°C this "saturation" of transducin activation occurred at a fractional bleach of approximately $7 \times 10^{-5}$. Extension of the linear $T_c$ versus $\ln I_t$ function above the transducin saturation bleaching level of $7 \times 10^{-5}$ implies that the slope of this function is a measure of the lifetime of an intermediate "upstream" from transducin, namely, $R^*$. Photocurrent recordings obtained from rat rods indicate that increasing the temperature from approximately 21°C to 36°C increases the half-saturating flash intensity, i.e., decreases flash sensitivity—by approximately threefold. Taking this as a rough indicator of the temperature dependence of transducin saturation, one obtains 0.02% (i.e., $3 \times 7 \times 10^{-5}$) as an estimate of the fractional bleach yielding transducin saturation in human rods in vivo. For all subjects examined in the current study, the upper branch of the $T_c$ versus $\ln I_t$ function was approximately linear over a significant range of flash intensities that exceeded 0.02% bleaching. On this basis, we hypothesize that the observed slope of this upper branch represents the in vivo lifetime of $R^*$. For the normal subjects and the subjects with rod monochro-
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10.9 In scot td-sec

2 sec ISI

4 sec ISI

16 sec ISI

120 sec ISI

25 μV

0 5 10 15 10 15

13.1 In scot td-sec

16 sec ISI

32 sec ISI

64 sec ISI

120 sec ISI

0 5 10 15

time (msec)

FIGURE 8. Rod-only responses to second (probe) flash (9.3 In scot td-sec) of two-flash paradigm in a patient with autosomal dominant retinitis pigmentosa. Dashed curves represent best fit of equation 1 with $S$ and $t_4$ held constant at values derived from fits to a full set of dark-adapted $a$-waves as in Figure 1. (left column) Responses at various interstimulus intervals (ISI) after a test flash of 10.9 In scot td-sec. (right column) Responses at various ISI after test flash of 13.1 In scot td-sec.

matism, the apparent $R^*$ lifetime was 2.3 seconds, a value remarkably similar to that determined in salamander rods.

Patients with the pro-23-his mutation had large decreases in maximum rod amplitude ($R_{mp}$). Clearly, these patients have decreased numbers of functioning outer segment disks because of cell loss, shortened outer segments of remaining cells, or both. However, decreased numbers of disks alone cannot explain either the abnormally low gain of activation ($S$) or the slowed inactivation, as indexed by a measured upper-branch slope of 12.1 seconds (approximately five times the normal value). If the density of cGMP-gated channels per unit rod outer segment length is normal in the patients tested here, the decrease in $S$ implies abnormalities within the activation stages of transduction.\textsuperscript{9} Moreover, to the extent that the deactivation kinetics of $R^*$ determine the upper-branch $\Delta T_A/\Delta \ln I_t$ slope, our findings suggest a prolonged lifetime of $R^*$ in the form of adRP associated with the pro-23-his mutation. These abnormalities in transduction may directly reflect the abnormal function of the pro-23-his mutant rhodopsin. It has been shown in studies of 9-desmethyl rhodopsin, an analog visual pigment, that an alteration of the pigment molecule itself can underlie both reduced activation efficiency and sluggish inactivation of the rod response.\textsuperscript{5,15–17} However, we cannot rule out the possibility that the pro-23-his mutant rhodopsin alters the function of the normal transduction machinery. For example, the mutant pigment could somehow cause deactivation of a transduction intermediate downstream from $R^*$ (e.g., PDE*) to become the rate-limiting step in determining the upper-branch slope. Alternatively, the mutant rhodopsin might act to impede the lateral diffusion of normal rhodopsin in the disk membrane (thereby reducing...
amplification), or to inhibit the diffusion of soluble components such as arrestin (thereby slowing recovery). Conceivably, abnormalities in the normal transduction components could be induced by the pro-23-his mutant pigment even if this protein were expressed at low levels or were incapable of reaching the outer segment.

Numerous psychophysical experiments have attempted to measure the persistence of rod signals and to relate these measures to the kinetics of phototransduction. Typically, these studies used a two-flash (probe plus flash) paradigm developed to measure the response functions of the human visual system. In this paradigm, a probe flash is presented as an increment on a flash of light, and the subject must distinguish between the probe plus flash versus the flash alone. At high flash intensities, the visual system saturates. At first, the probe plus flash is indistinguishable from the flash, but it becomes distinguishable during the short-term afterimage. Studies have measured the time at which the probe becomes discriminable by either measuring reaction times for probe detection or by adjusting a tone to coincide with the detection of the probe. The solid squares in Figure 11 show the data from seven normal subjects in the Benzschawel and Massof study measuring reaction time, and the pluses are the data from three subjects in the Adelson study adjusting a tone. The open circles represent the data from Figure 7 of the current study. Both ERG and behavioral data show evidence for two branches. In both studies, data were fitted with two exponentials with time constants of 0.26 and 1.6 seconds (Adelson) and of 0.19 and 1.2 seconds (Benzschawel and Massof). These are close to our values of 0.2 and 2.3 seconds. The solid lines show that the agreement is good when our values are fitted to their data. The behavioral measures, however, appear displaced on the flash energy axis relative to our measures by approximately 2.3 In units. Although it is accepted that the probe plus flash data can be influenced by postreceptoral nonlinearities, there is a simple explanation for this discrepancy that is consistent with a receptor origin of the delay in the detectable signal. According to Geisler's theoretical analysis, the signal is most detectable when the response returns to approximately 50% of its maximum value. Thus, the behavioral measures should be compared to our estimate of the time at which the response to our second flash returns to 50% of its maximum value. The open squares are the estimates of these values. The agreement between the behavioral and the ERG data are remarkable given the differences in techniques and assumptions, as well as possible variations in calibrations.

Psychophysical techniques also were used to study recovery kinetics in patients of undisclosed genetic type with retinitis pigmentosa. Three showed rod afterimage data that were at least qualitatively similar to those reported here for patients with the pro-23-his mutation. Each patient showed a normal slope
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for the lower branch of the recovery function and a markedly elevated higher branch. It now appears that this time-constant measured psychophysically in previous studies and electrophysiologically in the current study reflects a defect in inactivation leading to a prolonged lifetime of R*.

Previous studies have documented that rod b-waves are reduced in amplitude and delayed in implicit time in the pro-23-his form of adRP.28 The current results extend the analysis of the phenotype to the rod a-wave, and they suggest that the gain of the activation stage of transduction is abnormally low in these patients and that recovery from activation during early dark adaptation is abnormal in patients after test flashes that bleach < 12% of the visual pigment. It is of interest that abnormalities also have been reported in the time-course of dark adaptation after a more extensive bleach in patients with the pro-23-his mutation.29 Although the delays in dark adaptation and the delays reported here involve the recovery of rod sensitivity, the mechanisms are different. The abnormalities in dark adaptation exist for more than an hour and reflect the kinetics of rhodopsin regeneration, rather than the inactivation of R*. Nevertheless, it is possible that both abnormalities reflect conformational changes in the opsin molecule resulting from the pro-23-his mutation.

Key Words
electroretinography, inactivation, gene mutations, retinitis pigmentosa, transduction

References
16. Corson DW, Cornwall MC, MacNichol EF, et al. Relief


