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Selective reduction of S-cone response and on-response in the cone electroretinograms of patients with X-linked retinoschisis

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Abstract *Purpose:* To examine the electroretinograms (ERGs) of the short-wavelength-sensitive (S-) and the mixed long- and middle-wavelength-sensitive (L,M-) cones, and the ON- and OFF-responses of the cone ERGs in three patients with X-linked juvenile retinoschisis (XLRS). *Methods:* Cone ERGs elicited by different color flashes and those elicited by long duration stimuli under Ganzfeld conditions were recorded from three patients with XLRS. *Results:* The S-cone b-waves were undetectable to short-wavelength stimuli in all three XLRS patients, while the L,M-cone ERG b-waves were within the normal range. To long-duration white stimuli, the ON-response (b-wave) was reduced and delayed in all patients compared with that of the normal subjects, while the d-wave or OFF-response appeared normal in amplitude and implicit time. *Conclusions:* These results support the hypothesis that the normal S-cone ERG arises primarily from the ON-pathway of the cone ERGs and the hypothesis that ON-bipolar cells are predominant in the S-cone system.

Introduction

X-linked juvenile retinoschisis (XLRS) is an inherited retinal disorder characterized by an intraretinal splitting in the macular area and the peripheral retina [4]. The results of previous electrophysiological studies have indicated that the primary defect is located in the Müller cells because the b-wave amplitudes of the electroretinograms (ERGs) elicited by bright-flash stimuli were significantly reduced with normal or subnormal a-wave amplitudes [9, 16]. A recent study reported that the *RS1* gene, the disease-causing gene for XLRS, is expressed in rod and cone photoreceptors as well as in the bipolar cells [14]. Another study showed that the protein product of *RS1* is present both in the photoreceptors and within the inner layers of the retina [7]. These findings suggest that the protein may be produced by the photoreceptors but it may also have a functional role within the inner retinal layers.

Recently, two research groups independently found that the amplitude of the b-wave to light onset was reduced more than that of the d-wave to light offset, resulting in a reduced b-wave to d-wave ratio in patients with XLRS [2, 17]. Their results suggested that there is a somewhat greater dysfunction of the ON-bipolar pathway of the cone system. Several other retinal diseases, such as the complete type of congenital stationary night blindness (CSNB) [13], melanoma-associated retinopathy (MAR) [1], and acquired night blindness [3], also have defects of the ON-response. In some of these retinal disorders, responses of the short-wavelength-sensitive (S-) cone are also selectively reduced compared with those of the middle- (M-) and long-wavelength-sensitive (L-) cones. This suggests that the S-cone bipolar system may be ON-dominant [10, 12].

Because of the close relationship between the ON-pathway alterations and S-cone defect in these diseases, we hypothesized that XLRS patients with defective ON-responses will also have a defect of the S-cone ERGs. To test this hypothesis, we recorded the S-cone and the L,M-cone ERGs elicited by color stimuli, and the ON- and OFF-responses elicited by long-duration

Table 1 Amplitudes and implicit times of the S-cone and L,M-cone ERG b-waves (*ND* nondetectable)

Patient	S-cone b-wave to 450 nm stimuli		L,M-cone b-wave to 633 nm stimuli	
	Amplitude (μV)	Implicit time (ms)	Amplitude (μV)	Implicit time (ms)
1	ND	ND	1.85	28.8
2	ND	ND	1.45	30.0
3	ND	ND	1.1	32.5
Normal range[a]	0.7~3.1	40.1~45.8	1.0~3.2	24.9~27.2

[a] The calculated 2.5 and 97.5 percentiles for 12 normal subjects

stimuli in patients with XLRS. We shall show that the ON-responses were depressed as reported, and in support of our hypothesis, the S-cone component was unrecordable in XLRS patients.

Patients and methods

Three male patients from unrelated families and diagnosed with XLRS were studied. The patients' ages were 7 years (patient 1), 20 years (patient 2), and 34 years (patient 3). The corrected visual acuities in the tested eye (the right eye for all patients) were 20/50, 20/100, and 20/100, respectively. All patients had microcystic lesions with a radial spoke-like appearance in the fovea and peripheral retinoschisis. The single bright flash ERG under dark-adapted conditions consisted of a reduced b-wave and a negative-type waveform. These ERGs are typical of those reported for XLRS patients. The color vision as tested by the Farnsworth Panel D-15 was normal in all patients. The results of genetic studies have not been completed yet. Twelve age-similar normal subjects (6–45 years) served as controls.

Informed consent was obtained from all subjects after the nature and possible consequences of the study had been fully explained.

The method used for eliciting and recording the S-cone ERGs was first reported by Gouras and associates [5, 6] and has been described in detail earlier [8, 19]. In brief, the subject's pupils were fully dilated with 0.5% tropicamide, and the ERGs were recorded from the right eye with a bipolar Burian–Allen contact lens electrode. A Ganzfeld stimulator provided full-field flash stimuli (5 cd/m^2/s) on a white background of 50 cd/m^2.

Spectral stimuli were produced by Kodak Wratten color filters #98 (450 nm) and #29 (633 nm) (Eastman Kodak, Rochester, N.Y., USA) on the same white background. The stimulus frequency was 5 Hz and 500 responses were averaged. We routinely recorded responses with the maximum flash intensity available at 450 nm and then dimmed the other spectral stimuli with neutral-density filters to produce approximately equal mixed L,M-cone b-waves, because longer-wavelength stimuli had more effective energy for the mixed L,M-cones.

Under these conditions, the ERGs elicited by the blue (450 nm) stimulus from normal subjects consisted of a b-wave made up of two peaks (Fig. 1). Extensive studies have shown that the action spectrum of the second b-wave had a relatively narrow spectrum that peaked in the blue region of the spectrum. Another reason why this second peak is considered to originate from S-cones is that an S-cone achromat had exactly the same response to bright white flashes under light-adapted conditions [6]. Although the amplitude of the S-cone b-wave, the second peak, by this method is relatively small, both S- and L,M-cone mechanisms can be examined simultaneously at the same state of retinal adaptation.

A monopolar contact lens electrode with a built-in light-emitting diode (LED) was used for recording the ON- and OFF-responses of the cone ERGs [17]. A reference electrode was placed on the forehead, and the ground electrode was attached to the earlobe. The stimulus had a luminance of 300 cd/m^2, a duration of 100 ms, and was presented on a steady background of 40 cd/m^2. Flashes were presented at a rate of 3 Hz, and 50 responses were averaged.

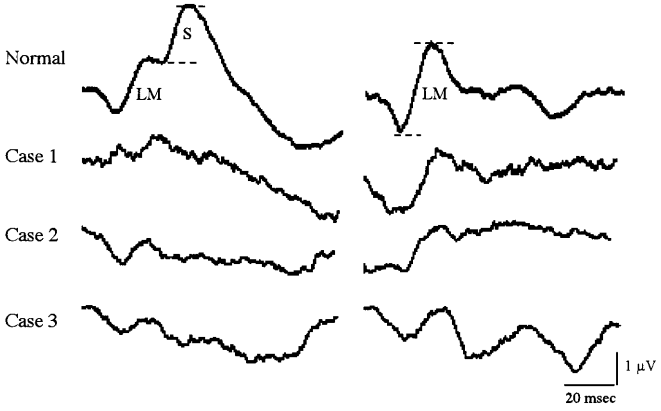

Fig. 1 Cone ERGs elicited by 450-nm flash stimuli (*left*) and by 633-nm flash stimuli (*right*) from a normal subject and three patients with XLRS in the presence of a bright white background. *S* S-cone b-waves, *L,M* mixed L,M-cone b-waves. The calibration marker represents 1 μV vertically and 20 ms horizontally

Results

In normal eyes, the S-cone component of the ERG elicited by short-wavelength stimuli (450 nm) appears as a separate b-wave riding on a shorter-latency mixed L,M-cone b-wave (Fig. 1). The S-cone b-wave was measured from its initial appearance, after the peak of the mixed L,M-cone b-wave, to its peak. The mean S-cone ERG amplitude was 1.38±0.56 μV (± standard deviation), against a 2.5 to 97.5 percentile range of 0.7–3.1 μV in the normal controls. Only the mixed L,M-cone b-wave appeared to long-wavelength stimuli (633 nm). The mean amplitude of the L,M-cone b-wave to the 633-nm stimuli was 2.30±0.71 μV, against a 2.5 to 97.5 percentile range of 1.0–3.2 μV in the normal controls.

The S-cone b-waves were not detectable in the three XLRS patients, while the amplitudes of the L,M-cone b-waves were within the normal range but with prolonged implicit times (Fig. 1, Table 1).

The ERGs elicited by long-duration stimuli from a normal subject and the patients with XLRS are shown in Fig. 2. The amplitudes of the a-, b-, and d-waves

Table 2 Amplitudes and implicit times of the a-, b-, and d-waves of the long-duration flash ERGs

Patient	Amplitude (μV)					Implicit time (ms)		
	a-wave	b-wave	d-wave	b/a ratio	b/d ratio	a-wave	b-wave	d-wave
1	30.8	14.1	44.5	0.46	0.32	19.7	41.2	123
2	43.8	35.0	68.3	0.80	0.51	18.0	36.5	119
3	31.2	20.8	41.6	0.67	0.50	18.2	37.6	120
Normal range[a]	19.0~49.8	24.7~78.7	21.6~60.9	1.02~3.13	0.95~1.66	17.2~20.5	30.4~36.0	120~123

[a] The calculated 2.5 and 97.5 percentiles for 12 normal subjects

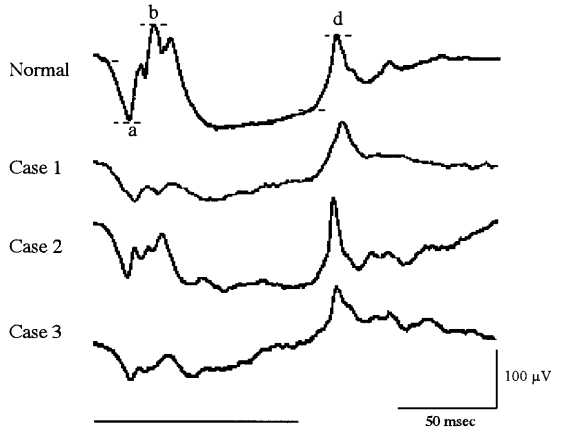

Fig. 2 Cone ERGs elicited by long-duration stimuli from a normal subjects and three patients with XLRS. *a* a-wave, *b* b-wave, *d* d-wave. The calibration marker represent 100 μV vertically and 50 ms horizontally

were measured from the baseline to the negative trough, from the negative trough to the positive peak, and from the plateau to the positive peak after light off, respectively. The b-waves were reduced and delayed in all patients compared with that of the normal subjects, while the a- and d-waves were comparable with those of the normals in amplitude and implicit time. The amplitude ratios of the b-wave to the a-wave and those of the b-wave to the d-wave were markedly reduced in all patients compared with those in normal subjects (Table 2).

Discussion

A decreased b/d-wave ratio was first reported in one patient with XLRS by Sieving [18]. Recently, Shinoda et al. reported that the photopic ERGs to long-duration stimuli suggested a considerable impairment of the ON-pathway [17]. They also found no significant correlation between the ERG responses and the locus of the gene mutation. Alexander and co-workers, using a sawtooth flicker stimuli, also reported a reduction of the b/d-wave ratio in patients with XLRS and concluded that a relative dysfunction of the cone ON-bipolar cell pathway existed [2]. Our ERG results to long-duration stimuli are consistent with these earlier findings that there is a defect in the ON-pathway in patients with XLRS.

It is well known that cone signals in the retina are processed through dual pathways; one is the ON-pathway involving ON-center bipolar cells, and the other is the OFF-pathway involving OFF-center bipolar cells [18]. The S-cone pathway is less well studied, although there is some evidence of a preponderance of S-cone ON-units at the ganglion cell level [11]. MacKay et al. and Kamiyama et al. reported that the S-cone b-wave and the rod b-wave were undetectable in the complete type of CSNB [10, 12]. Because the ON-pathway of both the cone and rod systems was selectively defective in CSNB, these earlier results strongly suggested that the ON-pathway is dominant in the S-cone bipolar system.

Our current study demonstrated that the S-cone component of the ERGs was more reduced than the L,M-cone component in eyes with XLRS. A greater reduction of the b-wave of the ON-response than the d-wave of the OFF-response of the cone system was also observed previously [2, 17]. These results supported the hypothesis that the ON-pathway is dominant in the S-cone system.

The exact mechanism(s) for the cone ON-pathway defect in XLRS has not been determined. As noted, the *RS1* gene is expressed primarily in cone and rod photoreceptors [7, 14], and the gene protein product has been reported to be associated not only with photoreceptors but also with cone bipolar membranes [14]. Therefore, there may be several possible explanations for the relationship between the *RS1* gene and the defective cone ON-response. In other retinal diseases in which the ON-pathway is selectively impaired, such as CSNB, MAR, and acquired nyctalopia, the rod ERGs are also severely decreased [1, 3, 10, 12, 13]. However, the reduction of the rod b-wave is not as severe in patients with XLRS as it is in patients with these other disorders. Furthermore, it has been reported that patients with

XLRS show normal or near-normal rod-mediated thresholds [15, 16]. To determine the reasons for the coincidental reduction of the ON-response and the S-cone amplitude as observed in our XLRS patients, a comparison of the ON-/OFF-responses of the S-cone and L,M-cone ERG would be interesting. Currently, however, there is no means of separating ON-/OFF-responses to chromatic stimuli. Additional studies are needed to determine the relationship between the gene defect and the ERG abnormalities in XLRS.

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