Oscillatory potentials (OPs) are wavelets that appear on the ascending limb of the b-wave in electroretinographic (ERG) responses to bright flashes and are thought to reflect feedback synaptic modulation of bipolar cell responses. In some patients with diabetic retinopathy or central retinal vein occlusion, these wavelets have been reported to be reduced in amplitude, without alteration of the a-wave, which is generated by photoreceptors. Since OPs are more susceptible than the a-wave to clamping of the retinal circulation, their reduction in patients with a normal a-wave has been interpreted as a sign of possible inner retinal ischemia.

Patients with diabetic retinopathy or central retinal vein occlusion frequently have small pupils and media opacities (cataracts, vitreous or subhyaloid hemorrhages, and retinal hemorrhages of variable extent) that may diminish the amount of light reaching the photoreceptors. Vitreous opacities, for example, can attenuate light up to 1000-fold. The presence of such “filters” in front of the photoreceptors makes it difficult to quantify the degree of inner retinal malfunction, even when OPs are detectable with bright flashes, because attenuation of the light reduces not only these responses, but also the a-wave on which they depend.

We tried to determine whether (in normal subjects) a relationship could be defined between OP amplitude, as a measurement of inner retinal function, and a-wave slope, as a measurement of photoreceptor function, that would take into account or even be independent of flash luminance. A-wave amplitude was not used as a measurement of photoreceptor function, because it is determined, in part, by onset of the b-wave, which is delayed with diminished retinal illumination. The OPs were quantified both in the time domain, by which amplitude has been conventionally assessed, and in the frequency domain, by which mid frequency (ie, 50–100 Hz) and high-frequency (ie, 100–200 Hz) bands have been separated, to determine to what extent either measurement was related to the a-wave slope.

Materials and Methods

Basic Studies

The primary subjects were six healthy volunteers (age range, 29–51 years) who had normal ophthalmic examinations and <6 diopters of myopia. Informed consent was obtained from these and other subjects before examination. The pupil of one eye of each subject was dilated with 2.5% phenylephrine hydrochloride and 1% cyclopentolate hydrochloride, and the eye was dark-adapted for 45 min. Full-field ERGs were then elicited routinely in darkness with xenon flashes of white light ranging in ascending integrated luminance from −3.66 to 2.34 log ft.L.-sec in 0.5-log
unit steps and presented once per minute, one flash per step. These flashes were generated by a bright-flash stimulator (L.K.C., Gaithersburg, MD) positioned above a diffuser in a Ganzfeld dome and were varied in luminance with gelatin neutral-density filters (Wratten #96, Eastman Kodak Company, Rochester, NY).

In pilot experiments, OPs in response to the first flash of a series of four of equal luminance, presented at 1-min intervals after 45 min of dark adaptation, were found to be smaller than those in the subsequent three responses for luminances ≥ 1.34 log ft.L.-sec. In other words, for this luminance range, the first flash conditioned the response to the second flash, and the second and third flashes had no additional effect on the subsequent responses. Over the four responses, a-waves showed no change. A conditioning effect on OPs in a succeeding response was even seen when the luminance of the first of two flashes was 0.5 log unit lower than the luminance of the second flash. Therefore, in our routine protocol of single flashes of ascending luminance at 1-min intervals, OPs to luminances ≥ 1.34 log ft.L.-sec were considered to be conditioned by prior flashes. Conditioning of OPs elicited with bright flashes has been used to maximize OP amplitudes of patients with diabetic retinopathy.

Responses were monitored with a bipolar Burian-Allen contact lens electrode (Hansen, Iowa City, IA) coated with 1% methylcellulose and placed on the cornea, topically anesthetized with 0.5% proparacaine hydrochloride. A ground electrode was placed on the forehead. Fixation was facilitated by a red light-emitting diode in the rear of the dome. Responses were amplified differentially at a gain of 1000 (−3 dB at 2 Hz and 1000 Hz) and digitized at a sampling rate of 521 Hz over a time window of 492 msec by an analog-to-digital data acquisition module (MacADIOS 411; G. W. Instruments, Cambridge, MA). Waveforms were then saved to a diskette.

After testing, responses were displayed and analyzed by computer (Macintosh SE; Apple, Cupertino, CA). A-wave slopes, quantified as amplitude from initial baseline to the major cornea-negative peak divided by the time interval between a-wave onset and peak, were then calculated after the examiner had designated these locations with a screen cursor. Although the photoflash unit generated a variable-sized photovoltaic artifact that partially obscured the a-wave, this artifact was eliminated from each ERG response by subtracting a digitized replica of the artifact automatically scaled in amplitude to that present in the initial 2 msec of the response (Fig. 1A).

The OP amplitudes were quantified in the time domain between the a-wave peak (or, if absent, onset of the b-wave) and major cornea-positive b-wave peak, after excluding from analysis any responses with blink artifacts on or preceding the b-wave peak. Quantitation of OPs was done even for those responses in which OPs could not be detected by inspection, in which cases the values were considered measures of noise. First, low-frequency components were reduced with a 62 Hz high-pass digital filter. Then, the mean of the absolute value of the amplitude variation above and below baseline for the several wavelets between the a-wave and b-wave peaks was computed (Fig. 1B).

The OP amplitudes were quantified in the frequency domain by next assigning zeros to points preceding the a-wave peak and following the b-wave peak and then multiplying the high-pass-filtered waveform between these peaks by a Hamming window. Over the four responses, a- and b-wave implicit times, respectively. The Hamming window was used to minimize voltage discontinuities preceding the peak of the a-wave and following the peak of the b-wave. A magnitude fast Fourier transform (FFT) was then done on the entire wave form, which generally revealed two distinct bands for the OPs, one peaking in the mid frequencies between 60–90 Hz and the other peaking in the high frequencies between 100–180 Hz (Fig. 1C). The amplitude of each of these peaks, when present, was then determined.

Time- and frequency-domain OP amplitudes, derived from ERG responses to the standard luminance series, were related to the respective a-wave slopes by linear regression. A similar analysis was applied to the b-wave, generated by Müller cells and whose amplitude can also be reduced preferentially compared with the a-wave amplitude in central retinal vein occlusion. B-wave amplitudes were quantified from the a-wave peak to the major cornea-positive peak and then related to a-wave slopes.

Clinical Studies

Since one of the analyses revealed a potentially useful measurement of inner retinal function independent of flash luminance, this measurement was then evaluated in a patient with a dense vitreous hemorrhage and presumed normal retinal function and in a patient with central retinal vein occlusion and clear media; the values were compared with values obtained from 26 normal subjects (age range, 19–76 years). For these clinical studies, ERGs were elicited with our standard diagnostic protocol for patients with known or suspected retinal vascular disease, which includes dim blue (λmax = 440 nm) flashes of −3.8 log ft.L.-sec to elicit rod-isolated responses, dim white flashes of −1.2 log ft.L.-sec to elicit mixed cone-rod responses, 30-Hz flashes of the same white light to
elicit cone-isolated responses, and bright white flashes of 1.85 log ft.L.-sec to elicit OPs. The responses to dim blue flashes were used to derive an estimate of OP noise.

**Results**

**Basic Studies**

Representative ERGs show that a-waves and, to a lesser extent, OPs were detectable for a flash of -1.66 log ft.L.-sec, and both appeared to increase in amplitude with increasing flash luminance (Fig. 2). At low luminances, the a-wave consisted of only a single deflection, presumably representative of rod function, whose implicit time decreased with increasing luminance. For a middle range of luminances, the a-wave consisted of a minor followed by a major deflection, thought to be indicative of superimposed cone and rod activity, respectively.38,39 For higher luminances, the a-wave became a single peak again, although both cone and rod contributions presumably remained but now had indistinguishable implicit times. The b-wave was detectable at a lower luminance than the a-wave or OPs, increased in amplitude with increasing flash luminance, and reached a maximum amplitude for the highest luminances.

Linear regression of OP amplitude on a-wave slope, based on the luminance range for which a-waves were detectable, revealed significant relationships for each subject (Table 1 and Fig. 3). Mean OP amplitude, minus the y-intercept for which a-wave slope was zero, equaled 24% of a-wave slope (Fig. 3). The y-intercept (ie, 10 μV) equaled the upper 95% limit for noise based on amplitudes calculated from those responses (n = 16) to lower luminance flashes for which a-waves and OPs were not detectable. This noise limit was subtracted from each OP amplitude for responses to higher luminances in which a-waves were detectable to derive the residual signal. The ratio of residual OP amplitude to a-wave slope was found to be independent of luminance for integrated luminances ≥ -1.2 log ft.L.-sec (Fig. 4).
Full-field ERGs from a normal subject

INTEGRATED LUMINANCE (LOG FT.L.-SEC.)

RESPONSE

2.34
1.85
1.34
0.85
0.34
-0.15
-0.66
-1.15
-1.66
-2.15
-2.66
-3.15
-3.66

400 μV

50 msec

Fig. 2. Time domain ERGs recorded from a normal subject in response to full-field flashes of varying integrated luminance. Traces begin at flash onset.

Linear regression of OP amplitude in the frequency domain on a-wave slope for each subject revealed weaker correlations than were found with time domain analysis (Table 2). On average, frequency domain OP amplitude, whether from mid- or high-frequency bands, equaled 4% of a-wave slope minus a y-intercept (Fig. 5). The higher variability seen in the frequency domain compared with the time domain was due, in part, to switching in the dominance of mid- and high-frequency peaks from one luminance step to the next. In some subjects, the mid-frequency peak was dominant for one luminance while the high-frequency peak was dominant for the next, and vice versa. Rarely, only a single broad peak near 100 Hz was present. Based on wave forms for which both peaks were evident, the peak high frequency was found, on average, to be the second harmonic of the peak mid frequency (Fig. 6).

In contrast to the linear relationships between OP amplitude and a-wave slope, a logarithmic (ie, non-linear) relationship was found between b-wave amplitude and a-wave slope (Fig. 7). B-wave amplitude had a value of ~310 μV for an a-wave slope approaching zero and appeared to saturate for the three highest flash luminances while a-wave slope continued to increase.

Clinical Studies

Based on our clinical series of recordings in 26 normal subjects, we set out to define normal confidence limits for the ratio of OP amplitude to a-wave slope and for the maximum interocular percentage difference in the ratio. The ratio was found to be inversely correlated with age (r = -0.398, P = 0.04, based on logarithmic data) as with many other absolute measures of ERG function, which prevented a practical estimation of confidence limits from this sample size. However, the interocular percentage difference in the ratio (ie, 1 - (smaller ratio / larger ratio)) showed no correlation with age (r = 0.005; P, not significant, based on logarithmic data), and a 95% confidence limit of 32% could be derived.

With respect to this normal limit, two case studies illustrate the potential application of quantifying the ratio of OP amplitude to a-wave slope to evaluate inner retinal function. The first was a 25-year-old woman with a dense vitreous hemorrhage in her left eye (OS). The patient had a 3-year history of chronic uveitis OS treated with topical and periocular corticosteroids. After a sub-Tenon’s injection, she had a sudden decrease in vision OS. Findings OS included hand-motions acuity, posterior synechiae limiting maximal dilation to 4 mm, and slight posterior subcapsular cataractous changes. The right eye was normal. B-scan ultrasonography revealed dispersed vitreous echoes and an attached retina. Her ERG responses to all four stimuli of our diagnostic series were subnormal and delayed OS relative to her right eye (OD). However, the ratio of OP amplitude to a-wave slope derived from her bright-flash ERGs was actually 17% smaller OD (0.79) than OS (0.95) (Fig. 8), an insignificant interocular percentage difference compatible with a diagnosis of preserved inner retinal function OS.

The second case was a 77-year-old woman with loss of vision OD secondary to central retinal vein occlusion.

Table 1. Time domain regression of oscillatory potential amplitude on A-wave slope

<table>
<thead>
<tr>
<th>Subject</th>
<th>Regression slope</th>
<th>Y-intercept</th>
<th>Correlation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.26</td>
<td>10.99</td>
<td>0.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B</td>
<td>0.24</td>
<td>7.82</td>
<td>0.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C</td>
<td>0.29</td>
<td>13.17</td>
<td>0.97</td>
<td>&lt;0.001</td>
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<tr>
<td>D</td>
<td>0.18</td>
<td>9.89</td>
<td>0.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E</td>
<td>0.28</td>
<td>9.15</td>
<td>0.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>F</td>
<td>0.19</td>
<td>8.94</td>
<td>0.96</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Based on data illustrated in Figure 3.
LINEAR REGRESSION OF TIME-DOMAIN OSCILLATORY POTENTIAL AMPLITUDE ON A-WAVE SLOPE IN NORMAL SUBJECTS

Fig. 3. Linear regression of time domain oscillatory potential amplitude on a-wave slope for each of six normal subjects and for the mean. Values were obtained for flashes of integrated luminance between $-1.66$ and $2.34 \log ft L$-sec. Regression line for the mean is $y = 0.24x + 10.02$, $r = 0.99$, $P < 0.001$. See Table 1 for regression coefficients and statistics for each of the six subjects.

A-WAVE SLOPE (µV/msec)

MEAN RATIO OF TIME-DOMAIN OSCILLATORY POTENTIAL AMPLITUDE TO A-WAVE SLOPE VS LUMINANCE IN NORMAL SUBJECTS

Fig. 4. Plot of mean ratio of time domain oscillatory potential amplitude, corrected for noise (see text), to a-wave slope versus log integrated flash luminance based on six subjects. Error bars designate ± 1 SEM. Solid line is linear regression of the ratio on log luminance, $y = -0.008x + 0.26$, $r = 0.16$, $P = NS$. The ratio for $-1.7 \log ft L$-sec, at which the a-wave was first detectable, fell significantly below the values for higher luminances (not illustrated).

A medical history was notable for Graves's disease and hypertension for which she was receiving levothyroxine, enalapril, and furosemide. Visual acuity was counting fingers OD and 20/30+ OS. Slit-lamp examination revealed slight nuclear and cortical haze OU. Ophthalmoscopy showed extensive intraretinal hemorrhages with dilated tortuous veins, macular hemorrhages, and edema OD and slight arteriolar narrowing OS. Fluorescein angiography was interpreted as inconclusive as to the extent of ischemia. Her ERG responses were reduced and delayed to all four stimuli OD relative to OS (Fig. 9). The ratio of OP amplitude to a-wave slope derived from her responses to bright flashes was 0.21 OD and 0.40 OS. Her 47% reduction OD relative to OS for the ratio fell outside our normal 95% confidence limit of 32%. Therefore, this patient's recordings are compatible with a diagnosis of inner retinal malfunction.
Table 2. Frequency domain regression of oscillatory potential amplitude for mid- (top) and high-frequency (bottom) peaks on A-wave slope

<table>
<thead>
<tr>
<th>Subject</th>
<th>Regression slope</th>
<th>Y-intercept</th>
<th>Correlation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
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<td>2.36</td>
<td>0.48</td>
<td>n.s.</td>
</tr>
<tr>
<td>B</td>
<td>0.062</td>
<td>1.55</td>
<td>0.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C</td>
<td>0.022</td>
<td>1.29</td>
<td>0.73</td>
<td>0.04</td>
</tr>
<tr>
<td>D</td>
<td>0.045</td>
<td>1.48</td>
<td>0.83</td>
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<td>E</td>
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<td>F</td>
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<td>0.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>0.051</td>
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<td>0.93</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>0.016</td>
<td>1.63</td>
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<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>0.060</td>
<td>1.12</td>
<td>0.88</td>
<td>0.004</td>
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<td>0.02</td>
</tr>
<tr>
<td></td>
<td>0.059</td>
<td>0.87</td>
<td>0.91</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>0.048</td>
<td>0.35</td>
<td>0.88</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Discussion

Our study shows that, in normal subjects, OP amplitude was proportional to a-wave slope for responses to flashes of varying luminance. On average, the ratio of OP amplitude quantified in the time domain to a-wave slope was independent of flash luminance from the highest luminance down to a 3.5 log-unit or ~3000-fold attenuation of luminance. Since OP amplitude is a measurement of inner retinal function, and the a-wave slope is a measurement of photoreceptor function, the constancy of this ratio over a wide range of flash luminances in normal subjects with clear media raises the possibility that an abnormally low ratio in patients with media opacities might be a reason to suspect that they have inner retinal malfunction.

Examination of two patients with unilateral disease using our diagnostic ERG protocol consisting of dim flashes to isolate cone and rod components and bright flashes to elicit OPs supported this idea. One patient with a dense vitreous hemorrhage had comparable ratios in her two eyes despite having subnormal and delayed responses to dim flashes and subnormal OPs to bright flashes in her affected eye. This finding could be explained by an attenuation of light incident on normal outer and inner retina. Conversely, a patient with central retinal vein occlusion and relatively clear media had a significantly smaller ratio, abnormal responses to dim flashes, and subnormal OPs in her...

Fig. 5. Linear regression of oscillatory potential amplitudes for mid- and high-frequency peaks on a-wave slope for mean of six subjects. Values were obtained for flashes of integrated luminance between −1.66 and 2.34 log ft L−sec. Regression lines are $y = 0.041 x + 1.25$, $r = 0.94$, $P < 0.001$ for the mid-frequency peak and $y = 0.044 x + 1.69$, $r = 0.98$, $P < 0.001$ for the high-frequency peak. See Table 2 for regression coefficients and statistics for each of the six subjects.

Fig. 6. Histogram of peak high-frequency divided by peak mid-frequency for oscillatory potentials in normal subjects. Observations are plotted against the ratio of peak high-frequency divided by peak mid-frequency. The mean ratio was 2.04 ± 0.04.

Fig. 7. Plot of mean of b-wave amplitude versus a-wave slope based on six subjects. Values were obtained for flashes of integrated luminance between −1.66 and 2.34 log ft L−sec. The data could be fitted by $y = 214.2 \log x + 310.11$, $r = 0.99$, $P < 0.001$ (not illustrated).
CASE STUDY: VITREOUS HEMORRHAGE OS

Fig. 8. ERG responses from a patient with a vitreous hemorrhage OS. Responses were elicited after 45 min of dark adaptation and two or three consecutive traces are superimposed. Oscillatory potential noise was derived from the average response to blue light. For the bright-flash recordings, asterisks designate the point on each b-wave up to which oscillatory potentials were quantified in order to exclude blink artifacts. Quantifications of oscillatory potential amplitude and a-wave slope were also based on the average waveform. For further details of methods, see text.

CASE STUDY: CENTRAL RETINAL VEIN OCCLUSION OD

Fig. 9. ERG responses for a patient with central retinal vein occlusion OD and relatively clear media. For details of recording and analysis, see Figure 8 legend and text.
affected eye. This finding was consistent with inner retinal malfunction and, possibly, ischemia.

The OP amplitudes quantified by Fourier analysis were also linearly proportional to a-wave slope, but the correlation coefficients for mid- and high-frequency peaks were smaller than those obtained from time-domain analysis. This resulted from the fact that, for the mid-frequency or high-frequency peak, FFT amplitude grew less systematically than time-domain amplitude with increasing luminance, probably due to shifting dominance between the two frequency peaks. Since the high-frequency peak averaged the second harmonic of the mid-frequency peak, both could reflect the output of the same retinal oscillators with the capacity for intermittent, variable full-wave rectification.\(^4\) In other words, the two frequency bands may arise from interdependent sources. This is supported by the finding that the ratios of both mid- and high-frequency peak amplitudes to a-wave slope were the same. Alternatively, since both cone and rod components were present in the a-wave, it is possible that shifting dominance may reflect a luminance-sensitive interaction between these two contributions to the OPs.

In contrast to OP amplitude, b-wave amplitude was not linearly correlated with a-wave slope. For high flash luminances, b-wave amplitude became stable and relatively independent of a-wave slope. This non-linear relationship indicates that b-wave amplitude relative to a-wave slope cannot serve as a useful index of inner retinal malfunction in patients with dense media opacities. For example, patients could have inner retinal ischemia, which may preferentially reduce b-wave amplitude, and a vitreous hemorrhage, which will preferentially reduce a-wave slope, resulting in a normal value for the ratio of b-wave amplitude to a-wave slope. Moreover, the large y-intercept (ie, \(\sim 310 \mu V\)) represents a second parameter that would have to be estimated for each patient. On the other hand, with the brightest flash used in this study, b-wave amplitude would be insensitive to opacities that attenuate light \(\leq\) tenfold and, in such cases, could be used by itself to assess inner retinal malfunction.

Digital subtraction of the photovoltaic artifact from each response before quantifying a-wave slope permitted use of a conventional bipolar Burian-Allen contact lens electrode with its unshielded metallic ring to record bright flash responses. This approach was adopted as a convenient alternative to developing a custom monopolar nonmetallic or light-shielded metallic electrode to minimize or prevent a photovoltaic artifact in bright flash recordings.\(^41-43\) Analysis of OPs only between the a-wave and b-wave peaks eliminated the problem of additional, erroneous amplitude due to blink artifacts subsequent to the b-wave peak, which might lie in the same frequency bands as OPs. Quantitation of OP amplitude as the mean absolute value of the area between each wavelet and baseline, unlike the "caliper-square" method of summing peak amplitudes,\(^4\) did not depend on the number of peaks thought to be present in the response.

Since decreased retinal illumination appears not to alter the ratio of OP amplitude to a-wave slope based on our findings in normal subjects and a patient with a vitreous hemorrhage, it would be interesting to study this ratio in patients with known inner retinal ischemia with or without intraretinal hemorrhages. Initially this work should be done in patients with presumed unilateral disease, since a normal confidence limit for the ratio has been defined up to now only with respect to an interocular comparison. The ratio should be abnormally low in the affected eyes but comparable in the two groups of patients, assuming that the intraretinal blood has not, itself, been toxic to\(^44\) or indirectly affected\(^45\) the outer or inner retina. If confirmed, the ratio of OP amplitude to a-wave slope may then be used in patients with presumed unilateral central retinal vein occlusion to evaluate possible inner retinal ischemia even in the presence of media opacities. If and when age-corrected limits have been set for normal eyes considered in isolation, then this approach also could be applied to both eyes of patients with diabetes mellitus.

Key words: a-wave, electroretinography, oscillatory potentials, retinal vascular disease, vitreous hemorrhage

References
