K⁺-evoked Müller cell depolarization generates *b*-wave of electroretinogram in toad retina

(K⁺-selective microelectrode/barium/potassium conductance)

RONG WEN* AND BURKS OAKLEY II*†‡

*Neural and Behavioral Biology Program and †Department of Electrical and Computer Engineering, University of Illinois at Urbana-Champaign, 1406 West Green Street, Urbana, IL 61801-2991

Communicated by John E. Dowling, January 2, 1990

ABSTRACT We tested the hypothesis that a light-evoked increase in [K⁺]_o produces a depolarization of the Müller cell membrane, which in turn generates the electroretinogram *b*-wave current. Using *Bufo marinus* isolated retinas and K⁺-selective microelectrodes, we recorded two distinct light-evoked increases in extracellular K⁺ concentration: one in the inner plexiform layer and the other near the outer plexiform layer; the “distal” K⁺ increase was found over only 10-μm depth and had a maximum amplitude of 0.3 mM. We also recorded the electroretinogram and the light-evoked responses of rods and Müller cells. After correction for the response time of the K⁺-selective microelectrode, the waveforms of all three of these responses were almost exactly as predicted by an earlier computer simulation of the K⁺/Müller cell hypothesis of the *b*-wave by Newman and Odette [Newman, E. A. & Odette, L. L. (1984) *J. Neurophysiol.* 51, 164–182]. The distal K⁺ increase and the *b*-wave varied in a similar manner as a function of stimulus irradiance. Superfusion with 0.2 mM Ba²⁺ attenuated both the Müller cell depolarization and the *b*-wave by ≈65% but had no significant effect upon the distal K⁺ increase. Because Ba²⁺ reduces K⁺ conductance of Müller cells, these results are very strong support of the K⁺/Müller cell hypothesis of the origin of the electroretinogram *b*-wave; the light-evoked increase in extracellular potassium concentration still is present during superfusion with Ba²⁺, but the K⁺-evoked Müller cell depolarization and the *b*-wave are decreased in amplitude because Müller cell K⁺ conductance is reduced.

The *b*-wave of the electroretinogram (ERG) is a corneal-positive field potential of retinal origin that is of clinical importance in the assessment of retinal function. Based on a current-source-density analysis, the geometry of the radially oriented Müller (glial) cells, and the dominant K⁺ conductance, *g*_K, of glial cells, Faber (1) and Miller and Dowling (2) hypothesized that the *b*-wave originated in the Müller cell response to a light-evoked increase in extracellular potassium concentration, [K⁺]_o, near the outer plexiform layer (OPL). Although there are several variations of the original “K⁺/Müller cell” hypothesis of the *b*-wave (3, 4), central to all of them is the notion that a light-evoked increase in [K⁺]_o near the OPL produces a localized depolarization of the Müller cell membrane, which acts as the dominant current sink and leads to the flow of *b*-wave currents through the extracellular space from current sources located on other parts of the Müller cell membrane. Because up to 95% of the membrane conductance in Müller cells is localized to the endfoot of the cell facing the vitreous humor (5, 6), the membrane at the endfoot acts as the primary current source, and a current pathway is established that leads to extracellular current flow

from the vitreal border of the retina to the OPL, resulting in the vitreal-positive *b*-wave (3).

Miller and Dowling (2) recorded from Müller cells and found that the light-evoked responses of Müller cells had many of the correct properties to be the cellular source of the *b*-wave, including latency, amplitude–intensity function, and waveform. Measurements of light-evoked changes in [K⁺]_o are, in general, consistent with the K⁺/Müller cell hypothesis of the *b*-wave (4, 7–11). However, several observations have challenged the validity of the K⁺/Müller cell hypothesis. The light-evoked increase in [K⁺]_o near the OPL appears to be too small and too slow to produce the *b*-wave (12). Although this “distal” K⁺ increase is augmented by conditions that increase the *b*-wave and is attenuated by conditions that decrease the *b*-wave (8–10), such results do not rule out the possibility that the distal K⁺ increase is due to the passive release of K⁺ by the Müller cells, as they depolarize in response to another transmitter. Recent experiments have found that Müller cells have both an electrogenic glutamate-uptake system (13, 14) and γ-aminobutyric acid (GABA) receptors (15), and it has been suggested that light-evoked changes in glutamate or GABA release near the OPL could depolarize Müller cells and thereby could contribute to *b*-wave currents (13–15). Finally, experiments designed to block *g*_K in Müller cells have produced results that are difficult to reconcile with the K⁺/Müller cell hypothesis of the *b*-wave. In mammalian retinas, the addition of Ba²⁺, which blocks resting *g*_K in amphibian Müller cells (16, 17), does not abolish the *b*-wave (18, 19). Nevertheless, low concentrations (0.2–1.0 mM) of Ba²⁺ significantly attenuate the *b*-wave in frog (20) and in tiger salamander (21).

In light of these challenges to the K⁺/Müller cell hypothesis of *b*-wave generation, we now have used K⁺-selective microelectrodes with very fine tips and rapid response times to perform several additional tests of this hypothesis in the toad retina. We have recorded light-evoked changes in [K⁺]_o at various retinal depths, as well as light-evoked changes in Müller cell-membrane voltage and the ERG. Our data provide strong support for the K⁺/Müller cell hypothesis.

MATERIALS AND METHODS

Preparation and Solutions. All experiments were performed on the isolated retina preparation of the toad *Bufo marinus*, as described in detail (22). Briefly, a dark-adapted toad was rapidly double-pithed, an eye was enucleated, and a small piece of retina was separated from the pigment epithelium. The isolated retina was pinned receptor-side up

Abbreviations: [K⁺]_o, extracellular potassium concentration; *V*_K, voltage between two barrels of potassium-selective microelectrode; *V*_m, membrane voltage; *g*_K, membrane conductance for potassium; ERG, electroretinogram; LERG, local ERG recorded by reference electrode; OPL, outer plexiform layer; GABA, γ-aminobutyric acid. ‡To whom reprint requests should be addressed.

in a small chamber and was superfused with various solutions (22°–24°C). The control solution contained 87.7 mM NaCl, 22.3 mM NaHCO₃, 2.4 mM KCl, 0.9 mM CaCl₂, 1.3 mM MgCl₂, 5.6 mM glucose, and 0.003 mM phenol red. This solution was equilibrated with 98% O₂/2% CO₂ and had a pH of 7.80 ± 0.02. Test solutions were made by adding up to 0.8 mM BaCl₂ to the control solution.

K⁺-Selective Microelectrodes. Double-barrel, K⁺-selective microelectrodes were used to measure [K⁺]_o; one barrel was an ion-selective electrode of the liquid membrane type and the other barrel was a reference electrode. These electrodes were made from thick-septum theta tubing (W-P Instruments, New Haven, CT), modified to contain a glass fiber in each barrel. The electrodes were pulled on a horizontal puller (P-77B, Sutter Instruments, San Rafael, CA) and had an extremely rapid taper. After pulling, the ion-selective barrel was silanized with trimethylsilyldimethylamine vapor (23). The silanized electrodes were beveled on a dry alumina plate (0.05 μm, Sutter Instruments) for 1–2 min at a 30° angle to the beveling surface, using an axial illumination technique to visualize the electrode tip (24). The beveled tips were 0.5–1.5 μm across the beveled surface, and the electrodes were <4 μm in diameter 20 μm back from the tip. The tip of the ion-selective barrel then was filled with K⁺-ion exchanger (#477317, Corning), and the rest of the barrel was backfilled with 150 mM KCl, so that the ion-exchanger column was ≈2 mm long. The reference barrel was filled with a solution containing 110 mM NaCl and 2.4 mM KCl.

Each microelectrode voltage was measured with respect to a grounded Ag/AgCl electrode, which was connected to the bathing solution by a KCl-agar bridge, using a differential amplifier with an input resistance of 1 PΩ (Axoprobe-1, Axon Instruments, Foster City, CA). This amplifier had self- and cross-capacitance compensation adjustment (9). With the electrode tip in the bathing solution, the capacitance compensation for the K⁺-selective barrel was adjusted to provide minimum rise time to a voltage step, and then the cross-capacitance compensation was adjusted to eliminate the voltage step from the differential voltage between the two barrels. This differential voltage (K⁺-barrel positive), which was termed V_K , was a logarithmic measure of [K⁺]_o. The electrodes were calibrated before and after an experiment (22); they responded to changes in [K⁺]_o with a logarithmic slope of 56–58 mV per decade and had a 70–80:1 selectivity for K⁺ over Na⁺. The electrode selectivity for K⁺ over Ba²⁺ was ≈100:1, and the addition of 0.2 mM Ba²⁺ had negligible effect upon the electrode response to K⁺.

The response times of the K⁺-selective microelectrodes were studied in detail. The response to a change in [K⁺]_o was measured by moving the electrode tip rapidly (16 μm in ≈25 ms) between two streams having different [K⁺]_o values; the electrodes responded to an increase in [K⁺]_o from 1.0 to 3.0 mM with a latency of ≈10 ms from the time the electrode movement began, and 50% of the total response was registered in 50–65 ms. The form of this step response was approximately exponential with a time constant of 70–95 ms (eight electrodes). The electrical time constant of the K⁺-selective barrel was <2 ms (with optimal self- and cross-capacitance compensation), and the resistance of this barrel was in the range of 4–8 GΩ. Although the cross-capacitance compensation reduced the electrical time constant of the K⁺-selective barrel, it did not affect the chemical response time of this barrel.

Other Techniques. With the use of a piezoelectric advancer (Burleigh Instruments, Fishers, NY), microelectrodes were advanced into the retina from above at an angle of 30° to the receptor surface; the step size in the direction of advancement was 2 μm. Single-barrel micropipette electrodes were used to record intracellularly from rods and from Müller cells. Müller cells were penetrated near the OPL and had

resting membrane voltages of approximately –85 mV. A microelectrode with a large beveled tip (≈3 μm) was advanced through the retina to the vitreal surface, and the ERG was recorded between this microelectrode and the grounded reference electrode in the bathing solution. All electrode voltages were recorded and processed as described (22). The retina was stimulated (full-field) by flashes of 500-nm light (22).

RESULTS

The depth profile of the light-evoked change in [K⁺]_o was characterized as a K⁺-selective microelectrode was advanced into the isolated retina from the receptor surface. As shown on the left side of Fig. 1, a 100-ms flash of light evoked three distinct changes in [K⁺]_o: a K⁺ decrease, which peaked 95 μm below the receptor surface (4, 7, 22), a distal K⁺ increase, which peaked 112 μm below the receptor surface (4, 7–10), and a proximal K⁺ increase, which peaked 165 μm below the receptor surface (10, 25). While the K⁺ decrease and the proximal K⁺ increase could be recorded over a large range of depths, the distal K⁺ increase was localized within only 10 μm, and its amplitude varied significantly with only a 1- to 2-μm electrode advancement (note that the waveforms in Fig. 1 are from very unevenly spaced depths).

The distal K⁺ increase was seen to be a different component from the proximal K⁺ increase because at depths just proximal to the amplitude maximum of the distal K⁺ increase (114–115 μm in Fig. 1), the light-evoked increase in V_K was smaller and had a slower rise time than at the amplitude maximum. This result eliminates the possibility that the distal K⁺ increase was due to diffusion of K⁺ from the proximal source (10). In 23 retinas, the amplitude maximum for the

FIG. 1. Depth profile of the light-evoked change in V_K and the LERG. Each pair of waveforms (V_K , LERG) was recorded simultaneously as the tip of a K⁺-selective microelectrode was advanced into the retina from the receptor surface; the depth of penetration into the retina (measured normal to the receptor surface) is indicated to the left of each pair of waveforms. These responses were evoked by 100-ms flashes of light (indicated by the light monitor waveform, LM); each flash produced 60 quanta absorbed per rod. In this and in all other figures, the baseline level of [K⁺]_o in darkness was 2.5 mM (22), and it did not vary by >±0.1 mM throughout the retina. At 2.5 mM [K⁺]_o, a 1.0-mV increase in V_K corresponded to an increase in [K⁺]_o of 0.17 mM [based upon external Na⁺ concentration = 110 mM, an electrode slope of 57 mV per decade, and a selectivity for K⁺ over Na⁺ of 70:1 (22)].

distal K^+ increase was found at depths ranging from 100 to 125 μm . In some cases, however, the electrode might have dimpled the retinal surface before actually penetrating, so the amount of electrode advancement would have overestimated the actual penetration into the retina. In all these experiments, the distal K^+ increase always was found over a range of depths of $<12 \mu\text{m}$. At the amplitude maximum for the distal K^+ increase, the light-evoked increase in $[K^+]_o$ was followed in time by a slow decrease in $[K^+]_o$, which presumably was due to diffusion of K^+ away from the recording site towards the depth where the light-evoked decrease in $[K^+]_o$ was maximal (7, 22). In several experiments, the light-evoked increase in V_K at the amplitude maximum of the distal K^+ increase was 1.8 mV, corresponding to an increase in $[K^+]_o$ of 0.31 mM, from 2.50 mM to 2.81 mM. The depth profile of the distal K^+ increase in the toad retina is similar to that of the response that has been implicated in the generation of the b -wave in other species (4, 7–10).

The LERG (local ERG recorded by the reference barrel of the K^+ -selective microelectrode) also varied as a function of retinal depth, as shown on the right side of Fig. 1. The LERG contained a positive b -wave in the proximal retina but only a negative potential in the distal retina. In all experiments, the positive b -wave in the LERG was only recorded proximal to the depth where the distal K^+ increase was maximal.

According to the K^+ /Müller cell hypothesis of the b -wave, the distal K^+ increase produces the Müller cell depolarization, which in turn generates the b -wave current. We recorded intracellularly from Müller cells, and we compared the Müller cell depolarization to both the distal K^+ increase and the b -wave. To perform a rigorous test of this hypothesis, we varied the irradiance of a 1.0-s flash of light, so that the flashes produced between 0.1 and 6000 quanta absorbed per rod. In support of the hypothesis, the distal K^+ increase and the b -wave both varied in amplitude in a similar manner over the entire range of irradiance tested, as shown in Fig. 2. By comparison, the proximal K^+ increase varied in a much different manner, with its half-maximal amplitude occurring at about a 10-fold lower stimulus irradiance than that of the b -wave. The peak Müller cell depolarization varied with irradiance in a way that was intermediate between that of the distal and proximal K^+ increases, presumably because it was caused, in part, by both of these K^+ increases (3). Similar relationships between the distal K^+ increase and the b -wave

FIG. 2. Effects of stimulus irradiance upon the distal K^+ increase, the Müller cell depolarization, the ERG b -wave, and the proximal K^+ increase. These responses were evoked by 1.0-s flashes of light (LM, light monitor waveform), which produced the number of isomerized rhodopsins (Rh^*) per rod per flash indicated to the left of each row of responses. The Müller cell membrane voltage (V_m) and the ERG were recorded simultaneously from one retina, whereas each type of V_K waveform was recorded from a different retina. All waveforms are averages of two or three similar responses. The light-evoked changes in Müller cell V_m were superimposed upon the resting membrane voltage of -84.0 mV .

(4, 7), the Müller cell depolarization and the b -wave (2), and the proximal K^+ increase and the b -wave (25) were observed previously in a variety of species.

We next examined in greater detail the waveforms of the distal K^+ increase, the Müller cell depolarization, and the b -wave. A Müller cell response and an ERG (recorded simultaneously) are shown in Fig. 3A. The initial parts of these waveforms were normalized to have the same peak amplitudes, and the normalized waveforms are shown on an expanded time scale in Fig. 3B. The b -wave and the Müller cell depolarization had comparable latencies, but the b -wave reached its peak earlier and decayed more rapidly than the Müller cell depolarization. The temporal relationship between these waveforms is similar to that predicted by Newman and Odette (3) in a computer simulation of the K^+ /Müller cell hypothesis.

Newman and Odette's simulation also predicted that the waveforms of the b -wave and the distal K^+ increase would be identical in time course (see figures 3 and 4 of ref. 3). We therefore compared the waveforms of the distal K^+ increase and the b -wave in Fig. 3C (each of these waveforms was recorded from the same retina within a 15-min period). The initial part of each of these waveforms is shown on an expanded time scale in Fig. 3D. The b -wave had a much shorter latency and reached its peak much earlier than the distal K^+ increase, and both of these results would appear inconsistent with the K^+ /Müller cell hypothesis. However, the waveform of the light-evoked change in V_K was not a true measure of the light-evoked increase in $[K^+]_o$ because the V_K waveform was slowed by the chemical response time of the

FIG. 3. Comparison of the waveforms of the light-evoked change in Müller cell membrane voltage, the ERG b -wave, and the distal K^+ increase. All illustrated responses were evoked by 1.0-s flashes of light (LM, light monitor waveform), each of which produced 600 quanta absorbed per rod. All waveforms are averages of two or three similar responses. (A) The light-evoked change in Müller cell membrane voltage and the ERG were recorded simultaneously. The Müller cell responses were recorded from the same cell as in Fig. 2. (B) The waveforms from part A were normalized to have the same peak amplitude, and the initial portion of each response is shown on an expanded time scale. (C) The light-evoked change in V_K and the ERG were recorded sequentially from the same retina within a period of 20 min. The V_K waveform was recorded at the amplitude maximum for the distal K^+ increase. (D) The initial portions of the responses from C are displayed on an expanded time scale. The ERG waveform is shown both with high-frequency components present (-3 -decibel frequency of 160 Hz, as in A and C of this figure) and also with high-frequency components absent (the waveform from C was filtered by a single-pole low-pass filter having a -3 -decibel frequency of 2.1 Hz; equivalent to a 75-ms time constant). The low-pass-filtered ERG and the distal V_K waveform were normalized to have the same peak amplitudes and are displayed superimposed.

K⁺-selective microelectrode, which was approximately first-order with a time constant of ≈ 70 –95 ms (see *Materials and Methods*). Therefore, we filtered the ERG waveform with a single-pole low-pass filter having a 75-ms time constant, corresponding to a -3 -decibel frequency of 2.1 Hz. Although the choice of this time constant was arbitrary, using other values between 70 and 95 ms (the range of electrode time constants we observed) did not noticeably affect the waveform of the filtered ERG on the time scale shown in Fig. 3C (data not shown). The filtered ERG had both a longer latency and a later time-to-peak than did the original ERG. The filtered ERG and the distal V_K waveform were normalized to have the same peak amplitudes, and when these waveforms were superimposed, they were very similar in time course, as shown in Fig. 3D.

Based upon our measurements of the response times of the K⁺-selective microelectrodes, we know that the V_K waveform must have been slower and smaller than the actual change in $[K^+]_o$, just as the b -wave of the low-pass-filtered ERG was slower and smaller than the actual b -wave. Consequently, from the analysis in Fig. 3D, we have strong reason to believe that the actual distal K⁺ increase had the same time course (latency, rise time, time-to-peak) as the b -wave. Our results shown in Fig. 3, therefore, are consistent with the simulation of Newman and Odette (3) and are strong support of the K⁺/Müller cell hypothesis.

Superfusion with 0.2 mM Ba²⁺ decreases the amplitude of the b -wave in amphibian retina (20). Because Ba²⁺ blocks g_K in amphibian Müller cells (17), we hypothesized that Ba²⁺ produces its effects upon the b -wave by decreasing the Müller cell depolarization evoked by the distal K⁺ increase. Representative effects of 0.2 mM Ba²⁺ are illustrated in Fig. 4. Ba²⁺ increased the amplitude and duration of the rod photoresponse and caused oscillations of membrane voltage during the recovery phase, as reported by others (26, 27). Ba²⁺ had no significant effect upon the resting level of $[K^+]_o$ in the isolated retina (22). At 0.2 mM, Ba²⁺ produced $<10\%$ decline in the amplitude of the distal K⁺ increase, while it

more than doubled the amplitude of the K⁺ decrease throughout the distal retina. In contrast to its lack of effect on the distal K⁺ increase, 0.2 mM Ba²⁺ depolarized Müller cells by 4–6 mV and decreased both the amplitude of the light-evoked depolarization of the Müller cell and the b -wave of the ERG by $\approx 65\%$ (the b -wave amplitude was measured from the pre-stimulus baseline to the peak). In three other experiments, 0.2 mM Ba²⁺ decreased both the amplitude of the light-evoked depolarization of the Müller cell and the b -wave by similar amounts, ranging from 60 to 75%. During superfusion with 0.2 mM Ba²⁺, the proximal K⁺ increase was smaller and had a much longer time-to-peak (data not illustrated). These results are consistent with the hypothesis that Ba²⁺ produces its effects upon the b -wave by decreasing the Müller cell depolarization evoked by the distal K⁺ increase. All of the effects of Ba²⁺ shown in Fig. 4 were reversible with periods of Ba²⁺ superfusion of <15 min.

In other experiments (data not shown), superfusion of the retina with 0.1 mM 2-amino-4-phosphonobutyrate, a glutamate analogue that blocks light-evoked responses in ON-bipolar cells (28), rapidly abolished the light-evoked K⁺ increases in both the distal and proximal retina and the light-evoked Müller cell depolarization, as well as the b -wave. When 10-s flashes of light were used to stimulate the retina, there was an off-K⁺ increase in the proximal retina, an off-depolarization of the Müller cell membrane, and a positive off-response in the ERG; 0.1 mM amino-4-phosphonobutyrate was without significant effect upon these off-responses. Similar effects of amino-4-phosphonobutyrate have been observed by others (29, 30). Even though the proximal K⁺ increase likely produces the off-response in the ERG, it appears only to make a small contribution to the rising portion of the b -wave (3).

DISCUSSION

The results of the present experiments provide strong support of the K⁺/Müller cell hypothesis of the origin of the ERG b -wave. We characterized the light-evoked increase in $[K^+]_o$ at the retinal depth corresponding to the major current sink of the b -wave, and we compared its properties to the light-evoked Müller cell depolarization and the b -wave. In the toad retina, the OPL is a very narrow band of processes ≈ 100 μ m from the tips of the rods (23). The distal K⁺ was found in various experiments to be of maximum amplitude when the electrode was advanced 100–125 μ m below the receptor surface; because the electrode may have dimpled the retina during penetration, we cannot distinguish this depth from the OPL. The limited depth over which the distal K⁺ increase can be recorded may indicate that it is generated within the OPL, as found by Karwowski *et al.* (10). Because the inner plexiform layer is 150–190 μ m from the tips of the rods (23), our measurements of the proximal K⁺ increase are consistent with it being generated within the inner plexiform layer.

The distal K⁺ increase and the b -wave both vary with stimulus irradiance in a similar manner. After correction for the low-pass filtering characteristics of the K⁺-selective microelectrodes, the distal K⁺ increase appears to have the same time course as the b -wave, as predicted by a simulation of the K⁺/Müller cell hypothesis (3). We have found that the distal K⁺ increase is as large as 0.31 mM, even when measured with K⁺-selective microelectrodes that would have attenuated the maximum amplitude due to significant limitations in their frequency response, and this result again is consistent with the simulation that required $[K^+]_o$ to increase by ≈ 0.45 mM (3).

There are several factors that still may act to underestimate the actual amplitude of the distal K⁺ increase. A dead space of radius 3–5 μ m has been estimated to exist at the electrode tip, through which K⁺ must diffuse before it is sensed (11).

FIG. 4. Effects of 0.2 mM Ba²⁺ upon the light-evoked changes in rod and Müller cell membrane voltage, the distal K⁺ increase, and the b -wave of the ERG. All illustrated responses were evoked by 100-ms flashes of light (LM, light monitor waveform), each of which produced 60 quanta absorbed per rod. All waveforms except the rod responses are averages of two or three similar responses. Each type of response was recorded from a different retina. All waveforms in the right column were recorded under steady-state conditions after 4–7 min in 0.2 mM Ba²⁺ (similar responses were seen with superfusion of 0.2 mM Ba²⁺ up to 15 min). In the dark, the rod membrane voltage (V_m) was -32.3 mV and the Müller cell V_m was -87.2 mV. Superfusion with 0.2 mM Ba²⁺ was without significant effect upon the rod V_m in darkness, whereas it depolarized the Müller cell by 4.4 mV.

The proximal K^+ increase occurs over a range of retinal depths $>30\ \mu\text{m}$, so this dead space would have had only a minimal impact on the measured response, as K^+ could diffuse to the electrode tip from all directions. Because the distal K^+ increase is found only over a range of retinal depths of $\approx 10\ \mu\text{m}$, the sources of K^+ proximal and distal to the electrode tip could be within the dead space when the electrode tip is in the OPL. Therefore, K^+ would have to diffuse to the electrode tip from lateral regions. In addition, diffusion of K^+ through an increased extracellular space along the electrode track might be expected to have a greater effect in the distal retina than in the proximal retina because in the distal retina, the gradient of the light-evoked change in $[K^+]_o$ along the electrode track is much larger than in the proximal retina.

The original simulation of the K^+ /Müller cell hypothesis (3) showed that if the distal K^+ increase was assumed to be faster and larger than the response that had been measured by others at that time, it could generate a pattern of b -wave currents consistent with current-source-density measurements (31). In this simulation, the distal K^+ increase was required to have essentially the same time course as the b -wave. A revised simulation of this hypothesis (12) used actual measurements of light-evoked changes in retinal $[K^+]_o$ and the b -wave, in which the distal K^+ increase peaked 0.75–1.0 s from light onset, whereas the b -wave peaked at ≈ 0.3 s (10). It is not surprising, therefore, that this revised simulation provided results that were inconsistent with the b -wave current-source-density measurements. This revised simulation challenged the validity of the K^+ /Müller cell hypothesis of b -wave generation. However, we now have shown that the distal K^+ increase in toad is probably as large and as fast as was assumed in the original simulation (3), and this ionic response now seems to have all necessary properties to produce the Müller cell depolarization that generates the b -wave.

The distal K^+ increase that we measure in toad retina is very similar in waveform to the distal K^+ increase measured by Kline *et al.* (4, 7) in skate (*Raja erinacea* and *Raja ocellata*) retina. In many retinas, the distal K^+ increase is seen only when the electrode is advanced in small steps (4, 9), and this fact is consistent with our observations that the distal K^+ increase is found over a range of depths $<12\ \mu\text{m}$.

Newman (17) found that 0.05–0.5 mM Ba^{2+} blocks g_K in amphibian Müller cells in a dose-dependent manner. Because 0.2 mM Ba^{2+} decreases both the Müller cell depolarization and the b -wave by $\approx 65\%$ but has little effect on the distal K^+ increase, we believe that these results are strong support of the K^+ /Müller cell hypothesis of the b -wave. Electrogenic glutamate uptake by Müller cells is not affected by 6.0 mM Ba^{2+} (13, 14), and thus our results are inconsistent with a primary role for electrogenic glutamate uptake in the generation of the b -wave. However, we cannot exclude the possibility that mechanisms involving glutamate or GABA contribute to the fraction of b -wave current that is not blocked by 0.2 mM Ba^{2+} .

In mammalian retinas, Ba^{2+} may actually increase the b -wave amplitude when injected intravitreally (18, 19). The reason for this effect is unknown, but it is possible that any effect upon Müller cell g_K could be offset by a Ba^{2+} -induced enhancement of the distal K^+ increase. We look forward to the results of experiments similar to ours performed upon mammalian retinas.

We thank Drs. C. J. Karwoski, E. A. Newman, and R. H. Steinberg for helpful discussion. We gratefully acknowledge the expert technical assistance of Yiwen Li and J. L. Tucker. This work was supported by Grant EY04364 from the National Eye Institute, U.S. Public Health Service.

1. Faber, D. S. (1969) Dissertation (State University of New York, Buffalo, NY).
2. Miller, R. F. & Dowling, J. E. (1970) *J. Neurophysiol.* **33**, 323–341.
3. Newman, E. A. & Odette, L. L. (1984) *J. Neurophysiol.* **51**, 164–182.
4. Kline, R. P., Ripps, H. & Dowling, J. E. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5727–5731.
5. Newman, E. A. (1985) *J. Neurosci.* **5**, 2225–2239.
6. Brew, H., Gray, P. T. A., Mobbs, P. & Attwell, D. (1986) *Nature (London)* **324**, 466–468.
7. Kline, R. P., Ripps, H. & Dowling, J. E. (1985) *Neuroscience* **14**, 225–235.
8. Dick, E. & Miller, R. F. (1978) *Brain Res.* **154**, 388–394.
9. Dick, E. & Miller, R. F. (1985) *J. Gen. Physiol.* **85**, 885–909.
10. Karwoski, C. J., Newman, E. A., Shimazaki, H. & Proenza, L. M. (1985) *J. Gen. Physiol.* **86**, 189–213.
11. Newman, E. A. (1988) *Prog. Retinal Res.* **8**, 153–171.
12. Odette, L. L. & Newman, E. A. (1988) *Glia* **1**, 198–210.
13. Henshel, D. & Miller, R. F. (1986) *Soc. Neurosci. Abstr.* **12**, 169.
14. Brew, H. & Attwell, D. (1987) *Nature (London)* **327**, 707–709.
15. Malchow, R. P., Quian, H. & Ripps, H. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4326–4330.
16. Karwoski, C. J., Lu, H.-K. & Newman, E. A. (1989) *Science* **244**, 578–580.
17. Newman, E. A. (1989) *Brain Res.* **498**, 4326–4330.
18. Hu, K. G. & Marmor, M. F. (1984) *Vision Res.* **24**, 1153–1156.
19. Frishman, L. J. & Steinberg, R. H. (1989) *J. Neurophysiol.* **61**, 1221–1232.
20. Griff, E. R., Shirao, Y. & Steinberg, R. H. (1985) *J. Gen. Physiol.* **86**, 853–876.
21. Coleman, P. A., Carras, P. L. & Miller, R. F. (1987) *Neurosci. Lett.* **80**, 61–65.
22. Oakley, B., II (1983) *Vision Res.* **23**, 1325–1337.
23. Oakley, B., II, & Wen, R. (1989) *J. Physiol. (London)* **419**, 353–378.
24. Fox, J. A. (1985) *J. Neurosci. Methods* **15**, 239–241.
25. Oakley, B., II (1975) Dissertation (Univ. of Michigan, Ann Arbor, MI).
26. Brown, K. T. & Flaming, D. G. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1587–1590.
27. Fain, G. L., Gerschenfeld, H. M. & Quandt, F. N. (1980) *J. Physiol. (London)* **303**, 495–513.
28. Slaughter, M. M. & Miller, R. F. (1981) *Science* **211**, 182–185.
29. Witkovsky, P., Stone, S. & Ripps, H. (1985) *Brain Res.* **328**, 111–120.
30. Stockton, R. A. & Slaughter, M. M. (1989) *J. Gen. Physiol.* **93**, 101–122.
31. Newman, E. A. (1980) *J. Neurophysiol.* **43**, 1355–1366.