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Rod Photoreceptor Temporal Properties in Retinal Degenerative Diseases

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62.1 Introduction

Among the many genetic causes of retinal degenerative disease (RDD), some predominantly affect the rods, while others predominantly affect the cones (*RetNet*, <http://www.sph.uth.tmc.edu/RetNet/disease.htm>). Because rhodopsin, for example, is unique to the rods (Liebman et al. 1974), rhodopsin mutations cause the characteristic early night blindness typical of retinitis pigmentosa (RP). Initial loss of rods is followed by secondary cone degeneration as the disease progresses (Petters et al. 1997; Koenekoop 2009; Punzo et al. 2009).

In contrast to RP, patients with cone–rod dystrophy (CRD) initially exhibit dysfunction of the cone photoreceptors and rod-mediated losses are minimal in the early stages. However, in later stage of CRD, rods also degenerate, causing elevated dark-adapted thresholds and reduction in the amplitude of dark-adapted rod electroretinogram (ERG) (Fishman 1976).

Stargardt disease represents a variant of CRD, with dystrophy limited primarily to the posterior pole. It is predominantly an autosomal recessive macular dystrophy caused by ABCA4 (ATP-binding cassette, subfamily A, member 4) mutations (Allikmets et al. 1997a, b; Fishman 2010).

It is unknown whether rod photoresponse kinetics are altered in these human RDDs, although studies in laboratory models of RP showed earlier-than-normal rod photoresponse recovery (Niculescu 2004; Kraft et al. 2005; Wen et al. 2006; Wen 2008; Wen and Kraft 2008). Here, we use paired-flash full-field ERG to characterize the rod photoresponse recovery kinetics in patients with primary rod degeneration (RP), patients with secondary rod degeneration (CRD), and patients with degeneration limited to the posterior pole (Stargardt disease).

62.2 Materials and Methods

62.2.1 Subjects

Patients were recruited from the database of the Southwest Eye Registry at the Retina Foundation of the Southwest. They included 18 patients with autosomal dominant retinitis pigmentosa (adRP) reported previously (Wen et al. 2011), 5 patients with CRD, and 4 patients with Stargardt disease. The 18 adRP patients included 10 patients harboring rhodopsin mutations and 8 patients harboring peripherin/rds mutations. Included within the 5 patients diagnosed with CRD were two autosomal recessive/isolate CRD patients heterozygous for an ABCA4 mutation (ABCA4: Asn-965-Ser or Ala-192-Thr) and one autosomal dominant patient carrying a peripherin/rds mutation (Arg-172-Trp). Individuals with normal eye exams ($n = 13$) provided normative values.

In order to ensure reliable analysis of pair-flash ERG results, patients were recruited only if they retained a dark-adapted rod response amplitude of greater than 10 μ V to a ISCEV (International Society for Clinical Electrophysiology of Vision) standard rod stimulus. 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. All procedures were approved by the institutional review board of University of Texas Southwestern Medical Center, Dallas, TX.

62.2.2 Evaluation of the Rod Inactivation Kinetics Using Paired-Flash ERG

Prior to paired-flash ERG, the pupil of the left eye was dilated (1% tropicamide and 2.5% phenylephrine hydrochloride), and the patient was dark adapted for 45 min. Paired-flash ERG was recorded in a Ganzfeld dome. A bipolar contact lens (Burian-Allen electrode, Hansen Laboratories, Coralville, IA) was used to record the signals. Details of pair-flash ERG recording were previously described (Birch et al. 1995; Pepperberg et al. 1997). Briefly, a test flash (achromatic, 2.4 log scot td-s) was delivered prior to the probe flash ($\lambda_{\text{cut-off}} = 470$ nm, 4.2 log scot td-s) with variable inter-stimulus-interval (ISI). The cone response at short (i.e., 200 ms) ISIs was subtracted from the response to the probe flash to provide a rod-only response at each ISI.

62.3 Results

62.3.1 Rod Photoresponse Recovery Kinetics in RDDs

To determine whether rod photoresponse recovery kinetics in patients with RDDs is different from normal, we derived T_{sat} from these patients using the paired-flash paradigm. Figure 62.1a–d shows the rod-only response to probe flash at indicated ISI after a fixed test flash in a normal subject (Fig. 62.1a), a patient with CRD (Fig. 62.1b), a patient with Stargardt disease (Fig. 62.1c), and a patient with RP (Fig. 62.1d), respectively. Figure 62.2 shows 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). The relative recovery of rod photoresponse was fitted with an exponential recovery function ($A/A_{\text{max}} = \exp[-(t - T_{\text{sat}})/\tau]$), where T_{sat} indicates the initiation of rod recovery from saturation from the test flash. Technically, T_{sat} is the horizontal intercept of the exponential fit. In Fig. 62.2, the T_{sat} derived from the patient with RP is 254 ms, which is shorter than that derived from the normal subject ($T_{\text{sat}} = 555$ ms), the patient with CRD ($T_{\text{sat}} = 523$ ms) and the patient with Stargardt disease ($T_{\text{sat}} = 580$ ms).

Figure 62.3 and Table 62.1 show T_{sat} values for 13 normal subjects, 5 patients with CRD, 4 patients with Stargardt disease, and 18 patients with adRP. T_{sat} derived from 5 patients with CRD was 473 ± 113 (SD) ms, which is not different from the normal 544 ± 92 (SD) ms ($P = 0.26$) (Fig. 62.3; Table 62.1). T_{sat} derived from 4 patients with Stargardt disease was 491 ± 98 (SD) ms, which is not different from the normal 544 ± 92 (SD) ms ($P = 0.38$) (Fig. 62.3; Table 62.1). However, T_{sat} derived from 18 patients with adRP was 331 ± 99 (SD) ms, which is 39% shorter from the normal average ($P < 0.001$) (Fig. 62.3; Table 62.1). Two patients with adRP showed T_{sat} values (489 and 520 ms) within the 99% confidence interval (CI) of normal (Fig. 62.3). These two highest T_{sat} values were derived from two patients who carried peripherin/rds mutation but retained normal ERG amplitudes at the time of pair-flash ERG recording.

62.3.2 T_{sat} Is Correlated with Dark-Adapted Rod Amplitude in adRP

The ISCEV standard dark-adapted ERG rod response has been recognized as a surrogate marker for rod activity (Marmor et al. 2009). Figure 62.4 shows the relationship between T_{sat} and the ISCEV standard rod response amplitude in the 18 patients with adRP. A significant correlation exists between these two indices of rod dysfunction in patients with adRP (Pearson's Correlation Coefficient = 0.54, $P = 0.01$) (Fig. 62.4). Thus, as ISCEV rod response diminishes, T_{sat} generally becomes shorter in patients with adRP. All patients with adRP with reduced rod amplitude also showed faster than normal recovery times.

The results from the patients with CRD (solid triangles) and Stargardt disease (solid circles) suggest that the correlation does not hold in these diseases (Fig. 62.4). Note that three of these patients exhibited normal T_{sat} values despite rod amplitudes less than 75 μV , the lower limit of normal adult subjects (Birch and Anderson 1992).

62.4 Discussion

Previously, we reported that rods in retinitis pigmentosa, regardless of the inheritance pattern, recover earlier than normal (Wen et al. 2011). In this study, we found that patients with CRD and Stargardt disease show normal rod photoresponse recovery kinetics. This reinforces the suggestion that shortened rod photoresponse recovery is a result of primary rod degeneration in RP. This finding is consistent with previous invasive studies in laboratory animals. Kraft et al. (2005) showed that transgenic rods with pro-347-leu and pro-347-ser rhodopsin, both of which associated with human adRP, have earlier-than-normal time-to-peak, diminished sensitivity to light, and reduced integration time. In addition, it has been reported that rods with the Mertk mutation, which is associated with autosomal recessive RP in human (Gal et al. 2000), showed earlier-than-normal recovery (Niculescu 2004; Wen et al. 2006). Furthermore, rods that survived transient light damage in albino rat exhibited earlier-than-normal recovery (Wen 2008; Wen and Kraft 2008). Contrary to shortened rod recovery in RP, prolonged rod photoresponse recovery was observed in a type of cone dystrophy associated with GCAP1 mutations (Jiang et al. 2008). In this study, we found that CRD caused by ABCA4 mutations ($T_{\text{sat}} = 523$ and 499 ms) is not associated with either shortened or elongated rod photoresponse recovery.

Rod photoresponse recovery kinetics can be manipulated by genetic engineering in laboratory animals (He et al. 1998; Chen et al. 2000; Kraft et al. 2006; Krispel et al. 2006; Tsang et al. 2007). Moreover, it has been shown that rods are able to recover to normal recovery kinetics in a transient light damage model (Wen 2008; Wen and Kraft 2008). Because normal T_{sat} is specific to healthy rods, rod recovery kinetics could serve as biomarker for treatment efficacy in clinical studies and trials.

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References

- Allikmets R, Shroyer NF, Singh N, et al. Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. *Science*. 1997a; 277:1805–1807. [PubMed: 9295268]
- Allikmets R, Singh N, Sun H, et al. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. *Nat Genet*. 1997b; 15:236–246. [PubMed: 9054934]
- Birch DG, Anderson JL. Standardized full-field electroretinography. Normal values and their variation with age. *Arch Ophthalmol*. 1992; 110:1571–1576. [PubMed: 1444914]
- Birch DG, Hood DC, Nusinowitz S, et al. Abnormal activation and inactivation mechanisms of rod transduction in patients with autosomal dominant retinitis pigmentosa and the pro-23-his mutation. *Invest Ophthalmol Vis Sci*. 1995; 36:1603–1614. [PubMed: 7601641]
- Chen CK, Burns ME, He W, et al. Slowed recovery of rod photoresponse in mice lacking the GTPase accelerating protein RGS9-1. *Nature*. 2000; 403:557–560. [PubMed: 10676965]
- Fishman GA. Progressive human cone-rod dysfunction (dystrophy). *Trans Sect Ophthalmol Am Acad Ophthalmol Otolaryngol*. 1976; 81:OP716–724.
- Fishman GA. Historical evolution in the understanding of Stargardt macular dystrophy. *Ophthalmic Genet*. 2010; 31:183–189. [PubMed: 20809774]

- Gal A, Li Y, Thompson DA, et al. Mutations in MERTK, the human orthologue of the RCS rat retinal dystrophy gene, cause retinitis pigmentosa. *Nat Genet.* 2000; 26:270–271. [PubMed: 11062461]
- He W, Cowan CW, Wensel TG. RGS9, a GTPase accelerator for phototransduction. *Neuron.* 1998; 20:95–102. [PubMed: 9459445]
- Jiang L, Wheaton D, Bereta G, et al. A novel GCAP1(N104K) mutation in EF-hand 3 (EF3) linked to autosomal dominant cone dystrophy. *Vision Res.* 2008; 48:2425–2432. [PubMed: 18706439]
- Koenekoop RK. Why do cone photoreceptors die in rod-specific forms of retinal degenerations? *Ophthalmic Genet.* 2009; 30:152–154. [PubMed: 19941421]
- Kraft TW, Allen D, Petters RM, et al. Altered light responses of single rod photoreceptors in transgenic pigs expressing P347L or P347S rhodopsin. *Mol Vis.* 2005; 11:1246–1256. [PubMed: 16402026]
- Kraft, TW.; Sandoval, IM.; Boye, SL., et al. Society for Neuroscience. Atlanta, GA: 2006. Dramatically altered rod responses in rat retina overexpressing R9AP and RGS9-1.
- Krispel CM, Chen D, Melling N, et al. RGS expression rate-limits recovery of rod photoresponses. *Neuron.* 2006; 51:409–416. [PubMed: 16908407]
- Liebman PA, Jagger WS, Kaplan MW, et al. Membrane structure changes in rod outer segments associated with rhodopsin bleaching. *Nature.* 1974; 251:31–36. [PubMed: 4547251]
- Marmor MF, Fulton AB, Holder GE, et al. ISCEV Standard for full-field clinical electroretinography (2008 update). *Doc Ophthalmol.* 2009; 118:69–77. [PubMed: 19030905]
- Niculescu, DM. Physiological Optics. Birmingham, AL: University of Alabama at Birmingham; 2004. Physiological Characterization of the Light Response of Rod Photoreceptors in the Dystrophic Royal College of Surgeons Rat.
- Pepperberg DR, Birch DG, Hood DC. Photoresponses of human rods in vivo derived from paired-flash electroretinograms. *Vis Neurosci.* 1997; 14:73–82. [PubMed: 9057270]
- Petters RM, Alexander CA, Wells KD, et al. Genetically engineered large animal model for studying cone photoreceptor survival and degeneration in retinitis pigmentosa. *Nat Biotechnol.* 1997; 15:965–970. [PubMed: 9335046]
- Punzo C, Kornacker K, Cepko CL. Stimulation of the insulin/mTOR pathway delays cone death in a mouse model of retinitis pigmentosa. *Nat Neurosci.* 2009; 12:44–52. [PubMed: 19060896]
- Tsang SH, Woodruff ML, Janisch KM, et al. Removal of phosphorylation sites of gamma subunit of phosphodiesterase 6 alters rod light response. *J Physiol.* 2007; 579:303–312. [PubMed: 17138607]
- Wen, Y. Neurobiology. Birmingham, Alabama: University of Alabama at Birmingham; 2008. Physiological Characterization of the Light Response of Rod Photoreceptors in the Light Damaged Rat.
- Wen, Y.; Kraft, TW. Altered Light Response of Rod Photoreceptors Surviving Light Damage. ARVO 2008 Annual Meeting; Fort Lauderdale, Florida, USA. 2008.
- Wen, Y.; Niculescu, DM.; Kraft, TW. Society for Neuroscience. Atlanta, GA: 2006. Desensitization of retinal photoreceptors during disease: not a story of equivalent light.
- Wen Y, Locke KL, Hood DC, Birch DG. Rod photoreceptor temporal properties in retinitis pigmentosa. *Exp Eye Res.* 2011; 92(3):202–208. [PubMed: 21219898]

Fig. 62.1.

Representative photoresponse recovery mediated by rod photoreceptors in normal subject and retinal degenerative diseases (RDDs). Representative rod-only waveforms generated by a probe flash at various inter-stimulus-interval (ISI) after a fixed test flash recorded from a normal subject (a), a patient with cone-rod dystrophy (CRD) (b), a patient with Stargardt disease (c), and a patient with RP (d). The rod-mediated photoresponse shows little recovery at ISIs of 500–600 ms in normal, CRD, and Stargardt disease (a–c). However, by an ISI of 500 ms, the rod-mediated photoresponse is fully recovered in the patient with RP

Fig. 62.2.

Deriving T_{sat} from patients with RP, CRD, and Stargardt disease. T_{sat} was derived from the rod-mediated photoresponse recovery shown in Fig. 62.1 using an exponential fit model ($A/A_{\text{max}} = \exp[-(t - T_{\text{sat}})/\tau]$) (Birch et al. 1995; Pepperberg et al. 1997). T_{sat} derived from the patient with RP ($T_{\text{sat}} = 254$ ms) is shorter than normal ($T_{\text{sat}} = 555$ ms). However, T_{sat} derived from patients with CRD ($T_{\text{sat}} = 523$ ms) and Stargardt disease ($T_{\text{sat}} = 580$ ms) is comparable to normal ($T_{\text{sat}} = 555$ ms).

Fig. 62.3.

T_{sat} derived from normal subject and RDDs. T_{sat} derived from 13 normal subjects, 5 patients with CRD, 4 patients with Stargardt disease, and 18 patients with autosomal dominant retinitis pigmentosa (adRP). Average value and standard deviation of each group is shown next to the scatter plot. *Broken line*: 99% confidence interval of normal T_{sat} distribution

Fig. 62.4.

Relationship between T_{sat} and the ISCEV standard rod response amplitude in patients with CRD, Stargardt disease, and adRP. A significant correlation (*solid line*) exists between these two indices of rod dysfunction in 18 adRP (Pearson's Correlation Coefficient = 0.54, $P = 0.01$). These indices do not appear to be related in patients with CRD or Stargardt disease. *Broken line*: lower limit of normal T_{sat} and ISCEV rod amplitude (75 μ V)

Table 62.1

Characterization of rod recovery kinetics with T_{sat} in patients with RDDs and normal subjects

	Count	T_{sat} (ms)	Reduction	Significance (P)
Normal	13	544 $\pm$ 92 (SD)		
CRD	5	473 $\pm$ 113		0.26
Stargardt	4	491 $\pm$ 98		0.38
adRP	18	331 $\pm$ 99	39%	<0.001

This table presents the statistical results of T_{sat} in patients with CRD, Stargardt disease, or adRP. Statistical significance was calculated using two-tailed unpaired student t -test between sampled normal subjects and patients with RDDs