PIII and derived PII analysis in a patient with retinal dysfunction with supernormal scotopic ERG

Subtitle: Retinal dysfunction with supernormal scotopic ERG

Naoyuki Tanimoto, Tomoaki Usui, Mikio Ichibe, Mineo Takagi, Shigeru Hasegawa & Haruki Abe

Division of Ophthalmology and Visual Science, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan

Correspondence and reprint requests to: Naoyuki TANIMOTO, MD, PhD, Division of Ophthalmology and Visual Science, Graduate School of Medical and Dental Sciences, Niigata University, 1-757 Asahimachi, Niigata 951-8510, Japan
Tel: +81-25-227-2296, Fax: +81-25-227-0785.
Email: naoyuki.tanimoto@med.uni-tuebingen.de

Key Words: Cone, electroretinogram, phototransduction, retinal dysfunction, rod

Abstract

Purpose: To present electroretinographic (ERG) findings in a patient with retinal dysfunction with supernormal scotopic ERG, and to analyze rod and cone PIII components and rod inner nuclear layer (derived PII) responses. *Patient:* A Japanese 11-year-old girl complained of poor visual acuity. There was no parental consanguinity in her family. The corrected visual acuity was 0.7 in both eyes. No abnormal finding was observed in both fundi. *Methods:* The patient underwent full-field ERGs. Rod and cone a-waves were analyzed using photoreceptor models. The derived PII responses were analyzed using a technique described by Hood and Birch. *Results:* In the photopic ERG, responses to single flash and 30-Hz flicker were attenuated. In the scotopic ERG, b-wave was supernormal in amplitude in response to intense flashes, but smaller than normal and markedly delayed over a lower range of flash intensities. By the PIII analysis, phototransductions (values of *S*) of both rod and cone were remarkably decreased. The derived PII responses for this patient were larger than the responses for normal subjects, and the onset of the PII responses in this patient are significantly delayed compared to those in normal

subjects. *Conclusions:* The ophthalmological findings in this patient are consistent with previous publications of this disease. Although it has been reported that the sites of disease action were beyond the outer segment (values of *S* were within the normal range), our results suggest that photoreceptors could be involved in sites of disease action in at least some patients with this disease.

Abbreviations: ERG- electroretinogram; cGMP- cyclic guanosine monophosphate;

Introduction

Cone dystrophy with unusual rod electroretinographic (ERG) findings was first reported in two siblings by Gouras et al [1]. The scotopic flash ERG b-wave was supernormal in amplitude in response to intense flashes, but smaller than normal and markedly delayed over a lower range of flash intensities. The rectangular shapes of the scotopic standard combined ERG a-wave were also peculiar in the patients. Since the first report, similar patients have been reported by several authors [2-8]. Hood et al [8] suggested that this disease could be called “supernormal and delayed rod ERG syndrome”. The pathophysiological mechanism of this disease still remains unknown. Because similar ERG changes to this disease had been reported from rod receptors in which intracellular level of cyclic guanosine monophosphate (cGMP) had been elevated [9-11], Gouras et al [1] suggested that the basis for the abnormalities in the rod ERG lie in the rod photoreceptors. On the contrary, Hood et al [8] suggested that the sites of disease action were beyond the outer segment, since there was no evidence for a delayed activation and deactivation of transduction of the outer segment. Here we report one patient with this disease, who showed different rod and cone functions from those by Hood et al [8].

Patient

A Japanese 11-year-old girl complained of a poor visual acuity. There was no family history of inherited eye disease, amblyopia, or nystagmus. There was no parental consanguinity in her family.

Ophthalmological examinations: The corrected visual acuity was 0.7 in each eye, with optical correction (-0.5 axis 30, right eye; -0.75 axis 35, left eye). No nystagmus was observed. The cornea, lens, and vitreous were clear. No abnormal finding was observed in both fundi by an ophthalmoscope and by fluorescein angiography. Kinetic visual fields measured with a Goldmann perimeter showed mild constriction to V and I targets at intensity 4e. Color vision was abnormal with no specific axis (Farnsworth D-15). Electro-oculogram complied with the International Society for Clinical Electrophysiology of Vision (ISCEV) protocol [12] showed normal light rise (the ratio of light peak and dark baseline was 2.5 in the right eye and 2.6 in the left). The dark adaptation curve, examined with the Goldmann-Weekers adaptometer (HAAG-STREIT AG, Koelnz/Bern, Switzerland), showed normal timing of cone-rod break, although the cone threshold was slightly elevated by about 0.1 log unit and the final rod threshold was elevated by about 1 log unit (Figure 1). She has been aware of hemeralopia, although she denied nyctalopia.

Physical examinations: The patient did not show any abnormalities on

general physical and neurological examinations. The serum cGMP level of this patient was within the normal range (2.4 pMOL/ml; normal range 1.8-4.8 pMOL/ml). Brain magnetic resonance imaging showed no abnormality.

---- Figure 1 near here -----

Methods

ERG

The ERG procedure complied with the ISCEV standard protocol [13]. The methods were similar to those described in other reports [14, 15].

Both eyes were dilated with a mydriatic and subjects were dark-adapted for at least 45 minutes before testing. The responses were obtained from Burian-Allen bipolar electrodes (Hansen Ophthalmic Instruments, Iowa City, IA, USA). The stimulus was a 10-μs-xenon flash (ERG Photic Stimulator, SLS-4100, Nihon Kohden, Tokyo, Japan) delivered by means of a Ganzfeld dome (Sanso, Tokyo, Japan). Stimulus intensity was controlled by means of neutral density filters (Fuji Film, Tokyo, Japan). Scotopic rod ERG and scotopic standard combined ERG were recorded with a 0.5 to 100 Hz filter setting. Oscillatory potentials (OPs) were recorded with a 50 to 500 Hz filter setting. The flash intensities were 0.13 cd·s/m^2 (0.7 log scot td-s) for the

scotopic rod ERG, and 109.5 cd·s/m^2 (3.6 log scot td-s) for the scotopic standard combined ERG and OPs. The single flash cone ERG and 30-Hz flicker ERG were recorded under 30 cd/m^2 background illumination after at least 15 minutes of light adaptation. The flash intensity was 109.5 cd·s/m^2 (3.6 log scot td-s) for the single flash cone ERG. The light intensity of 1120 cd/m^2 was used for 30-Hz flicker ERG recording. Amplitudes and implicit times from the responses were calculated and compared with the values from 15 normal subjects aged 6-24 years (mean age = 16.1 years) (Table 1).

Intensity-response series: The dark-adapted ERGs in various intensities with a Ganzfeld stimulus (Sanso, Tokyo, Japan) were recorded by white flash stimuli with intensity from 0.0013 to 109.5 cd·s/m^2 (from -1.3 to 3.6 log scot td-s in approximately 0.3 log unit steps after 45 minutes of dark adaptation. The responses were recorded with a 0.5 to 100 Hz filter setting. Intensity-amplitude and intensity-implicit time curves were constructed.

The model of rod and cone phototransduction activations: We analyzed rod and cone a-waves by fitting them to a model proposed by Hood and Birch [16-18]. Rod-only responses were obtained by computer subtraction of photopic ERGs from scotopic ERGs (flashes range from 2.75 cd·s/m^2 to 109.5 cd·s/m^2 (form 2.0 to 3.6 log scot td-s) in approximately 0.3 log unit steps). Rod-only responses and cone responses to all flash energies were fitted to the

following equation by estimating one set of parameters: *S*, t_d and Rmp_3 for rods and cones [16-18]. The fits have done on the basis of a single response only.

$$P3(i,t)=\{1-\exp[-\ i\cdot S\cdot(t-\ t_d)^2]\}\cdot Rmp_3 \qquad \text{for } t > t_d$$

where i = flash energy (log scot troland-s), t_d = time delay, t = time after flash onset, *S* = sensitivity, and Rmp_3 = maximum response amplitude.

In this study, to compare the values of log *S* and log | Rmp_3 | from the patient with those from 15 normal subjects aged 6-24 years (mean age = 16.1 years), the values of rod t_d and cone t_d were fixed to the mean of the normal values (3.5 and 2.4 msec, respectively). (Table 1)

Derived PII response: To estimate the response of rod inner nuclear layer (derived PII), we used the technique described by Hood and Birch [19]. Derived PII response could be obtained by computer subtraction of PIII response from rod-only response. The derived PII responses were calculated for six of the flash energies from 2.75 cd·s/m^2 to 109.5 cd·s/m^2 (from 2.0 to 3.6 log scot td-s.

Informed consent was obtained from the patient and normal subjects for each of the procedures after the explanation of the nature and possible consequences of the study.

Results

The full-field ERG results from the patient and normal controls are summarized in Table 1. Standard ERGs could be performed in both eyes, however, intensity-response series recordings and PIII and derived PII analyses were possible only in the right eye, since the patient did not agree to have both eyes tested.

Standard ERGs: Standard ERG responses are shown in Figure 2. The ERGs were similar in both eyes in standard ERGs. In the scotopic standard combined ERG, the amplitude of a-wave was slightly reduced and the implicit time was significantly delayed. The shape of a-wave was broad and rectangular. The amplitude of the b-wave was supernormal, and the implicit time was delayed. The b/a-wave amplitude ratios were 3.54 in both eyes. The amplitude of the rod ERG b-wave was reduced, and the implicit time was significantly delayed. OP waves were very small. In the photopic single flash cone ERG, the amplitudes of a- and b-waves were reduced, and the implicit times of them were delayed. The response to 30-Hz flicker was decreased in amplitude and delayed in implicit time.

---- Figure 2 and Table 1 near here -----

Intensity-response series: The rod ERG abnormalities associated with this disorder are clearly seen in the response-intensity series (Figure 3 (A) and (B)). No response could be detectable to the weakest flashes. The threshold to yield a detectable response was elevated by about 1 log unit. When the b-wave is measurable in this patient, it is smaller than normal and considerably delayed. At the higher flash energies, the amplitudes of b-wave of this patient were larger than the normal range, and the implicit times of b-wave were delayed, although they became faster than those to dim flashes.

---- Figure 3 (A and B) near here -----

Activation of phototransduction: To assess the activation of rod and cone phototransduction in this patient, the equation described above was fitted to the leading edge of the rod and cone a-waves (Figure 4 (A) and (B), respectively). The dashed curves in Figure 4 (A) and (B) show the fit of the model to the records from the right eye of this patient (right panel) and from the normal subject (left panel). In this patient, log | Rmp_3 | of both rod and cone were within the normal range, however, log S of both rod and cone were remarkably decreased.

---- Figure 4 (A and B) near here -----

Derived PII response: The derived PII responses are shown in Figure 5 (A) for six of the flash energies. The derived PII responses for this patient (solid lines) were larger than the responses for the normal subject (dashed lines) and the onset of the responses for this patient were markedly delayed relative to those for the normal subject. The time to onset of activity of the derived PII responses are shown in Figure 5 (B). For this range of flash energies, the onset times of the derived PII responses of our patient were delayed by about 15 msec relative to the normal mean responses.

---- Figure 5 (A and B) near here -----

Discussion

In "supernormal and delayed rod ERG syndrome" designated by Hood et al [8], the ERG is requisite for the diagnosis [1-8]. The characteristic ERG findings are as follows: (1) The scotopic flash ERG b-wave is supernormal in amplitude in response to intense flashes, but smaller than normal and markedly delayed over a lower range of flash intensities. (2) The

single flash cone ERG is reduced in amplitude and the b-wave delayed in implicit time. (3) The scotopic standard combined ERG shows the broad and rectangular a-wave that was expressed as a step-like a-wave [3] or squaring of the a-wave [7]. Our patient clearly showed these three ERG findings, therefore, we diagnosed the patient as “supernormal and delayed rod ERG syndrome”.

Clinical characteristics

22 patients with essentially identical ERG waveforms have been described by several authors [1-8]. The age at initial visit to an eye hospital of the patients ranges from 4 to 54 years old, and 16 of 22 patients were younger than twenty, including our patient. In most cases, no obvious inheritance pattern was noted. Our patient showed better corrected visual acuities than those in previous reports (ranging from 0.05 to 0.5). The results in dark adaptometry were described in ten patients [1, 3, 4, 6, 7]. Only one patient showed the borderline subnormal final rod threshold [4], while others showed elevated final rod thresholds by about 2 log units [1, 3, 6, 7]. In our patient, the final threshold was elevated by about 1 log unit, although she denied nyctalopia. This inconsistency between subjective and objective sensation of light was also described by Kato et al [7].

It has been controversial whether this disease is stationary [3, 6] or progressive [1, 5]. During a 4-year follow-up period, subjective symptoms,

visual functions, and ERG findings of our patient were stationary. Early onset of this condition suggests that these clinical findings might be congenital in some patients.

The serum cGMP level of our patient was investigated, because similar ERG changes to this disease had been reported from rod receptors in which intracellular level of cGMP had been elevated [9-11]. Our case showed normal serum cGMP concentration. In the past, Yagasaki et al [3] described that serum cGMP levels were slightly elevated in two patients. However, five cases of this disease were also reported that they had normal serum cGMP [1, 7].

PIII of rod and cone

In this disorder, it is very difficult to determine scotopic a-wave latencies because of the peculiar a-wave shape, because the a-wave in this disorder shows a broad trough. Sandberg et al [5] estimated the a-wave slopes in patients of this disease with a linear regression, and noted that rod a-wave slopes were reduced 50% below normal, and suggested the photoreceptor involvement. However, we cannot consider precisely from this result whether photoreceptors are involved or not, because the a-wave slope is decreased not only when *S* is affected but also when Rmp_3 is decreased. Therefore, it would be valuable to analyze a-waves by fitting them to a model proposed by Hood and Birch [16-18]. PIII of rod and cone in this disease were investigated only by

Hood et al [8]. They suggested that the sites of disease action were beyond the outer segment, since there was no evidence for a delayed activation and deactivation of transduction of the outer segment. However, in the results of our patient, both rod and cone *S* were remarkably decreased. In five reports [1, 2, 4, 6, 7] out of eight [1-8], this disorder was considered as “cone dystrophy” or “cone dysfunction”, because patients showed clinical characters of “cone dystrophy” or “cone dysfunction” (decreased visual acuity, color vision abnormality, macular abnormality, and diminished and prolonged photopic ERGs). It is suggested that the abnormal phototransduction might exist in both rods and cones in our patient. Further, our patient was young, and no abnormal finding was observed in both fundi, therefore, the primary cause of this disease might lie in both rods and cones.

Derived PII

The steps between the outer segment and the post-synaptic generation of inner nuclear layer activity have been reported to be the candidates for disease action [4, 7, 8]. We used the technique described by Hood and Birch [19] to obtain a better estimate of the response of the inner nuclear layer. The gross response of the inner nuclear layer can be derived by computer subtracting the PIII response of the rod receptor from the rod-only response. This analysis was already used in this disorder [8], and our results were the

same as those in that report; greatly slower and larger than the responses for the normal subject. This characteristic of the derived PII response in “supernormal and delayed rod ERG syndrome” is very unique among disorders whose derived PII responses were analyzed [20, 21]. When our patient and the patients by Hood et al [8] are compared, it is quite interesting that the PIII components were completely different, yet the mean delays of the derived PII components were almost equivalent (our patient, about 15 msec; Hood et al, about 16 msec [8]). Although it has been reported that the sites of disease action were beyond the outer segment [8], our results (values of rod and cone *S* were decreased) suggest that photoreceptors could be involved in sites of disease action in at least some patients with this disease. It is curious that the change of the PIII components were similar between rods and cones, although the responses from the inner nuclear layer were completely different; scotopic b-waves at high stimulus intensities were larger than normals, but photopic b-waves at high stimulus intensities were smaller than normals. The mechanism of this disease is still unclear. Further investigations are required to elucidate the mechanisms of this disease.

Acknowledgment

The rod and cone a-wave fitting programs were provided by Dr. Donald C.

Hood.

References

1. Gouras P, Eggers HM, MacKay CJ. Cone dystrophy, nyctalopia, and supernormal rod responses. A new retinal degeneration. Arch Ophthalmol 1983;101:718-24.

2. Alexander KR, Fishman GA. Supernormal scotopic ERG in cone dystrophy. Br J Ophthalmol 1984;68:69-78.

3. Yagasaki K, Miyake Y, Litao RE, Ichikawa K. Two cases of retinal degeneration with an unusual form of electroretinogram. Doc Ophthalmol 1986;63:73-82.

4. Foerster MH, Kellner U, Wessing A. Cone dystrophy and supernormal dark-adapted b-waves in the electroretinogram. Graefe's Arch Clin Exp Ophthalmol 1990;228:116-9.

5. Sandberg MA, Miller S, Berson EL. Rod electroretinograms in an elevated cyclic guanosine monophosphate-type human retinal degeneration. Invest Ophthalmol Vis Sci 1990;31:2283-7.

6. Rosenberg T, Simonsen SE. Retinal cone dysfunction of supernormal rod ERG type. Acta Ophthalmol 1993;71:246-55.

7. Kato M, Kobayashi R, Watanabe I. Cone dysfunction and supernormal

scotopic electroretinogram with a high-intensity stimulus. A report of three cases. Doc Ophthalmol 1993;84:71-81.

8. Hood DC, Cideciyan AV, Halevy DA, Jacobson SG. Sites of disease action in a retinal dystrophy with supernormal and delayed rod electroretinogram b-waves. Vision Res 1996;36:889-901.

9. Ebery TG, Hood DC. The effects of cyclic nucleotide phosphodiesterase inhibitors on the frog rod receptor potential. In: Langer H, eds. Biochemistry and physiology of visual pigments. Berlin: Springer, 1973: 341-50.

10. Lipton SA, Rasmussen H, Dowling JE. Electrical and adaptive properties of rod photoreceptors in Bufo marinus. II. Effects of cyclic nucleotides and prostaglandins. J Gen Physiol 1977;70:771-91.

11. Nicol GD, Miller WH. Cyclic GMP injected into retinal rod outer segments increases latency and amplitude of response to illumination. Proc Natl Acad Sci USA 1978;75:5217-20.

12. Marmor MF, Zrenner E. Standard for clinical electro-oculography. Arch Ophthalmol 1993;111:601-4.

13. Marmor MF, Holder GE, Seeliger MW, Yamamoto S. Standard for clinical electrophysiology of vision (2004 update). Doc Ophthalmol 2004;108:107-14.

14. Usui T, Tanimoto N, Takagi M, Hasegawa S, Abe H. Rod and cone a-waves in three cases of Bietti crystalline chorioretinal dystrophy. Am J Ophthalmol

2001;132:395-402.

15. Tanimoto N, Usui T, Takagi M, Hasegawa S, Abe H, Sekiya K, Miyagawa Y, Nakazawa M. Electroretinographic findings in three family members with X-linked juvenile retinoschisis associated with a novel Pro192Thr mutation of the *XLRS1* gene. Jpn J Ophthalmol 2002;46:568-76.

16. Hood DC, Birch DG. Rod phototransduction in retinitis pigmentosa: estimation and interpretation of parameters derived from the rod a-wave. Invest Ophthalmol Vis Sci 1994;35:2948-61.

17. Hood DC, Birch DG. Assessing abnormal rod photoreceptor activity with the a-wave of the electroretinogram: applications and methods. Doc Ophthalmol 1996;92:253-67.

18. Hood DC, Birch DG. Phototransduction in human cones measured using the alpha-wave of the ERG. Vision Res 1995;35:2801-10.

19. Hood DC, Birch DG. A computational model of the amplitude and implicit time of the b-wave of the human ERG. Vis Neurosci 1992;8:107-26.

20. Cideciyan AV, Jacobson SG. Negative electroretinograms in retinitis pigmentosa. Invest Ophthalmol Vis Sci 1993;34:3253-63.

21. Cox GF, Hansen RM, Quinn N, Fulton AB. Retinal function in carriers of Bardet-Biedl syndrome. Arch Ophthalmol 2003;121:804-10.

Legends to figures & table

Figure 1

Dark adaptation curve measured with Goldmann-Weekers adaptometer. Shadow zone shows the normal range obtained from 12 normal control subjects. Closed circles; the right eye of the patient.

Figure 2

Standard ERGs in a normal subject and the patient.

Figure 3 (A)

Dark-adapted ERGs recorded by white flash stimuli with intensity from -1.3 to 3.6 log scot td-s in approximately 0.3 log unit steps in the normal subject and the right eye of the patient. Vertical numbers indicate intensities of stimulus.

Figure 3 (B)

Rod ERG b-wave amplitude (left panel) and implicit time (right panel) as functions of stimulus energy for the right eye of the patient (closed circle and solid line) and for the mean of a group of normal subjects (dotted line). Dashed lines represent the maximum and minimum of a group of normal subjects.

Figure 4 (A, B)

Results of the rod a-wave (A) and the cone a-wave (B) fitted to models proposed by Hood and Birch [16, 18] in a normal subject and the right eye of the patient. Solid curves are the raw data, and dashed curves are the models

after fitting to the leading edge of the rod and cone a-waves. *S* and *Rmp3* values are given in the panel.

Figure 5 (A)

Derived PII responses for a normal subject and the right eye of the patient are shown for six flash energies from 2.0 to 3.6 log scot td-s. These responses were obtained by subtracting the rod PIII response from the rod-only ERG..

Figure 5 (B)

The latencies of the derived PII responses are shown as a function of flash energy for the patient (closed circle and solid line) and for the mean of a group of normal subjects (open circle and dotted line). The error bars represent 2 standard deviations.

Table 1

Electroretinogram results of the patient and normal controls. Asterisk (*) indicates the value outside the normal range.