Generation of b-wave currents in the skate retina

[electroretinogram/Müller (glial) cell/potassium kinetics/ion-selective electrodes]

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ABSTRACT Potassium kinetics within the skate retina were monitored extracellularly with K⁺-selective electrodes. Two sources of K⁺ efflux were detected in response to photic stimulation: one in the distal retina in the region of the outer plexiform layer, and the other at a more proximal location near the border between the inner nuclear and inner plexiform layers. The magnitude of the K⁺ efflux at these retinal depths was affected differently by spot and full-field illumination, suggesting that the two sources originate from different classes of neuron. There is evidence that both sources are associated with current sinks provided by the Müller cells, thereby establishing radial current paths along the lengths of these elements. We have proposed a model in which asymmetries in the magnitudes of these currents give rise to the b-wave of the electroretinogram. Extracellular field potentials recorded differentially at various retinal depths, and in response to changes in stimulus configuration, were consistent with predictions of the model.

The electroretinogram (ERG) is a complex extracellular field potential that has been used extensively for the study of retinal function. The most prominent component of the normal ERG response is the b-wave, a cornea-positive potential that is known to arise within the neural (i.e., postreceptoral) layers of the retina.

Faber (1) was first to suggest that the b-wave reflects current flow associated with the Müller cell, a glial element that extends axially through much of the retinal depth. Intracellular recordings from Müller cells of the mudpuppy retina (2) showed a number of similarities between the Müller-cell potential and the extracellularly recorded b-wave. For example, the latency and rise time of the Müller-cell response and the b-wave were nearly identical over a wide range of stimulus intensities. In addition, the waveforms of the b-wave and Müller-cell potentials were similar when elicited in the dark-adapted retina with dim photic stimuli. Because glial cells are known to behave like potassium electrodes, Miller and Dowling (2) suggested that the stimuli may induce a neuronal release of K⁺ in the distal retina which, in turn, produces a depolarization of the Müller cell; the resulting transretinal current flow is recorded as the b-wave of the ERG.

Subsequent measurements with K⁺-sensitive electrodes demonstrated that changes in extracellular potassium concentrations, [K⁺]_o, do occur within the retina in response to light stimulation. However, the initial reports (3-5) were not consistent with the predictions of Miller and Dowling. A light-induced increase in [K⁺]_o was observed, but only in the proximal retina in the neighborhood of the amacrine cells; this location is too proximal to account for the generation of the b-wave currents. Furthermore, Karwoski and Proenza (4, 5) showed that a change in stimulus size exerted a different effect on the release of K⁺ compared with the change produced in b-wave amplitude. Spot illumination, for example, caused a greater

accumulation of K⁺ in the proximal retina than did full-field illumination, whereas b-wave amplitude was less with spot stimuli than with full-field stimulation. In the distal retina, on the other hand, only a decrease in [K⁺]_o was found. This change, recorded in the region of the photoreceptors, correlated well with the slow, pigment-epithelial response that forms the c-wave of the ERG (3).

More recently, however, Dick and Miller (6) detected a second light-activated source of K⁺ arising in distal regions of the mudpuppy retina. The distal source could be enhanced by certain pharmacological manipulations that also enhanced the b-wave. Moreover, the proximal accumulation of K⁺ was decreased under those conditions that enhanced the distal K⁺ efflux. We report here that there is a prominent source of K⁺ in the distal retina of the skate that can be distinguished from the proximal source on the basis of its kinetics and its response to changes in stimulus configuration. The results of intraretinal potential measurements under the various stimulus conditions suggest a model for the generation of the b-wave based upon the interaction of current flows set up around the Müller cell as a consequence of the two K⁺ sources.

MATERIALS AND METHODS

Small pieces of eyecup (≈1 cm square), excised under dim red light, from the tapetal region of a dark-adapted skate (either *Raja erinacea* or *R. ocellata*), were prepared and maintained as described (7). The tissue was placed on a Ringer's solution-soaked cotton pad in contact with a chlorided silver plate that served as a grounded reference electrode. In most experiments the eyecup was constantly superfused with elasmobranch Ringer's solution (240 mM NaCl/6 mM KCl/20 mM NaHCO₃/1 mM MgCl₂/4 mM CaCl₂/1 mM Na₂HPO₄/0.2 mM NaH₂PO₄/360 mM urea/10 mM glucose). The solution was constantly oxygenated; its pH was monitored and kept between 7.6 and 7.8. A small wick collar was used to lead excess fluid away from the ground electrode at the bottom of the eyecup.

[K⁺]_o was measured locally at the tip of a double-barrel micropipette fashioned from theta tubing (8). One barrel was K⁺-selective (9), but it responded also to fluctuations in the extracellular local electrical potential. The second barrel was a Ringer's solution-filled reference barrel that responded only to the local potential fluctuations (the local ERG); recording differentially across the two barrels gave the [K⁺]_o response (KRG). A local ERG could also be monitored across the retinal depth proximal to the K⁺ electrode tip by recording between the reference barrel of the K⁺ electrode and a vitreal electrode.

The recording pipette was mounted on a microdrive that

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Abbreviations: ERG, electroretinogram; [K⁺]_o, extracellular K⁺ concentration; KRG, [K⁺]_o response; LERG, local (intraretinal) ERG.

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FIG. 1. Drawing of the skate retina. Relative retinal thickness is based on the dimensions observed in OsO_4 -fixed sections (70% retinal depth corresponds to about $105 \mu\text{m}$). The Müller cell (stippled) extends from the internal limiting membrane (ILM) at the vitreal surface of the retina to the external limiting membrane (ELM) in the outer nuclear layer (ONL). Proposed current flow set up around the Müller cell is indicated by arrows. There is a distal current sink at the interface of regions I and II and a proximal current sink at the interface of regions II and III. Cell types: g, ganglion; a, amacrine; b, bipolar; h, horizontal; r, receptor. GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer.

enabled depth adjustment of the electrode in steps of $1 \mu\text{m}$; an estimate of electrode depth was also provided by changes in the waveform of the local ERG (LERG) (10). Signals from both barrels of the K^+ electrode were led via Ag/AgCl electrodes to the input stages of Metametrics amplifiers with input resistances of $10^{14} \Omega$. The signals were displayed on a four-channel storage oscilloscope and chart recorder.

The K^+ electrodes were filled with Corning 477317 ion-selective resin as described (8); the pipette tips were approximately $2\text{--}4 \mu\text{m}$ with an interbarrel spacing of $1\text{--}2 \mu\text{m}$ (center to center). Combined electrochemical response times of the electrodes were in the range of $15\text{--}20 \text{ msec}$ and the electrode selectivity was between $45:1$ and $60:1$ for K^+ over Na^+ (11, 12). By adjusting the gains of the amplifiers, common mode rejection was sufficient to remove almost all measurable effects on the KRG output from sustained test pulses applied to the bath with amplitudes 10-fold greater than that of the intraretinal ERG (LERG). A variable RC filter was used to reduce the response time of the reference barrel to match that of the high-impedance selective barrel.

FIG. 2. Variation of KRG and LERG with retinal depth; the latter is the potential measured between the reference barrel of the K^+ -selective electrode and a grounded lead at the sclera. Intraretinal locations were estimated from the readings of the micrometer drive from the retinal surface and from the shape of the LERG and the reversal point of the b-wave. Responses were elicited with 1-sec stimuli; the stimulus intensities were constant at $\log I = -5$. All stimuli uniformly illuminated the retinal surface. Voltage calibration for the LERG traces are the $200\text{-}\mu\text{V}$ bars; calibrations for the KRG traces indicate both the voltage and the equivalent change in $[\text{K}^+]_o$. These and the other responses shown in this paper were traced from the original chart records.

Light was projected onto the preparation from a photostimulator that utilized for its source a concentrated-filament tungsten-halogen lamp (Philips 7023) operated from a current stabilized direct current source (13). Stimulus intensity, duration, and size were controlled by means of neutral density filters, an electromagnetic shutter, and interchangeable apertures, respectively. $\log I$ values give the densities of the neutral filters interposed in the stimulus beam; the retinal irradiance of the unattenuated test field was $1.7 \text{ mW}\cdot\text{cm}^{-2}$. The stimulus field was positioned by means of X-Y micrometer drives and was centered over the penetrating electrodes by visual inspection under dim red illumination; final centering was done electrophysiologically by observing the effect of movement on intraretinal field potentials. In order to exclude ambient illumination and to reduce electrical interference, the final elements of the optical system, together with the preparation, viewing microscope, micromanipulator, and amplifier probe were housed in a shielded enclosure.

FIG. 3. KRG responses for full-field (Left) and spot (Right) illumination with the K^+ -selective electrode in the proximal retina (bottom traces) or in the distal retina (top traces); stimulus duration = 0.2 sec. The traces at the top were elicited with a stimulus intensity of $\log I = -6.5$ or -6.0 . The traces at the bottom were in response to $\log I = -6.0$. Undershoots following the distal responses in the pair of recordings elicited with the brighter flashes are due to the slow distal decrease. All the records are from the same experiment. The distal responses were obtained first; the electrode was then withdrawn into the proximal retina and the lower traces were recorded.

THE MODEL

If there are two sources of $[K^+]_o$ within the retina, it is likely that the Müller cells will be affected by both and that multiple intraretinal current loops will be generated. Fig. 1 shows a diagram of the skate retina, and the probable current flows induced in and around a Müller cell as a result of photic stimulation. The diagram is based on light microscope sections of the skate retina and, in terms of retinal depth, is drawn approximately to scale. Note that the Müller cell extends from the inner margin of the retina to the middle of the receptor cell nuclei (i.e., 70% of the retinal thickness) and that the distal margin of the inner plexiform layer is approximately midway between the extremes of the Müller cells.

The lines of current flow in Fig. 1 illustrate the essential features of our hypothesis concerning the generation of the transretinal b-wave. Two current sinks are positioned along the Müller cell in relation to the locations of the experimentally determined sources of $[K^+]_o$. A distal sink is therefore situated close to the distal end of the Müller cell (see *Results*). Because of its asymmetric location with respect to the length of the Müller cell, more current will flow in the extracellular space toward the distal sink from the proximal retina than from the distal retina. Thus, a relatively large net voltage will be generated across the retina as a result of the distal K^+ current sink. The proximal sink, on the other hand, is located more centrally along the length of the Müller cell. This sink will tend to generate more equal current flows from both ends of the retina, and a smaller net transretinal voltage will result.

The schematic in Fig. 1 shows also that, along the regions of the Müller cell labeled I and III, the current loops generated by the two sinks have the same polarity, and reinforce each other. In region II, however, the currents generated by the two sinks are of opposite polarity and will act to cancel each other. The net transretinal voltage recorded across the retina will depend, therefore, on the relative magnitudes of the current loops established within the retina as a result of the two current

FIG. 4. Local b-waves recorded across the proximal and distal regions of the retina during spot (Right) and full-field (Left) illumination. The upper and lower sets of records were obtained with the intraretinal electrode at two different positions. Schematic at bottom gives approximate locations of the vitreal electrode (V) and scleral electrode (S) and the two electrode tip locations (E_1 and E_2) within the retina. E_1 probably corresponds to the region near the interface of regions I and II ($\approx 60\%$ retinal depth) in Fig. 1. E_2 is just proximal to the b-wave reversal point for full-field illumination ($\approx 50\%$ retinal depth). The large positive potential following the b-wave of E_2-S is the c-wave of the ERG, which is prominent in some full-field recordings elicited with this stimulus intensity.

sinks. Any alteration in the strengths of the current sinks will alter the magnitude of the current loops and of the net transretinal potential (b-wave).

RESULTS

$[K^+]_o$, Intraretinal Potentials, and Retinal Depth. Fig. 2 shows the KRG and the LERG recorded at a number of different depths within the retina. The lowermost pair of traces, taken at a retinal depth corresponding to the inner part of the inner plexiform layer, shows that photic stimulation induced a small and relatively slow accumulation of K^+ ; a large positive b-wave was typically recorded at this locus. As the electrode penetrated deeper into the inner plexiform layer, the K^+ accumulation became larger and faster; large positive b-waves were also recorded at these depths. With still further penetration of the electrode, to the middle of the inner nuclear layer, the $[K^+]_o$ produced by the 1-sec flash was less, and the time course of the response was somewhat slower than at the more proximal location. The amplitude of the LERG also decreased as the electrode approached the reversal point of the b-wave.

FIG. 5. KRG of the proximal retina and LERG (referenced to a scleral ground) in response to flicker stimulation. The two responses exhibit comparable changes in amplitude as the rate of intermittence is varied, but note the accumulation of $[K^+]_o$ as the flicker-fusion frequency is approached.

Somewhat deeper, at the approximate level of the outer plexiform layer, the polarity of the local b-wave had inverted (i.e., at 59% retinal depth). The K^+ accumulation observed at this level was of greater magnitude than that seen in the inner nuclear layer, and the response showed a faster rise time and was more transient in nature. At this retinal depth, a decrease in $[K^+]_o$ was observed after the $[K^+]_o$ increase. This decrease arises in distal regions of the retina (3) and probably contributes to the transient quality of the distal K^+ response. When the electrode moved further distally, to the outer nuclear layer, the rapid transient $[K^+]_o$ increase could still be detected for some distance, but it was much smaller than at the outer plexiform layer. At these loci, the latency of the distal $[K^+]_o$ decrease was shorter and the response grew larger; in addition, the inverted b-wave reached its maximal amplitude as the external limiting membrane was approached.

Not in every penetration was the distal K^+ accumulation as large or as obvious as in the experiment shown in Fig. 2. Under which conditions the distal K^+ accumulation is best seen is not entirely clear. However, precise positioning of a fairly small recording pipette in the vicinity of the outer plexiform layer seems to be important. When a large distal K^+ increase was observed, its leading edge was measurably steeper than the rise time of the K^+ response seen more proximally in the retina (see Fig. 3). Furthermore, the peak amount of K^+ accumulation in the distal retina in such experiments was comparable to that observed in the proximal retina.

Effect of Stimulus Size. The magnitude of the slow proximal increase in $[K^+]_o$ depends upon the size of the test stimulus—i.e., a small test flash is more efficacious in this regard than is a full-field flash. The bottom set of traces in Fig. 3 shows that when the K^+ electrode was in the proximal retina the KRG was significantly larger in response to a 0.4-mm spot than to the full-field stimulus. However, when the electrode was located in a more distal locus (i.e., near the outer plexiform layer), this result was not observed—i.e., the KRG elicited with the full-field stimulus was of about the same magnitude as that evoked with the spot. It should be noted in this connection that full-field stimulation also enhanced the distal decrease; thus, it may have masked the full extent of the distal $[K^+]_o$ response to large field stimulation. In any case, the $[K^+]_o$ change originating in the distal retina was not significantly affected by the change in stimulus size, providing evidence that it arises from a different source than the K^+ efflux in the proximal retina.

Distal and Proximal Current Loops. If, as suggested above, the distal and proximal sources of $[K^+]_o$ are affected differentially by stimulus configuration, then a change from full-field

to spot illumination should alter the light-evoked extracellular currents in a predictable fashion, and this change should be reflected in the measured transretinal voltage. The differential depth recordings of Fig. 4 illustrate these effects. The local b-wave, recorded across the distal layers of the retina (E_1 -S), was measured from the tip of an electrode (E_1) located approximately at the interface of regions I and II of Fig. 1 to a ground behind the sclera (S) (see schematic at bottom of Fig. 4); the proximal local b-wave (V - E_1) was measured simultaneously from the vitreal electrode (V) to the tip of the penetrating electrode (the latter includes primarily regions II and III of Fig. 1). As can be seen in the top row of Fig. 4, the effect of changing from full-field to spot illumination is to decrease dramatically the potential generated across the inner retina while causing only minor changes in the distal local b-wave. These results indicate that the currents flowing across the distal retina are not significantly altered by stimulus configuration but that the net currents flowing across the proximal retina are markedly reduced by spot illumination. These findings fit nicely with the observation (Fig. 3) that spot illumination increases the K^+ efflux in the region of the proximal source. The resulting current flow from this source opposes the current flow across the proximal retina generated by the distal K^+ source, resulting in a smaller net transretinal voltage.

Our model for the current pathways for b-wave generation suggests further that the b-wave reversal point should have a different locus depending on the size of the stimulus field. The records at the bottom of Fig. 4 show that this is indeed the case. With the penetrating electrode placed approximately at position E_2 (lower pair of traces in Fig. 4), the penetrating electrode tip is just proximal to the reversal point of the intraretinal b-wave. The distal local b-wave (E_2 -S) is positive in response to a full-field stimulus, but it undergoes a reversal of polarity with spot illumination. Thus, the reversal point of the intraretinal b-wave was shifted more proximally by spot illumination, a result that is consistent with our claim that two K^+ sources give rise to the extracellular retinal currents and with the hypothetical light-induced current pathways of Fig. 1.

Changes in $[K^+]_o$ with Flickering Stimuli. Responses to intermittent stimulation provide a useful index for examining the degree of correspondence between K^+ fluxes and intraretinal potentials. Although there is evidence (4) that the light-induced Müller cell response is too slow to follow flicker stimulation at rates >0.6 Hz, the failure may be due in part to the composite nature of the intracellular Müller cell potential (see Discussion and Fig. 6). Fig. 5 shows recordings of the KRG and of the b-wave of the LERG obtained with the double-barrel

FIG. 6. Possible explanation for the different waveforms of the Müller cell response and the b-wave. The distal and proximal increases in $[K^+]_o$ sum to generate the intracellular Müller cell response [see Miller and Dowling (2)]. The extracellular current flows produced by each (see Fig. 1) partially cancel, thereby generating the transient b-wave (see text).

electrode in the proximal retina. It appears that the KRG and b-wave react in a similar way to flashes of varying frequency. As the interval between flashes was reduced, there was a concomitant decrease in the amplitudes of both responses until they became indistinguishable from the noise level of the recording; a brief period of darkness restored both to nearly full amplitude. Note also the shifts in baseline indicating that prolonged stimulation led to an accumulation of $[K^+]_o$ which was maximal at the highest flash rates.

DISCUSSION

Our experiments indicate the following. First, there appear to be two sources that release K^+ in the retina upon light stimulation. One source is located proximally in the vicinity of the amacrine cells. The amount of K^+ released at this site is dependent on stimulus configuration; more K^+ is released with spot stimulation than with full-field stimulation, reflecting perhaps the antagonistic influence of surround illumination on that source of $[K^+]_o$. The second source is located more distally in the retina, in the region of the outer plexiform layer. The elements that are likely to give rise to the distal K^+ efflux are the bipolar cells (6). The amount of K^+ released at this site is not as strongly dependent on stimulus configuration—i.e., both spot and full-field stimulation cause similar changes in $[K^+]_o$. Second, we have observed differences in the kinetics of the K^+ efflux at these two sites. The K^+ accumulation in the distal retina is faster and more transient in nature than is the K^+ released proximally.

The model that we propose for the generation of the ERG b-wave retains the original suggestion put forth by Miller and Dowling—namely, that the Müller (glial) cells are the element principally responsible for this transretinal potential. However, the present model takes into account results obtained with differential depth recording that indicate the presence of two sinks for extracellular current, one at each of the regions of K^+ efflux; we suggest that the currents associated with the K^+ activity at these two sites interact to provide the transretinal b-wave. The way in which this interaction could produce both the transient b-wave response and the more sustained waveform of the intracellularly recorded Müller cell potential (2, 4) is shown schematically in Fig. 6. We suggest that the rapid, transient, efflux of K^+ in the distal retina generates the extra-

cellular currents responsible for the fast positive potential that forms the leading edge of the b-wave. On the other hand, the accumulation of $[K^+]_o$ in the proximal retina is assumed to reduce the currents flowing distally in the retina; this contributes a counterpotential that may contribute to the falling phase of the response. Because both sources of $[K^+]_o$ induce a depolarization of the Müller cell, an intracellular recording from this glial element would exhibit the sum of the two depolarizations [i.e., a relatively sustained response (Fig. 6)].

If two sources are involved in generating the b-wave, current source density analysis of the b-wave potential should reveal current sinks at the retinal depths at which K^+ is released. Intraretinal recordings with paired electrodes in the rat retina led G. B. Arden (personal communication) to conclude that the b-wave could not be attributed to a single current sink, although a sharply localized sink in the outer plexiform layer did seem to be responsible for the leading edge of the potential. Proenza and Freeman (14) and, more recently, E. Newman (personal communication) also report the existence of a current sink in the region of the outer plexiform layer/outer nuclear layer that develops with the leading edge of the b-wave. In addition, these authors have found a second current sink in the inner plexiform layer/inner nuclear layer (in the region of maximal positivity of the proximal KRG). This proximally located current sink develops with a time course that corresponds to the falling phase of the b-wave (14). Thus, although a detailed quantitative assessment has yet to be performed, the transient features of the ERG b-wave appear to result from the interaction of extracellular currents that are associated with K^+ fluxes in the distal and proximal regions of the vertebrate retina.

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