Preservation of Macular Oscillatory Potentials in Eyes of Patients with Retinitis Pigmentosa and Normal Visual Acuity

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PURPOSE. To study the functional changes in the macula of the retina in the early stage of retinitis pigmentosa (RP), by analyzing each component of the focal macular electroretinogram (fmERG).

METHODS. fmERGs were recorded from 39 patients with RP with normal visual acuity (>1.0) under direct fundus observation using a modified infrared fundus camera and 5°, 10°, and 15° stimulus spots. The amplitudes and implicit times of the a-wave, b-wave, and oscillatory potentials (OPs) in the patients with RP were compared to those from 30 age-similar normal control subjects.

RESULTS. The amplitudes of the different components of the fmERGs in patients with RP ranged from severely reduced to normal. The degree of amplitude reduction increased as the size of the stimulus spot increased in the patients with RP. The relative amplitudes of the OPs (67% of the mean in normal subjects) were better preserved than that of the b-wave (46%) and the a-wave (39%) in a 10° spot in the patients with RP.

CONCLUSIONS. The relative preservation of the OPs in the patients with RP could be due to either the buffering effect of the large receptive fields of the OP generators or to the retinal remodeling after the progressive loss of photoreceptors. Recordings of each component of fmERG can provide important information on the different layers of the central retina in RP eyes and can add to the understanding of the pathophysiology of RP. (Invest Ophthalmol Vis Sci. 2007;48:3312–3317) DOI: 10.1167/iovs.06-1417

Retinitis pigmentosa (RP) is a group of inherited retinal degenerations characterized by progressive loss of photoreceptors and eventual widespread atrophy of the retina.1–4 The initial visual impairment in patients with RP is usually night blindness and visual field loss in the periphery; the central visual function is usually affected at the later stages of the disease. RP is genetically heterogeneous. At present, approximately 40 genes have been identified as causing RP (http://www.sph.uth.tmc.edu/retnet/ provided in the public domain by the University of Texas Houston Health Science Center, Houston, TX).

Because the central retinal function is relatively better preserved than the peripheral retina until the late stages of RP, it is important to evaluate the functional changes in the macular area of patients with RP, not only for visual prognosis but also for studying the pathophysiology of the disease processes. The full-field electroretinograms (ERGs) have traditionally been used to assess objectively the retinal function of patients with RP. However, the full-field ERG is a mass potential from the entire retina, and it is not known how the local macular function contributes to the full-field ERG. To overcome these problems, focal (f)ERGs5–9 and multifocal (m)ERGs10–17 have been used to assess the macular function of eyes with RP, because these techniques can elicit electrical activities from localized retinal areas. However at present, there are no results on the alterations of the a-wave, b-wave, and oscillatory potentials (OPs) in the maculae of patients with RP.

Thus, the purpose of this study was to determine the functional changes in the different retinal layers of the macular area by analyzing each component of the focal macular (fm)ERGs at a relatively early stage of RP. We wanted to determine whether the amplitudes of OPs are better preserved than those of the a- and b-waves in the maculae of patients with RP. Our results are the first clinical demonstration that neural activities from the inner retina are better preserved than those from the middle and outer retina in the macular area of patients with RP.

METHODS

Subjects

We retrospectively reviewed the fmERGs of 127 patients with RP (62 men, 65 women), that were recorded from 1987 to 2006 in the Department of Ophthalmology, Nagoya University Hospital. The clinical diagnosis of RP was based on the funduscopic findings, visual fields, and ISCEV (International Society for Clinical Electrophysiology of Vision) standard full-field ERGs.18 The inclusion criteria were patients with RP who had received a complete medical examination including best corrected visual acuity, fundus examination, Goldmann kinetic visual field, full-field ERGs, and fmERGs; patients whose best corrected visual acuity was 1.0 or better; patients whose Goldmann kinetic visual fields by the I4e target were of a >5° radius; and those whose amplitude in the fmERG for 15° stimulus spot was detectable (>0.4 μV). We used these inclusion criteria for the definition of early-stage RP, because most patients with RP whose visual acuity is <0.8 or whose Goldmann kinetic visual fields by 1e target were <5° have undetectable fmERGs. Waveform analysis was very difficult or impossible in these patients because of the severely reduced fmERGs.

The exclusion criteria were patients with atypical RP (e.g., central RP, sector RP, or unilateral RP), patients with opacities in the media including cataracts, and patients with cystoid macular edema. If the fmERG were recorded from both eyes with visual acuity of >1.0, the data from the right eye were used for the analyses. The Goldmann kinetic visual fields were determined with the V4e and 1e white test light against the standard white background of 31.5 apostilbs.

Based on the inclusion and exclusion criteria, the fmERGs of 39 eyes of 39 patients with RP (18 men, 21 women; mean age, 37.9 ± 15.4
years) were analyzed. The inheritance pattern was considered to be autosomal dominant in 7 (18%) eyes, autosomal recessive in 9 (23%) eyes, and sporadic in 23 (59%) eyes. None of the patients was believed to have X-linked RP. The mean logMAR (logarithm of the minimum angle of resolution) best-corrected visual acuity was 0.061 ± 0.069 (1.15; Snellen equivalent). The mean radius of the central visual field with the Goldmann 14e target (average value for upper, lower, nasal, and temporal directions) was 14.8° ± 12.0°.

For controls, fmERGs were recorded from 30 age-similar normal subjects (17 men, 13 women; age, 39.4 ± 16.1 years). None had known abnormalities of the visual system, and their visual acuity was 1.0 or better.

The research was conducted in accordance with the institutional guidelines of Nagoya University and conformed to the tenets of the World Medical Association’s Declaration of Helsinki. Informed consent was obtained after sufficient information was provided about the examinations.

Focal Macular Electroretinograms

The stimulating and recording systems used to record the fmERGs have been described in detail. Briefly, an infrared fundus camera equipped with a stimulus light, background illumination, and fixation target was used. The image from the camera was fed to a television monitor, and the examiner used the monitor to maintain the stimulus on the macula.

The size of the stimulus spots was selected to be 5°, 10°, and 15°, and they were centered on the fovea. The background light was delivered to the eye from the fundus camera at a visual angle of 45°. Additional background illumination outside the central 45° produced homogeneous background illumination for nearly the entire visual field. The luminances of the white stimulus light and background light were 29.46 and 2.89 cd/m², respectively.

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A Burian-Allen bipolar contact lens electrode was used for the recordings. This lens not only allowed a very low electrical noise, but also permitted a clear view of the fundus on the monitor during the recordings.

After the patients’ pupils were fully dilated with 0.5% tropicamide and 0.5% phenylephrine hydrochloride, fmERGs were elicited by 5-Hz rectangular stimuli (100-ms light on and 100-ms light off), and 512 responses were averaged by a signal processor. With this duration, the responses to the stimulus-onset was evaluated in this study, whereas the on and off responses are inseparable with the briefflash stimuli that are widely used in the conventional full-field ERGs. A time constant of 0.03 seconds with a 100-Hz cutoff filter on the amplifier was used for recording the a- and b-waves, and a time constant of 0.003 seconds with a 300-Hz cutoff filter was used for recording the OPs.

The amplitude of the a-wave was measured from the baseline to the first negative trough, and the amplitude of the b-wave was measured from the trough of the a-wave to the positive peak of the b-wave. The amplitude of the OPs was calculated for only the 10° and 15° stimulus spots, because the OPs elicited by the 5° spot were too small, even in normal eyes. The amplitude of the fmERG was considered to be nondetectable when it was less than the noise level (<0.4 μV).

The fmERGs elicited by this method have been shown to be generated by the cone system, and the responses elicited by spot stimuli of 5° to 15° have been shown to be local responses.

Statistical Analyses

The significance of the differences between patients with RP and normal control subjects was analyzed using the nonparametric Mann-Whitney test. Differences in the amplitude or implicit times between 5° and 10° spots or between 10° and 15° spots were analyzed using the Wilcoxon signed rank test. Differences were considered to be significant at P < 0.05.

RESULTS

Effect of Stimulus Spot Size

The fmERGs recorded from a normal subject and three representative patients with RP are shown in Figure 1. The a- and b-waves were recorded with a 0.03-second time constant (top trace) and the OPs by a 0.003-second time constant (bottom trace). Of the 39 patients with RP, only 2 had fmERG amplitudes within the normal range for all stimulus spot sizes (Fig. 1, Case 1). There were 37 patients whose fmERG amplitudes were detectable but smaller than the normal range for at least one stimulus size (Fig. 1, Case 2, Case 3).

The mean (±SD) of the amplitudes of the a-wave, b-wave, and OPs for the three stimulus spot sizes in the 39 patients with RP and 30 age-similar normal control subjects are shown in Table 1. The mean amplitudes of all ERG components were significantly smaller for all stimulus sizes in the patients with RP than in normal control subjects (P < 0.0001).

The amplitude of each component of the fmERGs in each patient was divided by the corresponding component of the normal control subject, and the relative data are plotted in Figure 2. We found that the degree of amplitude reduction became larger with increasing stimulus spot size for all components. The differences were statistically significant between 5° and 10° spots and between 10° and 15° spots for all components (P < 0.05, Fig. 2).

The mean (±SD) implicit times of all ERG components for all stimulus spots in our patients with RP and 30 age-similar normal control subjects are shown in Table 2. The data of some patients were not included in this table because it was difficult to measure their implicit time. The mean implicit times of all components in patients with RP were not significantly different from those in normal control subjects for the 5° and 10° spots, but were significantly delayed for the 15° spot except for OP3 (third positive wave of the OPs).

Waveform Changes of fmERGs in Patients with RP

We next studied the changes in the waveforms of fmERGs in our patients with RP. The amplitudes of all ERG components for the 39 patients with RP, expressed relative to the mean
amplitudes in normal control subjects, are plotted in Figure 3. In patients with RP, the amplitudes of the OPs were better preserved than those of the b-waves for the 10° spot, and the b-waves were better preserved than those of the b-waves for the 10° spot, and the.

**FIGURE 2.** Amplitude of fmERGs in 39 patients with RP relative to the mean in normal control subjects. Comparison of the (A) a-wave and (B) b-wave amplitudes for 5°, 10°, and 15° spots. (C) Comparison of the OP amplitude for 10° and 15° spot. Bar, SEM. *P < 0.05, **P < 0.01, ***P < 0.001.

**DISCUSSION**

Our results showed that the degree of amplitude reduction and delay of implicit time became greater with increasing stimulus size (Tables 1, 2, Fig. 2). These results are not surprising when one considers the typical pattern of retinal dysfunction in RP. In most patients with RP, the retina is progressively impaired from the periphery to the central area. Past electrophysiological results of studies in which fERGs or mfERGs were used6-17 are in accord with our results.

Although fERGs and mfERGs have been extensively investigated in patients with RP,3-17 there are only a few studies in which different ERG components that originate from different retinal layers were investigated. Falsini et al.9 analyzed the which different ERG components that originate from different retinal layers were investigated. Falsini et al.9 analyzed the data of normal control subjects. Comparison of the mean amplitudes in the normal control subjects, are plotted in Figure 3. The fmERGs of three representative patients with RP who had a larger than normal OP/b-wave ratio are shown in Figure 5. It is clear that in these patients, the OPs were better preserved than were the a- and b-waves.

The amplitudes of the OPs elicited by the 10° stimulus spot are plotted against the amplitude of the b-wave for all 39 patients with RP and 30 normal control subjects. As in Figure 4A, the shaded area shows the range in the normal control subjects, and the dotted line shows the mean amplitudes in the normal control subjects.

Ten (25.6%) patients with RP had normal OP amplitudes with reduced b-wave amplitude, whereas none of the patients had normal b-wave amplitude with reduced OP amplitudes. These results combined with the data of Figure 3 indicate that the amplitudes of the OPs were better preserved than those of the b-wave for a 10° spot size in the patients with RP. We also noticed that the amplitude of OPs was larger than the normal range of one RP patient (Fig. 4B, arrowhead).

The fmERGs of three representative patients with RP who had a larger than normal OP/b-wave ratio are shown in Figure 5. It is clear that in these patients, the OPs were better preserved than were the a- and b-waves.

**FIGURE 3.** Amplitude of the fmERG in 39 patients with RP relative to the mean in normal control subjects. Comparison of the amplitude of each component for the (A) 10° and (B) 15° spot. Bar, SEM. *P < 0.05, **P < 0.01, ***P < 0.001.
respectively. They found that the ratio of the amplitudes of the fundamental component to the second harmonic component was larger in the RP group than that of normal control subjects. From these results, they concluded that not only the outer retina but also the inner retina contributed to the macular dysfunction in eyes with RP. Other ERG studies using full-field stimuli also demonstrated that neural cells postsynaptic to the photoreceptors contributed to the retinal dysfunction in eyes with RP.21,22

A new, interesting finding in this study was that the amplitudes of the OPs were better preserved than those of the a- and b-waves for a 10° spot in our patients with early-stage RP. The relative amplitude of the mean value in normal control subjects for the 10° spot was 0.67 for the OPs, and this value was significantly larger than that for the b-wave (0.46), which in turn was larger than that of the a-wave (0.39; Fig. 3A). In addition, 26% of the patients with RP had normal OP amplitudes with reduced b-wave amplitude, whereas none of the patients had normal b-wave amplitude with reduced OPs amplitudes (Fig. 4B).

Although the retinal origin of each component of fmERG has not been completely determined, one can assume their origins based on recent experiments in primates. The initial photopic a-wave is thought to originate mainly from cone photoreceptors and the cone off-bipolar cells.23,24 The photopic b-wave is determined by the combined activities of the cone on- and off-bipolar cells.25–27 The origins of the OPs are thought to be from feedback neural pathways in the inner part of the retina, especially around the inner plexiform layer, including the amacrine cells and partly the ganglion cells.28–30 Recent studies28 –30 have suggested that the origins of OPs are dependent not only on individual wavelets, but also on response frequencies. Thus, our results strongly suggest that in our patients at a relatively early stage of RP, the retinal activities from the inner retinal layer (OPs) were better preserved than those from the middle and outer layers (the b- and a-waves).

The exact mechanism for the relative preservation of the macular OPs in early-stage patients with RP was not investigated in this study. We have studied the effect of stimulus intensity on the amplitude of each component of the fmERG and have found that with decreasing stimulus intensities, the a-wave, b-wave, and OPs decreased proportionally.31 These results suggest that the waveform changes of fmERGs seen in our patients with RP cannot be explained simply by a decrease in the sensitivity to light.

The preservation of macular OPs in patients with RP is quite interesting when considering the spatial distribution of macular OPs in normal subjects. We have studied the spatial distribution of OPs in the macular area for normal subjects using an annulus (center off) stimulus to elicit fmERGs and have found that in normal subjects, the amplitude of the OPs was greater in the parafovea and perifovea. Therefore, we initially expected that the amplitudes of OPs would be more reduced.
than those of the a- and b-waves for 10° and 15° spots in patients with RP, because the parafovea and perifovea was thought to be more damaged than the fovea in patients with RP. However, our results were just the opposite: The amplitudes of the OPs tended to be more preserved than those of the a- and b-waves for 10° and 15° spots in patients with RP (Fig. 3). Thus, it is interesting to consider why some patients with RP show such a selective preservation of OPs.

We have two hypotheses for the mechanism that preserves the macular OPs in patients with RP. First is the buffering effect of the large receptive fields of the OP generators, viz., the amacrine cells and ganglion cells. Histopathological studies have shown that a large percentage of the inner retinal neurons remain histologically intact even though most photoreceptors were lost in patients with RP.32–34 Sufficient electrical activity from the inner retina may be produced by the large receptive fields, even though the electrical activities from outer and middle retinal layers are decreased as a consequence of progression of the RP. However, it is difficult to explain all the present results only by this mechanism. For example, why did one of our patients with RP (Fig. 4B, arrowhead) have supernormal OPs, despite lower borderline amplitude of the b-wave?

The second hypothesis is that the preservation of OPs in patients with RP may be due to retinal remodeling after the progressive loss of photoreceptors, to compensate for decreasing signal input to middle and inner retina. Evidence has been accumulating to support this idea in animal models of retinal degeneration.34–40 Alman et al.36 demonstrated that there is a compensatory synaptogenesis in reaction to partial loss of photoreceptors in rats with a rhodopsin mutation. Jones et al.39 have shown that after loss of the outer nuclear layer, various kinds of retinal remodeling were observed not only in rodent models of photoreceptor degeneration but also in humans with RP. Our results may be a clinical demonstration that such compensatory changes can occur in some patients at the early stage of RP. However, such compensatory retinal remodeling may not occur in all patients with RP, because there was a large variation in the ratio of OPs/b-wave in our patients with RP (Fig. 4B). Such variations may be due to the different stages and severities of the disease, different genetic backgrounds, or different environmental effects.

There are some limitations to our study. First, the study was performed retrospectively, and the selection of patients was not performed randomly. Second, we did not perform molecular testing on our patients with RP and could not characterize the pattern of fMERG as a consequence of specific gene mutation. Third, we did not compare the findings of the fMERGs with detailed psychophysical results (e.g., rod and cone perimetry) or macular morphologic tests (e.g., optical coherence tomography). Further studies are needed to clarify the mechanism of the fMERGs waveform changes in patients with RP.

In conclusion, our results demonstrated that electrical activities from the inner retina are preserved better than those from middle and inner retinal layers in the central retina of patients with early-stage RP. These results can provide important information on the pathophysiology of and possible future treatment for RP.

References


