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Rod Photoreceptor Temporal Properties in Retinitis Pigmentosa

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Abstract

One of the characteristic signs of retinitis pigmentosa (RP) is the progressive loss of night vision. We have previously shown that the gain of rod photoreceptor activation is moderately reduced in some patients with RP, but this decrease in activation kinetics is not sufficient to account for the night blindness. Recently, single rod recording from animal models of RP showed rods under degeneration remain saturated for shorter periods than normal rods; i.e. are less able to sustain the rod photoresponse. Using paired-flash ERG, here we determine whether rod phototransduction inactivation parameters might also be abnormal in patients with RP. Inactivation parameters were derived from 13 subjects with normal vision, 16 patients with adRP, and 16 patients with autosomal recessive/isolate (rec/iso) RP. The adRP cases included 9 patients with rhodopsin mutations and 7 patients with peripherin/RDS mutations. The inactivation phase was derived using a double-flash paradigm, with a test flash of 2.7 log scot td-sec followed at varying intervals by a 4.2 log scot td-sec probe flash. Derived rod photoresponses to this just-saturating test flash in normal subjects exhibit a critical time to the initiation of recovery (T_{sat}) of 525 ± 90 (SD) msec. The values of T_{sat} were 336 ± 104 (SD) msec in patients with adRP ($P < 0.001$) and 271 ± 45 (SD) msec ($P < 0.001$) in patients with rec/iso RP. When T_{sat} values were categorized by mutations, the values were 294 ± 91 (SD) msec ($P < 0.001$) for rhodopsin mutations, and 389 ± 100 (SD) msec ($p = 0.01$) for peripherin/RDS mutations. Overall, T_{sat} in patients with RP was significantly correlated with the amplitude of ISCEV standard rod response ($r = 0.56$; $P < 0.001$) and the gain of the activation phase of phototransduction ($r = 0.6$, $P < 0.001$). T_{sat} may be a useful marker for therapeutic efficacy in future clinical trials in RP.

Keywords

Rod inactivation; ERG; phototransduction; RP; rhodopsin; peripherin/RDS

1. Introduction

Retinitis pigmentosa (RP) affects primarily the photoreceptors, especially the rods. Rods hyperpolarize to light, the biochemical basis of which is the rod phototransduction cascade. Phototransduction converts photonic energy into graded changes in rod membrane potential,

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which trigger electrical and chemical changes in other neuronal types in the retina. The resulting photoresponse is a representation of the electrical activities in the retina when a stimulus is presented. The human electroretinogram (ERG) is used clinically to record these photoresponses non-invasively. The ERG waveform represents the propagation of signal transduction in the retina and the a-wave in particular represents photoreceptor activities (Dowling, 1987; Hood and Birch, 1990a, b; Ogden, 1994; Steinberg, 1991). The kinetics of the rod a-wave reflects both the activation and inactivation stages of rod phototransduction.

Much is known about the biochemical basis of the activation steps in vertebrate rods (Pugh and Lamb, 1993). A mathematical model based on the known biochemical events has been shown to accurately index the gain of phototransduction from the membrane current of the outer segment of single rods (Kraft et al., 1993; Lamb and Pugh, 1992; Pugh and Lamb, 1993) as well as massed rod current indexed by the rod ERG a-wave (Breton et al., 1994; Cideciyan and Jacobson, 1996; Hagins et al., 1970; Hood and Birch, 1994). Reduction of the gain of rod activation ($\log S$) has been found in a sub-population of RP and other retina degeneration diseases (Birch et al., 2002; Birch et al., 1995; Hood and Birch, 1996; Jacobson et al., 2000; Shady et al., 1995; Tzekov et al., 2003).

Recent studies reported that rate of rod inactivation can be shifted to longer or shorter than normal times by specific mutations. Mouse rods lacking guanylate cyclase activating proteins GCAP1 and GCAP2 (GCAPs^(-/-)) showed delayed recovery of the membrane current recorded from single rod outer segments (Burns et al., 2002). Consistent with this result, we reported massed rod inactivation was delayed in patients of cone-rod dystrophy with mutations in guanylate cyclase-activating protein (GCAP1) (Jiang et al., 2008). In addition to GCAPs, slowed rod inactivation was reported in mice with a deletion in any component of the RGS9.Gbeta5.R9AP GTPase-activating (GAP) complex (Chen et al., 2000; Keresztes et al., 2004; Krispel et al., 2003; Krispel et al., 2006). On the contrary, upregulation of the expression of GAP complex lead to earlier-than normal photoreceptor recovery (Krispel et al., 2006; Nishiguchi et al., 2004). Recently, earlier-than-normal rod recovery was reported in multiple animal models of RP, including the dystrophic Royal College of Surgeons (RCS) rat (Niculescu, 2004), transgenic pigs expressing P347L or P347S rhodopsin (Kraft et al., 2005), and a mild light damage model using albino rat (Kraft et al., 2005; Niculescu, 2004; Wen, 2008).

The rate of rod inactivation is also determined by the intensity of the stimulating flash. A two-branched relationship between T_{sat} and $\ln(I_{\text{test}})$ had been reported in salamander and mice rod recordings (Burns et al., 2002; Pepperberg et al., 1992) and in the human ERG (Birch et al., 1995).

We previously found that patients with pro-23-his rhodopsin mutation showed slower than normal recovery when tested with intense flashes (i.e. on the upper branch of the $T_{\text{sat}} - \ln(I_{\text{test}})$ function (Birch et al., 1995). Here we sought to determine the extent to which abnormal rod phototransduction inactivation is present to test saturating flashes lie on the lower slope of the $T_{\text{sat}} - \ln(I_{\text{test}})$ function. Moreover, we sought to quantify the relationships among rod inactivation kinetics, rod activation kinetics, and the degree of rod degeneration as indexed by International Society for Clinical Electrophysiology of Vision (ISCEV) standard rod response amplitudes in a sample of patients with different genetic forms of RP.

2. Materials and methods

2.1. Subjects

The subjects included 13 normal controls, 16 patients with autosomal dominant RP (adRP), and 16 patients with an autosomal recessive/isolate (rec/iso RP). The 16 patients with adRP

consisted of 9 patients with rhodopsin (RHO) mutations and 7 patients with peripherin/RDS mutations. The mutations associated with the rec/iso cases are unknown. These patients were selected from a larger cohort referred for ERG testing by ophthalmologists specialized in retinal diseases. Genetic testing results were retrieved from the database of Southwest Eye Registry of the Retina Foundation of the Southwest. In order to ensure sufficient a-wave amplitude for reliable analysis, patients were included if their ISCEV standard rod response amplitude was greater than 10 μV . The tenets of the Declaration of Helsinki were followed and all subjects gave written informed consent after a full explanation of the procedures was given.

2.2. Evaluation of rod activation and inactivation kinetics

Human subjects were dark adapted for 45 minutes after pupil dilation (1% tropicamide and 2.5% phenylephrine hydrochloride). ERGs were recorded in a Ganzfeld dome with bipolar contact lens (Burian-Allen electrode, Hansen Laboratories, Coralville, IA). ISCEV Standard rod ERGs were recorded immediately after dark adaptation, followed by high-intensity ERGs, paired-flash ERGs and lastly, light-adapted ERGs.

Methods of evaluating the rod activation process and detailed analysis procedures are described elsewhere (Hood and Birch, 1994; Shady et al., 1995; Tzekov et al., 2003). Briefly, after 45 minutes of dilation and dark adaptation, achromatic flash (Model 2150, Novatron, Dallas, TX) was presented with at least 45 seconds between flashes. Three responses were averaged at each retinal illuminance (3.2, 3.6, 4.1, and 4.4 log scot td-sec). Rod-only a-waves were obtained by subtracting cone-only a-waves, which were recorded to the same four illuminances in the presence of a rod-saturating background light (3.3 log scot td) in the Ganzfeld dome. The leading edges of the a-waves were fitted simultaneously with a mathematical model based on the activation model of rod phototransduction (Breton et al., 1994; Lamb and Pugh, 1992). The sensitivity (log S) was derived from the fitting process to describe the phototransduction gain in the rod activation process (Hood and Birch, 1994, 1996; Shady et al., 1995; Tzekov et al., 2003).

Methods of pair-flash ERG recording and detailed analysis procedures are also described elsewhere (Birch et al., 1995; Pepperberg et al., 1997). Briefly, a test flash was paired with a probe flash on each trial, with test flash delivered first. The inter-stimulus-interval between the two flashes was variable. The test flash was an achromatic flash (2.7 log scot td-sec) delivered by the Grass Photostimulator (PS33 Plus, Grass Instruments, Warwick, RI). The probe flash ($\lambda_{\text{cut-off}} = 470 \text{ nm}$, 4.2 log scot td-sec) was delivered by a high-intensity flash lamp (Model 2150, Novatron, Dallas, TX). T_{sat} , the period prior to recovery from saturation from the test flash, was a derived value from an exponential recovery function: $A/A_{\text{max}} = 1 - \exp[-(t - T_{\text{sat}})/\tau]$, where A_{max} is the saturating amplitude of rods, and A is the derived rod response amplitude to the test flash at various time points (msec) after the test flash was given, and τ is the time constant of recovery.

Three subjects (one patient with rec/iso RP, one blue cone monochromat, and one normal control) were tested with a range of test flash retinal illuminances (2.7, 3.2, 3.6, and 4.1 log scot td-sec). For these higher test flash intensities, a second Model 2150 Novatron stimulator replaced the Grass Photostimulator.

2.3. Statistical analysis

All two-sample hypothesis tests were preceded by serial randomness test and F test for equal variance. Unpaired independent two-sample Student's t-test was used for samples with equal variance. The Wilcoxon Rank-Sum test was used for samples with unequal variance. All

statistical analyses were performed using Igor Pro 6.12 (WaveMetrics, Inc. Lake Oswego, Oregon).

3. Results

3.1. Time to initiation of rod recovery (T_{sat}) to a fixed test flash

The “paired flash” ERG paradigm is used to determine T_{sat} , the value of the inter-stimulus interval (ISI), after which the rods start to respond to the probe flash. That is, it is a measure of the duration of rod saturation caused by the test flash.

Figure 1A shows the ERG waveforms (solid traces) of a normal subject to probe flashes at different inter-stimulus-intervals (ISI)(0.2 sec to 1.2 sec) following the test flash. The probe response is small at shorter ISIs (0.2 sec to 0.6 sec). These small asymptotic probe response in Fig. 1A are cone mediated (Birch et al., 1995; Pepperberg et al., 1997), as the cones recover faster than the rods (Schnapf et al., 1987). However, as ISI lengthens, the probe responses grow in amplitude (Fig. 1A) due to rod recovery. When the ISI is 1.2 seconds (Fig. 1A), the amplitude of the rod response to the probe flash is equivalent to that when the probe flash is presented alone (Fig. 1A, broken line).

We assumed that the probe response at ISI of 200 msec (Fig. 1A) is a pure cone response to the probe flash and subtracted its waveform from all probe responses shown in Figure 1A. The resulting waveforms are shown in Figure 1B, which were taken as the pure rod responses at different ISIs to the probe flashes (Fig. 1B). As shown in Figure 1B, at ISI of 400 msec, the rods still had not responded to the probe flash; that is, the amplitude of the rod probe response was close to zero. However, the rods fully recovered and showed a maximum response to the probe flash when the ISI was 1.2 seconds. In other words, at ISIs of 200 msec and 400 msec, the rods were still saturated by the test flash, and therefore, could not respond to the probe flash. As the rods recovered from saturation, the response to the probe grew in amplitude until it was equivalent to the probe presented alone (Fig. 1B).

Figure 1C shows the data from this normal subject plotted as the relative recovery of the rod response to the test flash from saturation (A/A_{max}), where A is the derived rod response amplitude of the test flash at a particular time point ($t = \text{ISI}$) after the test flash ($A(t) = A_{\text{max}} - A_{\text{probe}}(t)$) (Pepperberg et al., 1997). Thus, Figure 1C shows that rods were initially saturated by the test flash, but recovered completely over the next second or so. We fit this relationship with an exponential recovery function ($A/A_{\text{max}} = 1 - \exp[-(t - T_{\text{sat}})/\tau]$), where T_{sat} indicates the initiation of rod recovery from saturation from the test flash. In Figure 1C, the solid curve is the best fit of the exponential recovery function and the derived T_{sat} value for this normal subject was 556 msec. For 13 normal subjects, the mean T_{sat} value was 525 ± 90 (SD) msec, and the mean τ value was 535 ± 186 (SD) msec.

3.2. Normal T_{sat} in blue-cone monochromat validates cone subtraction procedure

The fundamental premise of the “paired-flash” paradigm is that pure rod response can be derived from mixture of rod and cone responses by computer-based linear subtraction. This premise has been verified in previous studies (Birch et al., 1995; Pepperberg et al., 1997). For a blue-cone monochromat, every response to the light flashes should be a pure rod response. Thus, T_{sat} should be within normal limits even without the subtraction procedure to isolate the rod probe response.

The blue-cone monochromat (RFSW#355; age 65) had a normal dark-adapted threshold and a normal rod ERG amplitude. However, light-adapted cone responses and the 31 Hz flicker response (above rod fusion frequency) were minimally detectable. The probe responses from this blue cone monochromat are shown in Figure 1D and 1E as a function of ISI. The rods

did not respond to probe flashes at ISIs of 200 msec, 300 msec, or 400 msec, but grew in amplitude at more prolonged ISIs. The time course of photoresponse recovery to the 2.7 log sc td-sec test flash was equivalent to normal, with a T_{sat} value of 479 msec, and tau value of 392 msec (Fig. 1F).

3.3 Premature photoresponse recovery is linked to rod degeneration in RP

Fig. 2A shows rod-only responses from a 37 year-old patient with adRP (RHO pro-23-his mutation). The derived time course of recovery is shown in Fig 2B (filled circles) with a T_{sat} of 254 msec and tau of 113 msec. The representative normal subject (triangles) shows slower recovery, with a T_{sat} of 571 msec and tau of 485 msec. Also shown is the recovery function from a previously reported patient with cone-rod dystrophy (squares) due to a GCAP1 mutation (Jiang et al., 2008) and a T_{sat} of 802 msec and tau of 581 msec. Compared to the representative normal subject, the rod photoresponse recovers earlier in the RP patient with a RHO mutation and later in the patient with CRD (GCAP1 mutation).

The T_{sat} values for 16 patients with autosomal dominant RP and 16 patients with rec/iso RP are shown in Figure 2C. T_{sat} , derived from 16 patients with adRP, was 336 ± 104 (SD) msec, a 36% reduction from the normal 525 ± 90 (SD) msec. The reduction was statistically significant ($P < 0.001$). The 16 patients with adRP include 9 patients with rhodopsin mutations (four cases of pro-23-his, single cases of pro-170-arg, pro-210-arg, phe-9-val, ala-164-val, and cys-185-arg). The mean T_{sat} value for these patients was 294 ± 91 (SD) msec, as compared to 525 ± 90 (SD) msec for the 13 normal subjects (Fig. 2C). The 44% decrease from normal mean T_{sat} value was statistically significant ($P < 0.001$).

We next asked whether mutations in peripherin/RDS are associated with changes in T_{sat} . Peripherin is present in the rim of mature disks of rod and cone outer segments (Arikawa et al., 1992). The product of the peripherin/rds gene is a photoreceptor disc membrane-associated glycoprotein (Travis et al., 1991). Five of the seven patients with RDS/peripherin mutation had manifested RP phenotype, but the other two were asymptomatic at the time of testing. The mean T_{sat} in 7 patients with peripherin/RDS was 389 ± 100 (SD) msec, which was 26% smaller than the normal value of 525 ± 90 (SD) msec. This difference was statistically significant ($P = 0.01$). The T_{sat} values in the peripherin/RDS group ranged from 217 msec to 520 msec (Table 1). The two highest T_{sat} values (489 msec and 520 msec) were derived from the two patients who carried a peripherin/RDS mutation but were asymptomatic.

Finally, the mean T_{sat} in the 16 rec/iso RP cases was 271 ± 45 (SD) msec, 48% lower than the normal average ($P < 0.001$) (Fig. 2C). Thus, T_{sat} reductions appear to be common within the RP phenotype. The average T_{sat} in rec/iso RP (271 ± 45 msec) was shorter than that in the autosomal dominant RP (336 ± 104 msec) ($P = 0.03$). This difference probably resulted from the inclusion of the two asymptomatic patients with peripherin/RDS mutation in the adRP group. After removal of these two patients, no significant difference could be detected between the 16 patients with rec/iso RP (271 ± 45 msec) and the 14 patients with symptomatic adRP (311 ± 87 msec) ($P = 0.08$).

Table 1 also showed that tau values were significantly lower ($P = 0.007$) in patients with adRP (36% reduction), either associated with RHO mutation (35% reduction, $P = 0.04$) or peripherin/RDS mutation (38% reduction, $P = 0.01$). For patients with rec/iso RP, the average tau values were 21% lower than normal average, although the difference was not statistically significant ($P = 0.09$).

3.4 T_{sat} decreases when dark-adapted rod response amplitude decreases

The human ERG b-wave reflects the activity of bipolar cells within the nuclear layer (Hood and Birch, 1990a, 1992). With the assumption that rod-bipolar cell synaptic transmission remains unaffected by retinal degeneration, the dark-adapted ERG rod response (ISCEV standard) has been recognized as a surrogate marker for rod activity (Marmor et al., 2009). We examined the relationship between T_{sat} and the ISCEV standard rod response amplitude. As shown in Figure 3, there is a significant correlation between these two indices of rod dysfunction in 32 RP patients (Pearson's Correlation Coefficient = 0.56, $P < 0.001$). Thus, as ISCEV rod response diminishes, T_{sat} generally becomes shorter.

3.5 Effect of test flash intensity on T_{sat}

In single rod recordings, the relationship between T_{sat} and the natural logarithm ($\ln$) of test flash intensity (I_{test}) can be divided into two branches (Pepperberg et al., 1992). The slope of the linear regression of the lower branch is the dominant time constant and index as the rate-limiting step of photoreponse inactivation (Krispel et al., 2006). Similarly, a two-branched relationship between T_{sat} and $\ln(I_{\text{test}})$ was observed when T_{sat} is derived from paired-flash ERG in normal subjects and patients with pro-23-his associated adRP (Birch et al., 1995). In Figure 4A, we examined the lower branch of the T_{sat} and $\ln(I_{\text{test}})$ function (6.2–9.4 $\ln$ scot td-sec) derived from paired-flash ERG. Figure 4A shows the linear relationship between the T_{sat} and the natural logarithm ($\ln$) of test flash intensity (I_{test}) when I_{test} is between 6.2 and 9.4 $\ln$ scot td-sec, which is equivalent to 2.7 to 4.1 $\log$ scot td-sec. Decreasing the test flash intensity by a factor of 10 decreased T_{sat} by approximately 400 msec.

Earlier findings suggest that the dystrophic rods in patients with RP may have reduced phototransduction gain ($\log S$) (Birch et al., 2002; Birch et al., 1995; Hood and Birch, 1996; Jacobson et al., 2000; Shady et al., 1995; Tzekov et al., 2003). In other words, the dystrophic rods may not be as sensitive to the test stimulus as the healthy rods. Therefore, we determined the relationship between T_{sat} and phototransduction gain ($\log S$). Within the sample of 32 patients with RP tested for inactivation parameters, phototransduction gain ($\log S$) values were also available for 29 RP patients. A positive and significant correlation was found between T_{sat} and $\log$ rod S in these 29 patients with RP ($r = 0.6$, $P < 0.001$) (Fig. 4B). However, even patients with $\log$ rod S within the normal range showed lower than normal values of T_{sat} .

4. Discussion

We found that the rod photoresponse duration, as indexed by T_{sat} , is shorter in patients with RP than in normal subjects. Previously, we found the gain of the activation mechanism, as measured by the value of $\log S$, is reduced in a majority of patients with rhodopsin mutations (Birch et al., 1995). Biochemically, reduced gain and premature inactivation will result in decreased synaptic input to bipolar cells. Together with rod loss, this helps to explain the loss of sensitivity of the ISCEV standard rod b-wave response and the elevated dark-adapted threshold in patients with rod degeneration. Studies of single rod recording support this argument. Pro-347-leu and pro-347-ser mutations in rhodopsin, like the rhodopsin mutations included in this study, lead to autosomal dominant RP in human. By direct recording from single rod photoreceptors in pigs, Kraft et al. (Kraft et al., 2005) showed that transgenic rods with pro-347-leu and pro-347-ser rhodopsin have earlier-than-normal time-to-peak, diminished sensitivity to light, and reduced integration time. Degenerating rods associated with the Mertk mutation (Gal et al., 2000) in Royal College of Surgeons (RCS) rat showed earlier than normal recovery without changes in gain (Niculescu, 2004). Similar results were found in albino rat rods after mild light damage (Wen, 2008).

In contrast to the findings in RP, we previously found that rod inactivation was significantly delayed in two patients with CRD associated with mutations in guanylate cyclase-activating protein (GCAP1) (Jiang et al., 2008). The delayed rod inactivation possibly resulted from the inability of mutant GCAP1 to respond to intracellular $[Ca^{2+}]$ drop when cGMP-gated cation channel is closed (Jiang et al., 2008). In transgenic mouse models, mutations in other genes directly involved in inactivation steps can also lead to delayed recovery from activation (Chen et al., 2000; Keresztes et al., 2004; Krispel et al., 2003). Thus, it seems that delayed inactivation kinetics is specific to a small number of mutations and may be useful in guiding mutation screening.

Although a correlation exists between shortened T_{sat} and rod b-wave amplitude ($r = 0.56$), T_{sat} measures cannot be replaced by rod b-wave measures. Because the rod b-wave reflects the massed rod response, response amplitude depends on not just sensitivity of rods, but also surviving rod count. Being a kinetic measure, T_{sat} is not affected by surviving rod count in retinal degeneration.

T_{sat} and log S are correlated since both reflect severity of the disease. However, they are not interchangeable since inactivation kinetics is faster than normal even in patients with normal sensitivity (Fig. 4B).

Furthermore, kinetic measures such as T_{sat} may be a more sensitive marker for rod health than sensitivity measures. Recent studies show that rod photoresponse recovery kinetics shorten to degenerative stress (Niculescu, 2004; Wen, 2008). However, the shortened rod recovery reverts back to normal timing during recovery in a normal environment. Recovery is less pronounced in sensitivity measures (Wen, 2008).

A major obstacle in developing treatment for heterogeneous genetic forms of RP is the prolonged time course of the disease. The annual rate of decline in b-wave amplitude is slow so that trials typically lasts years (Berson et al., 1993; Hoffman et al., 2004). Here we have shown that T_{sat} is abnormally short in patients with different forms of RP. Further studies are needed to determine whether recovery of T_{sat} might be a useful biomarker for treatment efficacy.

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Figure 1.

T_{sat} values derived from a normal subject and a patient with blue cone monochromacy. (A) ERG waveforms of a normal subject to a probe flash after a fixed test flash at different inter-stimulus-interval (ISI). Response to probe alone is shown in broken line. (B) As rods recover from saturation, the rod-only response to probe grew in amplitude until it is equivalent to the probe presented alone. Rod-only responses were derived by subtracting the cone responses at 200 msec from responses at each ISI. (C) T_{sat} of 556 msec was derived from the rod photoresponse recovery to the test flash (A/A_{max}) in a normal subject, where A is the derived rod response amplitude of the test flash at a particular time point ($t = ISI$). $A(t) = A_{max} - A_{probe}(t)$ (Pepperberg et al., 1997). (D) For a blue-cone monochromat, every response to a light flash can be considered as pure rod response. The rods did not respond to probe flash at ISIs of 200 msec, 300 msec, or 400 msec, but grew in amplitude at more prolonged ISIs. Response to probe alone is shown in broken line. (E) ERG waveforms of a blue cone monochromat to a probe flash after a fixed test flash at different ISIs. (F) For a blue cone monochromat, the time course of photoresponse recovery to the 2.7 log sc td-sec test flash was equivalent to normal, with a T_{sat} value of 479 msec.

Figure 2.

T_{sat} for patients with RP. (A) Rod-only response from a 37 year-old patient with adRP (RHO pro-23-his mutation) (Fig. 2A). The T_{sat} derived from this patient was 254 msec with tau of 113 msec (filled circles, Fig. 2B). (B) Figure 2B compares the full time course of the rod photoresponse recovery in a representative patient with RP (RHO pro-23-his mutation) and a normal subject. Also shown is the recovery function from a previously reported patient with cone-rod dystrophy due to a GCAP1 mutation (squares) (Jiang et al., 2008). Compared to the representative normal subject, the rod photoresponse recovers earlier in the RP patient with RP and later in the CRD patient with a GCAP1 mutation. (C) Figure 2C shows T_{sat} results from 16 patients with autosomal dominant RP and 16 patients with rec/iso RP. Average T_{sat} derived from 16 patients with adRP was 336 ± 104 (SD) msec, a 36% reduction from the normal 525 ± 90 (SD) msec ($P < 0.001$). The mean T_{sat} in the 16 rec/iso RP cases was 271 ± 45 (SD) msec, 48% lower than the normal average ($P < 0.001$). In summary, T_{sat} were shorter in patients with RP. Statistical results of T_{sat} measure in all RPs are presented in Table 1.

Figure 3.

Relationship between T_{sat} and the ISCEV standard rod response amplitude in RP. There is a significant correlation between these two indices of rod dysfunction in 32 patients with RP (filled circles) (Pearson's Correlation Coefficient = 0.56, $P < 0.001$). Thus, as ISCEV rod response diminishes, T_{sat} generally become shorter. The relationship between T_{sat} and rod amplitude in 13 normals is also shown (unfilled circles).

Figure 4.

Reduced T_{sat} derived from paired-flash ERG is correlated to reduced phototransduction gain (S). (A) T_{sat} is known to vary with the intensity of the test flash and the T_{sat} -intensity function can be divided into upper and lower branches (Birch et al., 1995). Figure 4A shows the linear relationships in the lower branch between 6.2 and 9.4 ln scot td-sec (2.7 to 4.1 log scot td-sec). (B) Earlier findings suggest that the dystrophic rods in patients with RP may have reduced phototransduction gain (log S) (Birch et al., 1995; Shady et al., 1995; Hood and Birch, 1996; Jacobson et al., 2000; Birch et al., 2002; Tzekov et al., 2003). Figure 4B shows a positive and significant correlation was found between T_{sat} and log S in the 29 patients with RP ($r = 0.6$, $P < 0.001$) where both measures were available. Lines show linear fit to data.

Table 1

Characterization of rod recovery kinetics with T_{sat} and Tau in normal subjects and patients with RP.

	Count	T_{sat} (msec)	Reduction	Significance	Tau (msec)	Reduction	Significance
Normal	13	525 ± 90 (SD)			535±186 (SD)		
adRP	16	336±104	36%	P<0.001	340±173	36%	P=0.007
RHO	9	294±91	44%	P<0.001	347±203	35%	P=0.04
RDS	7	389±100	26%	P=0.01	331±140	38%	P=0.01
rec/iso RP	16	271±45	48%	P<0.001	422±160	21%	P=0.09

Table 1 presents the statistical results of T_{sat} and tau in patients with RP and associated mutations. Significance differences and percent reductions are relative to the normal average.