Selective Loss of the Photopic Negative Response in Patients With Optic Nerve Atrophy

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Objective: To determine how the photopic negative response (PhNR) is altered in patients with optic nerve atrophy.

Methods: Ten patients with optic nerve atrophy induced by compression, trauma, or inflammation were examined. There were 6 men and 4 women with a mean age of 52.4 years. Ten age-matched control subjects were examined with the same protocol. Full-field electroretinograms were recorded, and the retinal nerve fiber layer thickness surrounding the optic nerve head was measured by means of optical coherence tomography.

Results: The amplitudes of the rod, maximum, cone, and 30-Hz flicker electroretinograms of the affected eyes were not different from those of the corresponding waves of the contralateral unaffected eyes or control eyes. In contrast, the amplitude of the PhNR was significantly smaller in the affected eyes than in the contralateral (P=.005) or control (P<.001) eyes. The decrease in amplitude of the PhNR preceded thinning of the retinal nerve fiber layer. There was a significant correlation between the PhNR amplitude and retinal nerve fiber layer thickness in eyes with optic nerve atrophy (r=0.879; P<.001).

Conclusions: Selective reduction and loss of the PhNR amplitude was found in eyes with optic nerve atrophy, which suggests that the PhNR can be used to evaluate the function of ganglion cells or their axons.


It is generally believed that the activity of the third-order retinal neurons contributes little to the shaping of the corneal electroretinogram (ERG). However, a response has been newly identified that originates from the third-order neurons that receive signals from cones.1 This response was named the photopic negative response (PhNR),2 and it consists of a negative-going wave that appears immediately after the cone b-wave (Figure 1).

The PhNR is strongly attenuated in primate eyes with experimentally induced glaucoma and also in eyes injected with tetrodotoxin;2 a blocker of the neural activity of retinal ganglion cells, their axons, and amacrine cells.3,4 The amplitude of the PhNR was reduced in patients with primary open-angle glaucoma (POAG), and the reduction was correlated with the optic nerve damage represented by optic disc cupping and visual field loss.2,3 A red stimulus light on a blue background produced by light-emitting diodes was used to elicit the PhNR in the previous studies, whereas a white stimulus on a white background is recommended by the International Society for Clinical Electrophysiology of Vision to produce cone ERGs.6 We have demonstrated that a negative response, which was elicited from rats by means of a white stimulus on a white background, had characteristics similar to the PhNR recorded in primates and humans.7 In addition, the negative response was selectively reduced in patients with low-tension glaucoma, even when the ERGs were recorded with the International Society for Clinical Electrophysiology of Vision protocol.8 Colotto et al9 recorded focal cone ERGs with a white background in patients with POAG and showed that the negative response correlated with the sensitivity loss of the visual field. All of these findings indicated that the negative response elicited by means of a white stimulus on a white background is equivalent to the PhNR elicited by means of a red stimulus on a blue background.2,3 The purpose of this study was to determine how the PhNR is affected by optic nerve atrophy.

METHODS

PATIENTS

Ten patients with optic nerve atrophy induced by compression (n=5), trauma (n=3),
or inflammation (n=2) were examined (Table). The patients included 6 men and 4 women whose ages ranged from 39 to 67 years with a mean of 52.4 years. Compression optic neuropathy was attributed to surgically resected pituitary adenoma or sphenoidal cystocele. The patients with traumatic optic neuropathy had been treated with systemic corticosteroids with improvement of visual acuity and visual field but not of optic nerve atrophy.

The patients underwent comprehensive ophthalmological examination including Snellen visual acuity, slitlamp biomicroscopic examination but not of optic nerve atrophy.

The patients with traumatic optic neuropathy had been treated with systemic corticosteroids and determined in patient 10. Both patients were treated with systemic corticosteroids with multiple sclerosis in patient 9, but the cause could not be determined in patient 10. Both patients were treated with systemic corticosteroids and improvement of visual acuity and visual field but not of optic nerve atrophy.

The patients underwent comprehensive ophthalmological examination including Snellen visual acuity, slitlamp biomicroscopic examination but not of optic nerve atrophy.

After a full explanation of the nature of the study, informed consent was obtained from all patients. The procedures used in this study conformed to the tenets of the Declaration of Helsinki.

ELECTRORETINOGRAM RECORDINGS

After the pupils were maximally dilated with topical 0.5% tropicamide and 0.3% phenylephrine hydrochloride, all subjects underwent dark adaptation for at least 30 minutes. After topical anaesthesia was induced with oxybuprocaine hydrochloride, the contact lens electrode, containing light-emitting diodes (EW-102; Mayo, Nagoya, Japan), was inserted in dim red illumination. The light-emitting diodes provided homogeneous white (color temperature, 4000-9000 K) stimulus and background light. Luminance produced by the contact lens electrode at the corneal side was measured with a photometer (LS-100; Minolta, Tokyo, Japan). Details of the contact lens electrode have been previously described. The reference and ground electrodes were placed on the forehead and right earlobe, respectively.

After 30 minutes of dark adaptation, rod and maximum ERGs were produced with white stimuli of 0.3 and 3.3 log cd/m², respectively, with 3-millisecond duration. Then the cone ERGs and 30-Hz flicker ERGs were produced with 3.0 log cd/m² with 3-millisecond duration and 2.0 log cd/m² with 33.3-millisecond duration, respectively, with a diffuse white background of 40.0 cd/m². Subjects underwent light adaptation by means of the white background light of 40.0 cd/m² for at least 10 minutes before the photopic recordings. The stimulus intensity and duration of the light-emitting diodes were controlled by a stimulator (WLS-20, Mayo). Responses were amplified at 5000 gain and a band-pass filtered from 0.5 to 1000 Hz (Neupack MED 5210; Nihon Kohden, Tokyo, Japan). Three to 16 responses were computer averaged with flash intervals of 2 seconds and 2 minutes for the rod and maximum ERGs, respectively. Thirty-two responses were averaged for the photopic recording.

**Figure 1.** Representative electroretinograms recorded from patient 2 at 6 months after symptom onset of compression optic neuropathy. The photopic negative response (PhNR) amplitude (arrows) was measured from the baseline to the trough between the cone b-wave and the i-wave.

### Functional and Morphological Changes in Patients With Optic Disc Atrophy

<table>
<thead>
<tr>
<th>Patient No./ Sex/Age, y</th>
<th>Optic Neuropathy</th>
<th>Eye</th>
<th>Rod ERG B-Wave, µV</th>
<th>Maximum ERG, µV</th>
<th>Cone ERG B-Wave, µV</th>
<th>30-Hz Flicker ERG, µV</th>
<th>RNFLT, µm</th>
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<td>Cone</td>
<td>Maximum</td>
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<tr>
<td></td>
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<td>B-Wave, µV</td>
<td>A-Wave, µV</td>
<td>B-Wave, µV</td>
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<td>225</td>
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<td>25.0</td>
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<td>25.0</td>
<td>78.1</td>
</tr>
</tbody>
</table>

Abbreviations: A, affected; C, compression; ERG, electroretinogram; I, inflammation; PhNR, photopic negative response; RNFLT, retinal nerve fiber layer thickness; T, trauma; U, unaffected.

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The a-wave and b-wave amplitudes were measured from the baseline to the trough of the first negative response and from the first trough to the peak of the following positive wave, respectively. In previous articles, the PhNR was defined as a negative-going wave immediately after the cone b-wave. The i-wave became apparent and obscured the negative trough of the PhNR in light-adapted conditions. The negative trough of the PhNR appears before or after the i-wave (Figure 1). We measured the PhNR amplitude from the baseline to the negative trough between the cone b-wave and the i-wave, as well as just after the i-wave. However, the amplitude of the PhNR measured between the cone b-wave and the i-wave is shown in the figures and table. Although the PhNR is a negative-going wave, its amplitude was expressed as a positive value in this study.

**FUNDUS PHOTOGRAPHY AND OPTICAL COHERENCE TOMOGRAPHY**

After the pupils were fully dilated, fundus photographs were obtained in all patients with a fundus camera (IA-50; Topcon Corp, Tokyo, Japan). We subjectively compared the disc color of the affected eyes with that of the unaffected contralateral eyes to determine whether optic nerve atrophy was present.

An optical coherence tomography scanner (OCT 2000; Carl Zeiss Meditech; Dublin, Calif) was used to obtain cross-sectional tomograms of the eyes. The delay of the light backscattered from the different layers in the retina was determined by using low-coherence interferometry. To measure the retinal nerve fiber layer thickness (RNFLT) around the optic nerve head, we used circular scans 2.0 mm in radius (OCT Application Version A6.1, Carl Zeiss Meditech). Each image consisted of RNFLT measurements at 100 points along a 360° path around the optic disc. A mean RNFLT of the 100 points was used for the analysis.

**STATISTICAL ANALYSIS**

A 2-tailed t test was used to compare data from affected with that from unaffected contralateral eyes or normal control eyes. The Pearson correlation coefficient was calculated for the PhNR amplitude and RNFLT.

**RESULTS**

**ELECTRORETINOGRAM WAVEFORM DIFFERENCES BETWEEN AFFECTED AND UNAFFECTED EYES**

Representative ERGs recorded from the affected and unaffected contralateral eyes in patient 2 at 6 months after symptom onset of compression optic neuropathy are shown in Figure 1. There was no difference in the waveforms of the scotopic rod, maximum, and 30-Hz flicker ERGs between the affected and unaffected eyes. However, PhNR was not present in the cone ERG of the affected eye, but a distinct negative deflection of approximately 40 µV followed the b-wave was present in the unaffected eye.

The amplitudes of the rod, maximum, cone, and 30-Hz flicker ERGs for each patient are shown in the Table. All of the ERG recordings were obtained more than 6 months after symptom onset and at a time when the optic nerve head appeared atrophic. The amplitudes of the scotopic rod b-wave, maximum scotopic a-wave and b-wave, cone a-wave and b-wave, and 30-Hz flicker ERGs of the affected eyes did not differ from the corresponding components in the unaffected contralateral eyes. On the other hand, the amplitudes of the PhNR were significantly reduced in the affected eyes (patients 1-10), as compared with those in the unaffected contralateral eyes (patients 1-4, 6-8, and 10; P = .005) and normal control eyes (P < .001) (Figure 2).

The amplitudes of the PhNR varied considerably in the normal control eyes. In our control subjects, the PhNR amplitudes ranged from 28.1 to 68.7 µV, but none of the values from the affected eyes was within this range (Table). The amplitude of the PhNR measured after the i-wave was also significantly attenuated in the affected eyes, as compared with that in the unaffected contralateral eyes (P < .001) and normal control eyes (P < .001; data not shown).

**RNFLT AND PHNR AMPLITUDE CHANGE WITH TIME**

Focal defects in the retinal nerve fiber layer can be detected with optical coherence tomography in early to moderate stages of glaucoma. We were able to measure the RNFLT and the PhNR amplitude in patients 2, 4, 7, and 8 from the early stages to the advanced stages in which optic disc atrophy was complete. The fundus photographs and optical coherence tomographic images obtained at 1 week and 1, 3, and 6 months after symptom onset are shown for patient 4 in Figure 3. This patient had compression optic neuropathy.

The PhNR amplitude was normal at 1 week after symptom onset, even though visual acuity had decreased to light perception. At 1 month after onset, the PhNR amplitude was considerably reduced, but the RNFLT was only slightly thinner. This finding suggests that functional loss preceded RNFLT thinning (Figure 3).

Averaged values of the relative RNFLT and PhNR amplitudes in patients 2, 4, 7, and 8 are plotted against the time after symptom onset in Figure 4. The values of the RNFLT and the PhNR amplitude at 1 week after the onset were set at 100%. There was a large discrepancy between the loss of RNFLT and PhNR amplitude at 1 month. Although the RNFLT was preserved for the first month after the onset, the PhNR amplitude was significantly reduced (P < .01). Reduction of the RNFLT progressed until 3 months, after which there were no further changes.
CORRELATION BETWEEN PhNR AMPLITUDE AND RNFLT

All of the data of the PhNR amplitude and RNFLT from the 12 affected and 8 unaffected contralateral eyes in the 10 patients were plotted to determine if a correlation existed between PhNR amplitude and RNFLT (Figure 5). The PhNR amplitude and RNFLT were measured more than 6 months after symptom onset when optic nerve atrophy is probably complete. The reduction of PhNR amplitude significantly correlated with RNFLT thinning at this time (r=0.879; P<.001). The PhNR amplitudes of the affected eyes did not overlap with those of the unaffected contralateral eyes (Figure 5). We also found a significant correlation between the PhNR amplitude measured after the i-wave and the RNFLT (r=0.902; P<.001; data not shown).

Figure 3. Fundus photographs (A), optical coherence tomographic images (B), and cone electroretinograms (C) at 1 week and 1, 3, and 6 months after symptom onset in patient 4 with compression optic neuropathy. A, Color photographs of the optic nerve head are shown. B, The retinal nerve fiber layer thickness (RNFLT) around the optic nerve head was measured. The representative values are the mean RNFLT of the 100 points along a 360° path around the optic disc. The arrowheads indicate the retinal nerve fiber layer. NAS, indicates nasal; SUP, superior; TEMP, temporal; and INF, inferior. C, Photopic negative response (PhNR; arrows) is shown.

Figure 4. Changes in the mean of the retinal nerve fiber layer thickness (RNFLT) and photopic negative response (PhNR) amplitude in patients 2, 4, 7, and 8 after disease onset. Each value at 1, 3, and 6 months is expressed as the percentage of RNFLT or PhNR amplitude seen at 1 week. Error bars indicate standard error of the mean.

Figure 5. All of the data of the photopic negative response (PhNR) amplitude and retinal nerve fiber layer thickness (RNFLT) from 12 affected and 8 unaffected eyes in the 10 patients were plotted. The PhNR amplitude and RNFLT were measured more than 6 months after disease onset (r=0.879; P<.001).
Reduction of the PhNR is correlated with the loss of sensitivity of the visual field in patients with POAG. Our results provide additional evidence that the PhNR is strongly attenuated by diseases of the optic nerve other than POAG.

DIFFERENCES OF RECORDING TECHNIQUE BETWEEN THE CURRENT AND PREVIOUS STUDIES

In previous studies, red stimuli on a blue background were used to record the PhNR. This stimulus configuration was advocated because it enhances the PhNR amplitude. However, with white stimuli on a white background in the present study, the PhNR amplitude ranged from 28 to 69 µV with a mean of 43 µV in normal control eyes. This finding indicates that even a conventional white stimulus on a white background will elicit substantial PhNR amplitude as long as the recordings arise from the photopic system.

However, we noted a difference in the cone ERG waveform between previous recording conditions and ours. In our recordings, the PhNR was followed by the i-wave that obscured the trough of the PhNR. The i-wave contributed little to the cone ERG in previous recording conditions, which then allowed a clear observation of the trough of the PhNR. The i-wave represents mainly the off response that grows in amplitude with light adaptation. A narrow-wavelength band of blue background light adapts the rods but not the cones so that the i-wave is not enhanced by blue light adaptation.

In the present study, we measured PhNR amplitudes before and after the i-wave. Both measurements produced similar results, which indicates that either scoring method is useful to evaluate the remaining function of ganglion cells and their axons in optic nerve atrophy.

In our previous article, conventional cone ERGs were recorded in patients with low-tension glaucoma, and no correlation was found between the loss of sensitivity of the visual field and PhNR amplitude reduction. In contrast, Viswanathan et al demonstrated that PhNR amplitude was significantly correlated with visual field sensitivity, with a moderately high coefficient of correlation in patients with POAG. These findings imply that the i-wave intrusion in our study may prevent an accurate measurement of PhNR amplitude, although we cannot eliminate the possibility that the alterations in eyes with low-tension glaucoma may have contributed to the difference.

CLINICAL APPLICATION OF PhNR TO OPTIC NEUROPATHY

Our results showed that PhNR amplitude remained unchanged in the early stages of optic neuropathy, even though vision had already been severely impaired. However, PhNR amplitude was reduced or completely lost as RNFLT thinning progressed. This finding indicates that PhNR does not reflect an early functional loss of the optic nerve without retinal structural change. Therefore, PhNR is not a suitable component to evaluate optic nerve function during the early stages when the normal structure of the ganglion cells and RNFLT are still preserved.

Measuring visual acuity and visual fields and assessing pupillary light reflexes are quicker and easier than recording ERGs during the early stages.

Opacities of the ocular media have a great effect on results measured by means of visual acuity and visual field. Total hyphema could be one of the causes of a relative afferent pupillary defect, which is widely used as a sign of unilateral optic nerve dysfunction. Furthermore, corneal opacities prevent a precise assessment of the pupillary reflex in contrast, the waveform of the cone ERGs produced by means of a strong flash are not affected by opacities of the ocular media, which suggests that PhNR amplitude could be used to evaluate residual optic nerve function and determine surgical indications in cases of severe opacity of the ocular media.

Changes in visual acuity cannot be used to quantify optic nerve function. Visual acuity can be well preserved if the papillomacular bundle of the nerve fiber is not impaired, even if widespread damage to the optic nerve is present. Although Thompson et al described a method to quantify relative afferent pupillary defect by using neutral density filters, 26% to 50% loss of ganglion cells is necessary to cause relative afferent pupillary defect. This finding indicates that pupillary reflexes are not a sensitive estimate for detecting optic nerve dysfunction.

There was a significant correlation between RNFLT thinning and PhNR amplitude loss in patients with optic nerve atrophy (Figure 4). This finding indicates that PhNR is a good measure of the surviving ganglion cells and their axons at the advanced stage of optic nerve disease. In addition, PhNR amplitudes in diseased eyes were not within the normal range of unaffected contralateral or normal control eyes, which suggests that PhNR amplitude measurement can be used to differentiate diseased eyes with optic nerve atrophy from normal ones.

DIFFERENCE FROM OTHER ERG COMPONENTS REPRESENTING GANGLION CELL ACTIVITY

We recently demonstrated that the first-order kernel of the multifocal ERG contains a component probably originating from ganglion cells. During specific conditions of stimulation, such as a low temporal frequency and 50% contrast, a small positive wavelet appears on the descending part of the first positive wave (P1) and is designated the s-wave. This response is strongly attenuated in patients with optic neuritis and is correlated with psychophysiological functional measures, such as critical flicker frequency and visual acuity. Measuring s-wave amplitude has a great advantage over measuring PhNR amplitude in evaluating focal functional loss of ganglion cells. However, multifocal ERG is not practical in patients with poor visual acuity and opacities of the ocular media because unstable fixation and distortion of the retinal image affect multifocal ERG. Because full-field ERGs are not generally affected by these factors, PhNR is better than multifocal ERG for evaluation of ganglion cell function in selected patients with severe loss of visual acuity and with opacities in the ocular media.

The scotopic threshold response (STR) is elicited by means of dim stimuli (0.6-1.0 log unit above the psychophysical threshold) and is driven by the third-order neurons in the rod pathway. Frishman et al demonstrated...
stated that the STR is eliminated in monkeys with experimentally induced glaucoma and concluded that the STR originated from the ganglion cells. However, there is evidence that the STR is not eliminated in patients with traumatic optic neuropathy and in cats with optic nerve resection, which suggests that the STR mainly represents the activity of amacrine cells. In clinical practice, it is difficult to record the STR because suitable recordings require complete dark adaptation. Furthermore, patient cooperation is important during the recording because baseline deflection due to the blink reflex has a temporal aspect similar to that of the STR and an effect on the amplitude of the STR. Complex recording techniques and controversy about its cellular origin make the STR less valuable than the PhNR in evaluating the function of the ganglion cells or their axons for clinical examinations.

The pattern ERG is another response that reflects the activity of ganglion cells and their axons. The cellular origin of negative potentials (N95) of the pattern ERG is similar to that of the PhNR. However, from a practical standpoint, full-field ERGs are easier to record than pattern ERGs because they do not require refractive correction, clear ocular media, and exact foveal placement.

CORRELATION BETWEEN RNFLT AND PhNR

We found a good correlation between the reduction of the PhNR amplitude and RNFLT at the advanced stages of the disease. However, as shown in Figures 3 and 4, PhNR amplitude loss preceded RNFLT thinning at 1 and 3 months after disease onset. This discrepancy could be due to several factors. First, excessive glutamate released by optic nerve damage can lead to PhNR loss because of suppression of the synaptic transmission between the bipolar and ganglion cells. The N-methyl-d-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate receptors were involved in ganglion cell death after the optic nerve was crushed in rats. In addition, we previously reported that intravitreal injection of N-methyl-d-aspartate agonist eliminates the PhNR in rats.

Second, glial alterations induced by optic nerve damage may reduce the PhNR amplitude. Experimental evidence suggests that glial mediation generates PhNR: intravitreal injection of barium blocks the potassium current in glial cells, with the subsequent elimination of PhNR in cats. Glial cell changes also occur after optic nerve resection. All of this evidence suggests that glial involvement could contribute to reduction of PhNR at the early stage of disease.

Selective reduction and loss of the PhNR amplitude was found in patients with optic nerve atrophy. The presence and size of PhNR was used to discriminate between patients with optic nerve atrophy and healthy control subjects, which suggests that PhNR can be used to evaluate the function of ganglion cells or their axons. The PhNR seems to be a good measure for quantifying RNFLT loss in eyes with optic nerve atrophy.

Submitted for publication January 14, 2003; final revision received July 30, 2003; accepted August 25, 2003.

This study was supported by the Japanese Retinitis Pigmentosa Society, Chiba, Japan (Dr Machida).

We thank Duco I. Hamasaki, PhD, for editing the manuscript.

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