Local Multifocal Oscillatory Potential Abnormalities in Diabetes and Early Diabetic Retinopathy

Marcus A. Bearse, Jr, Ying Han, Marilyn E. Schneck, Shirin Barez, Carl Jacobsen, and Anthony J. Adams

PURPOSE. To study retinal dysfunction in diabetes and early nonproliferative diabetic retinopathy (NPDR) using a new method to analyze local multifocal electroretinogram oscillatory potentials (mfOPs).

METHODS. One eye of each of 26 normal subjects, 16 diabetic subjects without retinopathy (NoR), and 16 diabetic subjects with early NPDR was examined. Slow-flash multifocal electroretinograms (sf-mfERGs) were recorded from the central 45°, and stereo fundus photographs of the diabetic eyes were taken. The first-order (K1), induced first-order (K1i), and second-order (K2) response components were extracted from each retinal location, and K1i and K2 were added to create Ks2. Responses from 35 contiguous areas were digitally filtered 90 to 225 Hz to isolate the mfOPs. The signal-to-noise ratio (SNR) of the mfOPs was calculated, and abnormality was defined as SNR below the fifth percentile of the normal subjects.

RESULTS. Combining the K1i and K2 components to form Ks2 before isolation of the mfOPs by digital filtering increased the SNR. Mean Ks2 and K1 mfOP SNRs were abnormal in 25% and 19% of the NoR eyes, respectively, and both were abnormal in 62% of the NPDR eyes. The retinal distributions of the local Ks2 and K1 mfOP abnormalities overlapped, but they differed. Furthermore, local Ks2 mfOP abnormalities were preferentially associated with retinal sites containing NPDR but K1 mfOP abnormalities were not.

CONCLUSIONS. The cells that contribute to the generation of local mfOPs are affected by diabetes and, to a greater degree, by early NPDR. The results suggest that fast adaptive mechanisms influencing the mfOPs are most abnormal at retinal sites containing NPDR. (Invest Ophthalmol Vis Sci. 2004;45: 3259–3265) DOI:10.1167 iovs.04-0308

Functional abnormalities preceding and associated with a number of retinal diseases are increasingly being studied with the multifocal electroretinogram (mfERG), a technique for mapping retinal function objectively.1–7 Several recent reports have provided new information regarding retinal dysfunction associated with diabetes mellitus. Investigators who have analyzed mfERGs combined over relatively large retinal areas have reported abnormally reduced response amplitudes and/or delayed implicit times in diabetic subjects with retinopathy.8–11 and in diabetic subjects without signs of retinopathy.12–15 Analyses of local mfERGs have shown functional abnormalities in eyes with diabetic retinopathy, both in retinal regions corresponding to retinopathy and in areas without signs of it.14–16 Local mfERG abnormalities also occur in some diabetic eyes that do not have evidence of retinopathy.14,16–18 Recently, we found that mfERG implicit time delays are associated with retinal locations in which nonproliferative diabetic retinopathy (NPDR) develops 1 year later.17 The mfERG, therefore, clearly has value for the study of diabetic retinal dysfunction.

Many of the electrophysiological manifestations of retinal dysfunction in diabetes suggest that the inner retina is most affected. Several full-field ERG studies have shown that the oscillatory potentials (OPs), which are largely generated by activity of the amacrine cells, are preferentially affected by diabetes and diabetic retinopathy.19–23 To date, however, most mfERG studies of diabetes have used “standard” 75-Hz pseudo-random flicker stimulation.8–10,14,15,17,18 The mfERGs evoked by this stimulus reflect predominantly the activity of bipolar cells with relatively minor contributions from the cone photoreceptors and amacrine and ganglion cells.24–26 It is possible, then, that nonstandard modes for stimulation and analysis of mfERGs emphasizing inner retinal contributions could provide greater sensitivity to, and better understanding of, diabetic retinal dysfunction than the “standard” mfERG.

In a few studies of diabetic eyes, researchers have used mfERG stimuli designed to accentuate inner retinal contributions to the responses. Shimada et al.13 studied diabetic eyes without retinopathy by using a “global flash” stimulus that was expected to evoke relatively large inner retinal contributions to an induced component of the first-order kernel.13 They reported, however, that the induced component was highly variable among their control eyes and that it was not abnormal in diabetes. A slow-flash mfERG (sf-mfERG) stimulus, which separates focal flashes by extended minimum periods of darkness, emphasizes the oscillatory content of, and inner retinal contributions to, the mfERG.27–31 In two studies of diabetic eyes variations of the sf-mfERG were used and multifocal oscillatory potentials (mfOPs) were extracted from the retinal signals. Kurtenbach et al.12 studied patients with type 1 diabetes without signs of retinopathy and reported that some mfOP implicit times were delayed. Onozu and Yamamoto11 reported mfOP amplitude reductions and implicit time delays in their group of diabetic subjects with proliferative diabetic retinopathy. However, these two mfOP studies analyzed responses that were averaged over relatively large retinal areas (quadrants and/or rings concentric with the fovea) rather than local responses, and groups of subjects rather than individuals were compared to visually normal subjects.

In the present study, we investigated inner retinal response components generated within 35 relatively small patches of retina. A new technique was used to combine mfOPs isolated from the first- and second-order sf-mfERG kernels to increase their signal-to-noise ratio (Bearse MA, et al. IOVS 2003;44: ARVO E-Abstract 2696). The amplitudes of the resultant local mfOPs were examined in normal eyes, in diabetic eyes without signs of retinopathy, and in those with NPDR. Abnormalities of
both first-order and induced mfOPs were examined and compared. We also examine whether mfOP abnormalities are preferentially associated with retinal sites of retinopathy in the subjects with NPDR.

METHODS

Subjects

One eye of each subject was studied. Twenty-six visually normal subjects (42.0 ± 11.3 years old; mean ± SD), 16 diabetic patients without retinopathy (NoR group; 50.1 ± 10.8 years old) and 16 with predominantly early NPDR (NPDR group; 53.1 ± 7.0 years old) were examined. Of the NoR subjects, 4 (25%) had type 1 and 12 (75%) had type 2 diabetes. Two (12.5%) of the patients with NPDR had type 1 and 14 (87.5%) had type 2.

In addition to dilated ophthalmic examinations, the diabetic subjects had central stereoscopic 50° fundus photographs taken within 1 month of sf-mfERG recording. The photographs were examined and graded according to Early Treatment Diabetic Retinopathy Study (ETDRS) criteria by a retinal specialist who was masked to the sf-mfERG results.32 The 50° (ETDRS) criteria by a retinal specialist who was masked to the sf-mfERG graded according to Early Treatment Diabetic Retinopathy Study (ETDRS) criteria by a retinal specialist who was masked to the sf-mfERG results.32 The 50° (ETDRS) criteria by a retinal specialist who was masked to the sf-mfERG graded according to Early Treatment Diabetic Retinopathy Study (ETDRS) criteria by a retinal specialist who was masked to the sf-mfERG results.32 The 50° (ETDRS) criteria by a retinal specialist who was masked to the sf-mfERG graded according to Early Treatment Diabetic Retinopathy Study (ETDRS) criteria by a retinal specialist who was masked to the sf-mfERG results.32

Four of the subjects with NPDR had signs consistent with mild retinopathy (microaneurysms and dot hemorrhages), four subjects had these plus at least one hard or soft exudate, seven subjects had these plus a single patch of edema, and one subject had these plus three patches of edema. Each instance of NPDR was confined to an area that was smaller than a single stimulated patch of the retina.

All subjects had corrected visual acuity of 20/25 or better. Subjects with cataracts graded moderate or worse, those with or suspected of having other ocular complications, and high (>6.0 D) myopes were not included in the study. Informed consent was obtained from all subjects after the experimental procedures were described to them. Approval of this research was obtained from the UCB Committee for the Protection of Human Subjects, and the tenets of the Declaration of Helsinki were observed.

Visual Stimulation

The stimulation and recording protocols were essentially the same as those used in our earlier sf-mfERG study of subjects with diabetes.16 A response imaging system (Visual Evoked Response Imaging System with refractor/camera; VERIS Science 4.3; EDI Inc., San Mateo, CA) was used to present an array of 103 hexagons scaled with eccentricity (diameter ~ 45°; scaling factor = 10.46) to the central retina. We used an sf-mfERG stimulus in which the shortest possible interval between focal flashes was 53.3 ms. That is, each step in the m-sequence used to drive the luminance of the hexagons was four video frames long (Fig. 1A). In the first frame, each patch had an equal probability of appearing at a luminance of 100 cd/m² or remaining dark (<2 cd/m²). In the next three frames, all hexagons remained dark. The display surrounding the hexagonal array and a central fixation cross were maintained at 50 cd/m². The luminance of the wall behind the stimulus display was also ~50 cd/m², to suppress stray light responses.36-37

Response Recording

The pupil of the tested eye was maximally dilated with 1% tropicamide and 2.5% phenylephrine hydrochloride. Retinal potentials were recorded using a bipolar Burian-Allen contact lens electrode applied after the cornea was anesthetized with 0.5% proparacaine, and a ground electrode was attached to the right earlobe. The fellow eye was occluded with light pressure to prevent blinks. Stability of fixation and the contact lens electrode position were monitored during recording, using the refractor/camera. Retinal signals were band-pass filtered 10 to 300 Hz, amplified 100,000 times and sampled every 0.833 ms. A recording was approximately 7.5 minutes long (215 - 1 m-sequence steps), separated into 16 segments for subject comfort. If loss of fixation was observed or significant artifacts occurred, the affected segment was discarded and replaced.

Signal Processing and Measurement

At each of the 103 stimulated locations, three response epochs containing mfOP sources were processed with one iteration of artifact
Local mfOP Abnormalities in Diabetes and NPDR

RESULTS

mfOP Waveforms and the Combination of K1i and K2

Examples of K1, K1i, and K2 mfOPs obtained from the temporal retina of a normal subject at 8° eccentricity (response group 15 in Fig. 1B) are shown on the left side of Figure 2A. Whereas the K1i and K2 mfOP waveforms are very similar, the K1 mfOP waveform differs, especially at the beginning of the oscillations, where it appears to be out of phase (Fig. 2A, arrows). This and additional K1 mfOP waveform differences were observed over most of the tested retina. This is not entirely unexpected, because the K1i and K2 mfOPs largely reflect the effects of adaptive processes acting on the K1 oscillatory components. 27-29

In contrast, the similarity of the local K1i and K2 mfOPs has an important practical significance because their combination could increase their SNR. Indeed, in the absence of lateral effects that could contribute to K1i but not to K2, the two mfOPs should be identical, because they are both calculated as the difference between a local mfOP preceded 53.3 ms by a flash and a local mfOP preceded 53.3 ms by a dark stimulus. 29

The right side of Figure 2A demonstrates the similarity of the K1i and K2 mfOPs at a single retinal location (location 15) by summing them (labeled Ks2) and differencing them (labeled K1i-K2). Indeed, whereas K1i tended to be larger in amplitude than K2 at most retinal locations, there were no other systematic differences between them. (It should be noted that, in practice, Ks2 is calculated by adding the K1i and K2 components at each of the 35 retinal locations before digital filtering is applied to extract the Ks2 mfOPs.) The entire Ks2 mfOP array obtained from the normal subject’s right eye is shown, plotted in retinal view, in Figure 2B. In this figure, the central Ks2 mfOP is displaced slightly upward to avoid overlap with neighbors. Note the pronounced nasal-temporal difference in amplitude which had been observed previously for focal OPs 27-29 and mfOPs. 27-30

The effect of combining K1i and K2 was examined in a preliminary study of the first 13 examined normal subjects. In each eye, the mean SNR was computed for the 35 K1i, K2, and Ks2 mfOPs. As shown in Figure 3, the mean SNR across subjects for the K1i mfOP (2.82 dB) was larger than that of K2 (2.26 dB), and the Ks2 combination was largest (3.58 dB). This corresponds to an improvement in SNR of 27% for K2 relative to K1i, and 44% for Ks2 relative to K2, approximating the 41% improvement one would expect if K1i and K2 were identical except for uncorrelated noise. In the rest of this study, we will concentrate on the Ks2 and K1 mfOPs.
K1 and Ks2 mfOPs in Normal and Diabetic Subjects

As a first step to examine the effects of diabetes and NPDR on mfOPs, the means of each subject’s 35 K1 and Ks2 mfOP SNRs were calculated. The resultant distributions of mean SNR in the three subject groups are shown in Figure 4. Each box plot in these graphs shows, as horizontal lines from top to bottom, the 95th, 75th, 50th (median), 25th, and 5th percentiles for the different subject groups and mfOPs. The K1 and Ks2 mfOPs (Figs. 4A and 4B, respectively) obtained from the normal subjects tended to have the highest SNRs, the subjects with NPDR tended to have the lowest, and the NoR diabetic group was intermediate. The distributions of mean SNR in the normal and NPDR subjects differed significantly for both K1 and Ks2 mfOPs (Mann-Whitney test, \( P < 0.01 \)).

The mean K1 mfOP SNR was abnormal (i.e., below the 5th percentile of the normal subjects) in 3 (18.8%) of the NoR and 10 (62.5%) of the NPDR eyes (Fig. 4A), and the mean Ks2 mfOP SNR was abnormal in 4 (25%) of the NoR and 10 (62.5%) of the NPDR eyes (Fig. 4B). The K1 and Ks2 mfOPs did not differ significantly in the number of eyes with abnormal mean SNRs (\( \chi^2 \) test, \( P = 0.15 \)). Thus, whereas many of the eyes had abnormal mean K1 and Ks2 mfOP SNRs, the number of abnormal eyes did not depend on the type of mfOP that was measured.

It should also be noted that subject age did not appear to contribute to mfERG findings in the present study, a potential concern because the mean ages of the diabetic subject groups were greater than that of the normal group. There was no decrease of mean mfOP SNR with increasing age in our group of 26 normal subjects. In fact, mean K1 mfOP SNR increased marginally with age (\( r^2 = 0.15, P = 0.052 \)), and Ks2 mfOP SNR was not related to age (\( r^2 = 0.01, P = 0.657 \)).

Analysis of Local mfOP SNR

Our first analysis of local mfOPs was to examine the total number of abnormal K1 and Ks2 mfOPs obtained from the NoR and NPDR groups. As shown in Figure 5A, there were 98 (17.5%) abnormal K1 mfOPs and 99 (17.7%) abnormal Ks2 mfOPs in the NoR subjects and 174 (31.1%) abnormal K1 mfOPs and 205 (36.6%) abnormal Ks2 mfOPs in the subjects with NPDR (Table 1). The total number of abnormal locations detected in each of the diabetic subject groups did not depend on whether K1 or Ks2 mfOPs were measured (\( \chi^2 \) test, \( P > 0.37 \)).

Differences between the K1 and Ks2 mfOPs became apparent when the correspondences between them were examined at each of the 35 retinal locations in each eye. As Table 1 shows, in the NoR eyes K1 and Ks2 mfOPs were both normal in 396 (70.7%) of the retinal locations and were both abnormal in 35 (5.9%). Although the K1 and Ks2 mfOP classifications are statistically related (\( \chi^2 \) test, \( P < 0.001 \)), most of this relationship is due to the large number (369) of locations classified as normal by both mfOPs. If we examine only the 65 + 66 + 33 = 164 abnormal retinal locations, only 33 (20.1%) of them are identified by both K1 and Ks2 as abnormal, and there is disagreement at 65 + 66 = 131 (79.9%) of the locations. Thus, in the NoR eyes, classification of retinal locations as normal or abnormal by measurement of K1 and Ks2 mfOP SNR differed.

The local results obtained in the NPDR eyes were similar to those in the NoR eyes. As shown in Table 1, K1 and Ks2 mfOPs were both normal in 283 (50.5%) of the retinal locations and were both abnormal in 102 (18.2%) of the locations. Again, although the K1 and Ks2 mfOP classifications are statistically related (\( \chi^2 \) test, \( P < 0.001 \)), most of this agreement is due to the large number (283) of locations classified as normal by both mfOPs. If only the 72 + 103 + 102 = 277 abnormal retinal locations are examined, only 102 (36.8%) of them are identified by both K1 and Ks2 as abnormal and there is disagreement at 72 + 103 = 175 (63.2%) of the locations. Thus, in both the NoR and NPDR eyes, classification of retinal loca-
in the preceding list was assigned to that location. It is important to note that each instance of retinopathy covered a much smaller area than the retinal location (shown in Fig. 1B) to which it was assigned.

The percentage of retinal locations with abnormal mfOP SNRs are shown for each retinopathy type in Figure 5B. The Ks2 mfOPs were more often abnormal than the K1 mfOPs in locations containing MA, HE, and edema, and the two mfOPs were equally abnormal at the six SE sites. The largest discrepancy was at locations with edema: 7 of the 10 sites had abnormal Ks2 mfOPs, whereas only three had abnormal K1 mfOPs. Across all 113 locations with retinopathy (labeled “All” in Fig. 5B), Ks2 mfOPs were abnormal at 54 (47.8%), and K1 mfOPs were abnormal at 42 (37.2%). A χ² analysis showed that the Ks2 mfOP abnormalities were associated with the presence of retinopathy in the NPDR eyes (P < 0.006), but the K1 mfOP abnormalities were not (P > 0.11).

**DISCUSSION**

This study establishes the value of the new techniques used to process and measure local mfOPs. Combining the K1i and K2 components to form Ks2 before isolation of the mfOPs by digital filtering increased their SNR. The results show that the retinal cells that contribute to the generation of mfOPs in local retinal areas are affected by diabetes and, to a greater degree, by early NPDR. Although both the Ks2 and K1 mfOPs were frequently abnormal in the diabetic eyes, there were interesting differences between them. The retinal distributions of Ks2 and K1 mfOP abnormalities overlapped but differed, both in diabetic eyes without retinopathy and in eyes with early NPDR. Furthermore, whereas Ks2 mfOP abnormalities were significantly associated with local retinal sites containing signs of NPDR, the K1 mfOP abnormalities were not.

The K1i and K2 mfOP waveforms obtained in the present experiments differed only slightly in amplitude. In the absence of horizontal retinal effects such as lateral inhibition, the K1i and K2 components should be identical except for the influence of noise. Therefore, the observation of small amplitude differences between the K1i and K2 mfOPs suggests that relatively small lateral retinal effects affected the mfOPs under the experimental conditions used in our study. A positive practical consequence of their waveform similarity was, of course, that the K1i and K2 components could be combined to produce a Ks2 with enhanced SNR.

In contrast, the observed differences between the Ks2 and K1 mfOP waveforms and the different retinal distributions of their SNR abnormalities in the diabetic eyes is probably related to the fact that the two mfOPs do not represent the same aspects of signal processing in the retina. Whereas the K1 mfOPs represent the average local responses (first-order effects), the Ks2 mfOPs primarily represent the effects of fast adaptive mechanisms acting on the generators of the local

**TABLE 1. Ks2 and K1 mfOP Agreement in NoR and NPDR Subjects**

<table>
<thead>
<tr>
<th></th>
<th>Ks2 Norm.</th>
<th>Ks2 Abn.</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>NoR</td>
<td>396</td>
<td>66</td>
<td>462</td>
</tr>
<tr>
<td>K1 Norm.</td>
<td>65</td>
<td>33</td>
<td>98</td>
</tr>
<tr>
<td>Total</td>
<td>461</td>
<td>99</td>
<td>560</td>
</tr>
<tr>
<td>NPDR</td>
<td>283</td>
<td>103</td>
<td>386</td>
</tr>
<tr>
<td>K1 Norm.</td>
<td>72</td>
<td>102</td>
<td>174</td>
</tr>
<tr>
<td>Total</td>
<td>355</td>
<td>205</td>
<td>560</td>
</tr>
</tbody>
</table>

Norm., normal mfOP SNR; Abn., abnormal mfOP SNR.
responses (second-order effects).27,20,42 Therefore, the observation that the Ks2 mfOP abnormalities were more associated with retinal sites of NPDR than were K1 mfOP abnormalities suggests that adaptation is more likely to be abnormal at early retinopathy sites than at locations in the same eye without retinopathy. In the future, it will be important in longitudinal studies to determine the temporal relationship between the development of local mfOP abnormalities and the appearance of retinopathy. Assuming that mfOP abnormalities precede development of retinopathy signs, which seems reasonable given the presence of abnormalities in locations and eyes without NPDR, it will be important to determine whether either local Ks2 or K1 mfOP abnormalities more often precede the appearance of NPDR. The two types of mfOPs may differ in their power to predict the appearance of retinopathy.

The local mfOP abnormalities that we observed do not necessarily indicate that only inner retinal neurons (amacrine cells) are dysfunctional. Dysfunction of neurons such as the photoreceptors or bipolar cells in more distal retina could contribute to the reduction of mfOP amplitude as a consequence of their additive effects. Early clinical (fundoscopic) evidence of diabetic retinopathy is largely the result of defects of retinal capillaries within the inner nuclear layer.43,44 Therefore, localized retinal blood flow abnormalities and ischemia could affect both the bipolar and amacrine cell bodies, which are in close proximity.

In the present study, the SNR (amplitude) of local mfOPs was examined. The SNR measure was chosen primarily because of the technical challenges related to obtaining reliable measures of the implicit times of the multiple small local mfOP peaks, especially when their amplitudes are severely reduced by disease. However, several studies indicate that retinal dysfunction related to diabetes and early NPDR, as reflected in “standard” local first-order mfERG kernels, is more apparent in measurements of implicit time rather than measurements of amplitude.14,18 Therefore, whereas mfOP SNR was clearly affected by diabetes and NPDR, it is possible that a method to reliably measure local mfOP implicit times could also provide a sensitive metric of abnormality.

In conclusion, local mfOP abnormalities exist in diabetic eyes without retinopathy and in eyes with early NPDR. The enhancement of mfOP SNR obtained by combining the induced first- and second-order components made it possible to establish relationships between the Ks2 mfOP abnormalities and retinal sites of NPDR. Methods similar to those used in the present study might also be of value in the study of inner retinal (ganglion cell) diseases such as glaucoma. In human glaucoma an oscillatory component is lost in the temporal retina and, in monkey, high-frequency and oscillatory components of the mfERGs are reduced by experimental glaucoma, tetrodotoxin (TTX) treatment, and induced nerve fiber layer defects.31,44–47 In the future, it will be interesting to compare the local mfOPs with first-order mfERG components known to be generated in other (earlier) retinal layers so that sites of diabetic dysfunction can be localized in retinal depth as well as lateral extent.

Acknowledgments

The authors thank Jenny Myung for assistance with data processing and Ken Hue for technical assistance.

References