Light-evoked Increases in Extracellular K^+ in the Plexiform Layers of Amphibian Retinas

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ABSTRACT   Recordings of light-evoked changes in extracellular K^+ concentration ($\Delta[K^+]_o$) were obtained in the retinas of frog and mudpuppy. In eyecup preparations, various recording approaches were used and provided evidence for a K increase near the outer plexiform layer (distal K increase). This distal K increase could be pharmacologically dissociated from the well-known, large K increase in the proximal retina by the application of ethanol and γ-aminobutyric acid. The distal K increase also often showed surround antagonism. A retinal slice preparation was used to permit electrode placement into the desired retinal layers under direct visual control and without the risk of electrode damage to adjacent layers. In the slice, a distinct distal K increase was found in the outer plexiform layer, in addition to the prominent K increase in the inner plexiform layer. Compared with eyecups, only weak K increases were found in the nuclear layers of the slice. This suggests that the K responses observed in the nuclear layers of eyecups may be generated by K^+ diffusing along the electrode track from the plexiform layers. In the context of current models of ERG b-wave generation, the magnitude of the recorded distal K increase, compared with the proximal K increase, seems too small to give rise to the b-wave. However, the distal K increase may be differentially depressed by electrode dead space. It is also possible that if certain aspects of the models of b-wave generation were modified, then the observed distal K increase could give rise to the b-wave.

INTRODUCTION

Of the various components of the electroretinogram (ERG), the b-wave has been particularly useful to ophthalmic clinicians and investigators because it is a relatively large, low-threshold response that can be routinely recorded and that serves as a noninvasive index of retinal activity proximal to the photoreceptors. Unfortunately, results derived from b-wave studies are sometimes limited because

the mechanisms of b-wave generation are not clearly understood. At the present time, the most widely accepted theory suggests that the b-wave is indirectly linked to light-evoked neuronal activity in the outer plexiform layer. This theory holds that light evokes an increase in the extracellular concentration of K ions ($[K^+]_o$) that locally depolarizes the membrane of Müller cells. This depolarization in turn generates radially directed currents that can be recorded extracellularly as the b-wave. This general scheme of events was first proposed by Faber (1969) and Miller and Dowling (1970) and has since received considerable support. Several variations of this original "Müller cell hypothesis" have recently evolved (Dick and Miller, 1978; Kline et al., 1978; Newman, 1980; Newman and Odette, 1984). In addition, the possibility of a substantial contribution to the b-wave from currents directly generated by bipolar cells has not been conclusively ruled out.

Substantiation of the Müller cell hypothesis of b-wave generation (sometimes referred to as the K^+ hypothesis) requires two critical observations: first, that a light-evoked increase in $[K^+]_o$ occur near the level of the outer plexiform layer; second, that the properties (e.g., amplitude, time course, etc.) of this increase be compatible with known properties of Müller cell responses and the b-wave. The initial studies, which measured the retinal depth profile of light-evoked changes in $[K^+]_o$, showed a prominent increase in $[K^+]_o$ only at the level of the inner plexiform layer, i.e., a "proximal K increase" (Oakley and Green, 1974, 1976; Oakley, 1975; Karwoski and Proenza, 1978; Vogel, 1980). While all subsequent studies have confirmed that this proximal response is the largest light-evoked K increase in the retina, several studies have also reported observations of an increase in $[K^+]_o$ more distal in the retina near the outer plexiform layer, i.e., a "distal K increase" (Dick and Miller, 1978; Kline et al., 1978; Dick, 1979; Karwoski et al., 1979, 1982; Shimazaki et al., 1984). Some of these studies showed that the distal K increase could be dissociated from the proximal K increase by pharmacological criteria, but there remain three aspects of the distal K increase that make its characterization and interpretation difficult: (a) it is not reliably recorded, (b) it is spatially continuous with the proximal K increase, and (c) it is generally much smaller in amplitude than the proximal K increase. Thus, while the existence of a distal K increase in the vicinity of the outer plexiform layer seems to have been demonstrated, there remains a paucity of data concerning the specific properties of this response.

In this paper, we begin to clarify certain aspects of the distal K increase by specifying the exact retinal layer that generates this response, by examining some of the factors that may influence its recording, and by quantifying some of its response properties. Particular attention is paid to the relative amplitudes of the distal and proximal K increases, with the goal of assessing the extent to which the distal K increase may be involved in b-wave generation. We conclude that a distal K increase can indeed be resolved at the level of the outer plexiform layer, but that its total magnitude as measured by K^+-selective microelectrodes cannot as yet be demonstrated to be large enough to lead to the generation of the b-wave by the mechanisms outlined in current versions of the Müller cell hypothesis.

METHODS

Experiments were done on mudpuppy (*Necturus maculosus*) and frog (*Rana pipiens pipiens* and *R. p. berlandieri*) eyecups at the University of Georgia, Athens, GA, and on slices of isolated frog (*R. p. pipiens*) retinas at the Eye Research Institute, Boston, MA.

Eyecups

Eyecups were prepared as previously described (Burkhardt, 1970; Proenza and Burkhardt, 1973). Some eyecups were superfused with an amphibian Ringer's solution buffered with either bicarbonate or HEPES, and, in some experiments, ethanol (3%) and γ-aminobutyric acid (GABA) (2.0 mM) were added to the superfusate (Shimazaki, 1983). When nonsuperfused eyecups were used, moistened oxygen was gently blown over the preparation. The light stimuli consisted of flashes of white light, with intensities ranging from 0.01 (near threshold) to 7,500 lm/m^2 (maximum output of the system), and these were superimposed upon a diffuse background of 0.2 lm/m^2. Recordings were referenced to either an Ag/AgCl pellet behind the eye or a separate micropipette in the vitreous humor. Responses were stored on tape and later played back via computer to an *X-Y* plotter.

Retinal Slices

Frog eyecups were cut into pieces that were placed vitreous-side down on filter paper. The sclera, choroid, and pigment epithelium were then peeled off, leaving isolated retinas that were cut into 300-μm-thick sections with a tissue chopper. These sections, which were 2–3 mm in length, were oriented with one of the cut sides facing upward. The slices were superfused with a bicarbonate-buffered Ringer's solution containing 2.5 mM K^+ (Newman, 1984) and viewed under infrared illumination during the course of an experiment. Light stimuli were normally flashes of white light at 0.5 lm/m^2 (~4 log units above threshold) with no background illumination present. Recordings were referred to an Ag/AgCl wire in the bath, and responses were viewed on a digital oscilloscope, stored on computer, and played back on an *X-Y* plotter.

Electrodes

In both eyecup and retinal slice experiments, recordings were made with K ion-selective microelectrodes (K-ISMs). Double-barreled pipettes were pulled from "theta-tubing" (R&D Scientific Glass Co., Spencerville, MD), and the tips were broken to 0.5–1.5 μm. The taper of these pipettes was such that at 25 μm from the tip, the shape of the electrodes described an oval whose average dimensions were 2.0 × 2.5 μm, while at 125 μm from the tip, the dimensions of the oval had increased to 6 × 10 μm. One barrel of this pipette was then silanized with *N,N*-(dimethylamino)trimethylsilane (Deyhimi and Coles, 1982) according to the method of Coles and Tsacopoulos (1977, 1979). The tapered portion of the silanized barrel was filled with liquid ion-exchange resin (477317, Corning Medical and Scientific, Medfield, MA), both barrels were backfilled with 120 mM NaCl plus 3.0 mM KCl (the ion-selective barrel was sometimes backfilled with 100 mM KCl), and an Ag/AgCl wire was sealed into the back end of each barrel. The ion-selective barrel of the electrode assembly had DC resistances ranging between 8 × 10^9 and 5 × 10^{10} Ω (measured as in Coles and Tsacopoulos, 1979) and exhibited electrical time constants (time to 63% of final value) of ~50–200 ms after optimal adjustment of negative capacitance.

In some eyecup experiments, K-ISMs made with valinomycin-based liquid membranes were used. Initial experiments in nonsuperfused frog eyecups used the liquid membrane described by Oehme and Simon (1976) and Wuhrmann et al. (1979), whereas in experiments with superfused mudpuppy eyecups, a slightly different liquid membrane (compo-

sition: 5% valinomycin, 2% K tetra-p-chlorophenyl borate, 93% 2,3-dimethylnitroben-zene) was employed (kindly supplied by Dr. Daniel Ammann, Swiss Federal Institute of Technology, Zurich, Switzerland). With the use of either type of valinomycin-based liquid membrane, the relative selectivity for K^+ over acetylcholine (ACh) is increased by at least a factor of 10,000, and for K^+ over Na^+ by a factor of more than 50, as compared with the selectivity of the K-ISMs made with the Corning exchanger (Oehme and Simon, 1976; Wuhrmann et al., 1979; Shimazaki, 1983).

A disadvantage of the valinomycin-based K-ISMs (valinomycin electrodes) was that they had very high resistances (up to 10^{12} Ω), which resulted in an increased noise level and in electrical time constants that averaged ~650 ms. These differences in relative selectivities and time constants had the practical consequence that light-evoked electrical K signals (ΔV_K) recorded with valinomycin electrodes were of larger amplitude and slower rise time than the signals from K-ISMs made with the Corning exchanger. Although ΔV_K is increased with valinomycin electrodes, $\Delta[K^+]_o$ may nevertheless appear to be decreased because the valinomycin electrode behaves as a low-pass filter that "smoothes" transient fluctuations in V_K. Therefore, to make a fairer (although still approximate) comparison of $\Delta[K^+]_o$ obtained with the two types of ISMs, ΔV_K's recorded with the K-ISMs made with the Corning exchanger (which have a short rise time compared with valinomycin electrodes) were first passed through an active low-pass filter having a rise time (650 ms) comparable to that of the valinomycin ISMs. This filter consisted of an operational amplifier (AM 501, Tektronix, Inc., Beaverton, OR) with an input resistor of 10 $M\Omega$ and with negative feedback through a resistor (4.7 $M\Omega$) and capacitor (0.15 μF) in parallel.

All K-ISMs were calibrated in Ringer's in which NaCl was replaced by equimolar amounts of KCl to yield solutions containing 1.0, 3.0, and 10.0 mM KCl. A curve based on a form of the Nicolsky equation used by Coles and Tsacopoulos (1979) was fit by computer to the resultant values, and V_K values (measured in millivolts) obtained during an experiment were then converted to corresponding values of $[K^+]_o$ (expressed in millimolar). After an experiment, calibrations of the K-ISMs containing the Corning exchanger were often identical to calibrations obtained prior to the experiment. Calibrations of valinomycin electrodes, however, were less stable, sometimes shifting up to several millivolts in 2 h. Results with valinomycin electrodes were discarded when drift was such that calibrations before and after an experiment resulted in values for $[K^+]_o$ that differed by >10%.

RESULTS

Eyecup Preparation

STANDARD DEPTH PROFILES   These were obtained by carefully advancing the electrode through the retina until the pigment epithelium was reached or slightly penetrated. From that point, the electrode was withdrawn in constant steps of 8–16 μm, and responses were collected at each depth. This procedure has been used previously by a number of investigators (e.g., Oakley and Green, 1976; Karwoski and Proenza, 1978; Dick and Miller, 1978) to obtain laminar profiles of the light-evoked ΔV_K in the retina, because electrode withdrawals are generally "smoother" than electrode penetrations, and because the recording of responses at constant steps facilitates the computation of current and ion source-density profiles (Vogel, 1980; Karwoski et al., 1982).

21 standard depth profiles in 16 frog retinas and 17 profiles in 13 mudpuppy retinas were analyzed for the presence of a distal K increase. For both small spot

and diffuse illumination, the depth profile of the light-evoked ΔV_K typically showed an increase throughout the proximal 50–70% of the retina, so that it was not clear whether a K increase seen at the level of the outer plexiform layer (OPL) was due to a distinct K source at that depth, or merely due to K^+ that diffused to the OPL from the larger, more proximal source. If a light-evoked K^+ source at the depth of the OPL were separate and distinct from the K increase at the inner plexiform layer (IPL), it would be expected to generate a response whose amplitude and rate of increase show relative maxima and whose latency
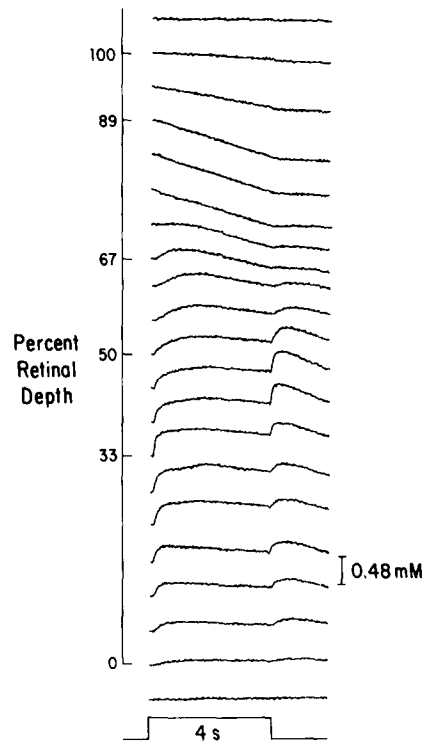

FIGURE 1. A standard depth profile of light-evoked ΔV_K obtained in a non-superfused frog retina. The inner limiting membrane corresponds to 0% retinal depth, and the pigment epithelium to 100%. Resting V_K corresponds to a $[K^+]_o$ of 2.9 mM.

shows a relative minimum when compared with responses observed at sites slightly more proximal. Such evidence for a distal K increase was indeed found in 6 of the 21 frog profiles and in 8 of the 17 mudpuppy profiles.

The records of Fig. 1 are from a depth profile in a frog retina in which responses were obtained to stimuli of several different intensities. The responses show some well-known features that were typically observed in frog profiles: light-evoked K increases over roughly the proximal 70% of the retina, and a K decrease over the distal third of the retina. The profile of Fig. 1 (obtained with a high intensity of 750 lm/m²) is also one of our more obvious examples of a

distal K increase, in that the response at 67% depth (approximately the OPL) seems better developed than responses at adjacent depths in the series. As shown in Fig. 2, quantitative comparisons bear this out: the response at 67% has a relatively shorter latency, a more rapid rate of increase, and a larger amplitude than responses at depths just proximal to it. However, it is also clear from Fig. 2 that, in absolute terms, the shortest-latency, fastest-rising, and largest-amplitude responses are in the proximal retina, at ~33% retinal depth. Moreover, the absolute latency of the proximal K increase can closely approach that of the b-
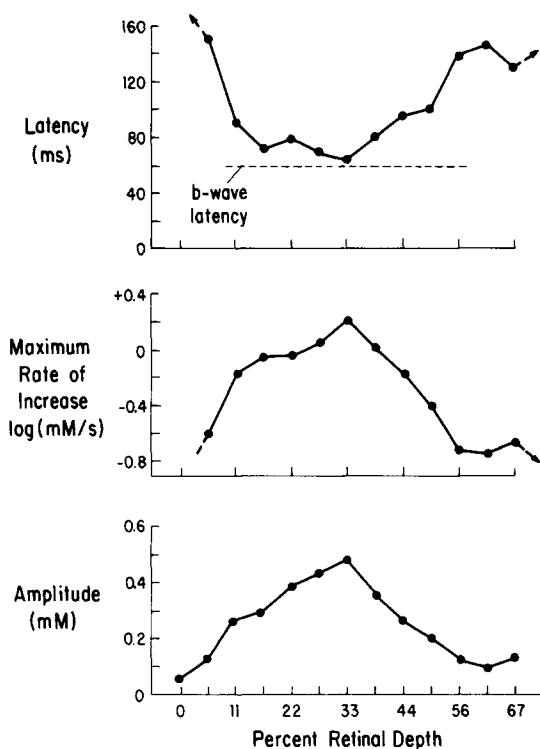

FIGURE 2. Quantitative measures for the depth profile of ΔV_K shown in Fig. 1. Only the K increases (not K decreases) were analyzed. Latencies were measured to the time at which V_K began increasing. The maximum rate of increase is a measure of the maximum light-evoked change in $[K^+]_o$ per unit time. The amplitudes of the K increases (ΔV_K) were measured at 0.5 s after light onset and converted to $\Delta[K^+]_o$.

wave, as previously described (Karwoski and Proenza, 1980). In some recordings of the distal K increase obtained in standard depth profiles, as well as in some with careful depth probing (see below), there was a separate K increase at light offset (see also Dick, 1979). This response was more reliably observed in mudpuppy than in frog, but even in mudpuppy it was exceptionally small and therefore was not studied in any detail.

A quantitative summary of specific aspects of the "on" K increases in both the distal and proximal retina is presented in Table I. Here, and also in Tables II

TABLE I

*Comparison of Proximal and Distal K Increases Obtained in
Standard Depth Profiles in Retinas of Mudpuppy and Frog Eyecups**

	Proximal K increase	Distal K increase
Mudpuppy		
Depth of maximum responses	14–25% $(N = 8)$	49% (±3) $(N = 8)$
Latency (ms) to 3.0-mm spot	204±28 $(N = 8)$	288±34 $(N = 8)$
Maximum amplitude (mM)‡		
3.0-mm spot	0.20±0.04 $(N = 8)$	0.025±0.01 $(N = 8)$
0.3-mm spot	0.42±0.08 $(N = 6)$	0.07±0.01 $(N = 6)$
Frog		
Depth	17–39% $(N = 6)$	58% (±3) $(N = 6)$
Latency	133±21 $(N = 6)$	238±43 $(N = 6)$
Amplitude (mM)‡		
3.0-mm spot	0.18±0.04 $(N = 6)$	0.03±0.005 $(N = 6)$
0.3-mm spot	0.30±0.06 $(N = 5)$	0.07±0.01 $(N = 5)$

* Data are from only those profiles in which evidence for a distal K increase could be found. In both species, "depth of maximum response" refers to the percent retinal depth at which maximum responses were recorded: for the proximal K increase, the mean range of depths over which maximum responses were obtained is presented; for the distal K increase, which could be recorded at only one or two depths in any given preparation, only the depth ($\bar{X}$ ± SEM) at which this response was maximum is presented. Other data regarding response parameters are presented as $\bar{X}$ ± SEM.

‡ The amplitudes of the distal K increase are millimolar conversions direct from the recorded responses. These amplitudes are overestimates, to the extent that these recorded responses are contaminated by K^+ diffusing from the proximal K increase (see text for details).

and III, only responses obtained with our most commonly used stimulus (~10 lm/m²) were analyzed. In both frog and mudpuppy, the proximal K increase was largest over a broad range of depths corresponding approximately to the thickness of the IPL. On the other hand, the distal K increase was relatively large at usually only one depth, approximately that of the appreciably thinner OPL. In all cases, the proximal K increase had a shorter latency and larger amplitude than the distal K increase.

TABLE II

*Comparison of Proximal and Distal K Increases
Obtained with Careful Depth Probing in Mudpuppy**

	Spot diameter	Proximal K increase	Distal K increase
	mm	$(N = 10)$	$(N = 12)$
Latency (ms)	3.0	164±10	263±17‡
	0.3	190±9	268±14‡
Amplitude (mM)§	3.0	0.25±0.02	0.05±0.01‡
	0.3	0.54±0.04	0.09±0.03‡
Half rise time (ms)	3.0	182±21	268±45

* Data reported are $\bar{X}$ ± SEM.

‡ Multiple t tests showed that for both spot diameters, amplitude and latency comparisons (proximal vs. distal K increases) were significant at $P < 0.001$.

§ The amplitudes of the distal K increase are millimolar conversions direct from the recorded responses. These amplitudes are overestimates, to the extent that these recorded responses are contaminated by K^+ diffusing from the proximal K increase (see text for details).

Since the distal and proximal K increases generally appear to be continuous in depth, some fraction of the distal response may consist of K^+ that has diffused from the proximal retina. An upper-limit estimate of this contribution was made by means of the analysis outlined in Fig. 3, which shows responses from various depths in a mudpuppy retina, both before (Fig. 3A, and 3B, "original") and after
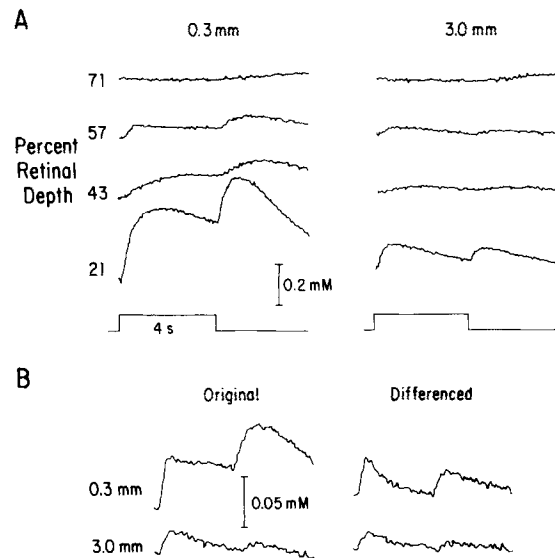

FIGURE 3.   (A) Selected light-evoked ΔV_K obtained at four depths in a standard depth profile in mudpuppy retina to 0.3-mm (left column) and 3.0-mm spots (right column). The numbers on the left refer to the percent retinal depth at which the recordings were made, and responses include those of the maximum proximal (21% depth) and distal (57%) K increases. If it is assumed that the 0.3-mm response at 43% depth is made up only of K^+ diffusing distally from the proximal retina, then as this K^+ further diffuses to 57%, its latency and rise time should become longer, and its amplitude smaller. To obtain an estimate of the distal K increase not contaminated by the proximal response, half the response at 43% was subtracted from the response at 57%. (Half was chosen in this case because the amplitude of the later portions of the on response at 57% did not exceed half that of the response at 43%. The technique thus gives an upper limit to the possible diffusional contribution from the proximal K increase.) (B) The responses at 57% depth to 0.3- and 3.0-mm spots are reproduced with increased gain on the left (original), and the differences, after subtracting half of the response at depth 43%, are shown on the right (differenced).

(Fig. 3B, "differenced") subtracting out the maximum possible influence of the proximal K increase on the distal K increase. The result of this analysis suggests that the recorded distal K increase can be influenced by K^+ diffusing from the proximal retina: in six different retinas, such upper-limit estimates indicated a maximum contribution of 10–40%. Thus, the amplitudes of the distal K increase presented in Tables I and II are probably somewhat inflated.

For both the proximal and distal K increases, there were greater responses to small, well-centered spots than to large spots (Fig. 3A). This surround antagonism in the distal K increase is probably the result of antagonism in neuronal responses, presumably in depolarizing bipolar cells, which generate the distal K increase (Dick and Miller, 1978; Dick, 1979). It is also conceivable that surround antagonism in the distal K increase arises simply because the K decrease in the subretinal space, which "cuts into" the distal K increase, is larger with large spots (Kline et al., 1978; Steinberg et al., 1980). However, little distal K decrease was usually observed in the light-adapted mudpuppy preparations, and there was little difference in the amplitude of the K decrease observed as a function of spot diameter in either mudpuppy (Fig. 3A, responses at 71% depth) or frog retinas. Thus, an interaction with the K decrease cannot explain surround antagonism in the distal K increase. Another mechanism that could influence surround antagonism in the distal K increase is diffusion of K^+ from the proximal K increase: since the proximal K increase exhibits strong surround antagonism, then, to the extent that some percentage of the distal K increase consists of ions that diffuse to the distal retina, the distal response will also exhibit antagonism. In Fig. 3B, however, even after differencing, the distal K increase to 0.3 mm has about twice the amplitude as the 3.0-mm response, which indicates that the distal K increase in this preparation does indeed exhibit surround antagonism. In a total of six preparations, surround antagonism in the differenced, or "uncontaminated," distal K increase was clear in four preparations, but was not evident in the other two. Therefore, in some retinas, surround antagonism in the distal K increase could be an artifact of diffusion of proximal K^+, but in others, the antagonism is a real property of the distal K increase.

It should be emphasized that the differencing analysis of Fig. 3B assumed a worst case, i.e., that all of the on response at 57% at 2–4 s was due to proximal K^+. The analysis also makes no allowance for the additional slowing of the proximal K increase that must occur at 57% relative to the response at 43%. However, the correction of neither of these would substantially affect the calculation of the maximum amplitude of the differenced responses, or alter the conclusions that some of the distal K increase, as recorded in eyecups, is due to diffusion of K^+ from the proximal K increase, and that the distal K increase usually exhibits surround antagonism.

CAREFUL DEPTH PROBING FOR DISTAL K INCREASE   In only a minority of standard depth profiles was evidence for a distal K increase found, and usually this response was extremely weak. Conceivably, the distal K increase is not reliably recorded because it arises from the thin OPL (~6 μm thick in frog, as measured in live retinal slices; see below), so that electrode steps of 8–16 μm would sometimes miss this layer. Alternatively, the insertion of an electrode into the retina may produce along the electrode track a relatively large-diameter cylinder of damaged tissue that would act to dilute and slow down K responses (Hertz et al., 1969; Newman and Odette, 1984), especially responses recorded as the ISM tip is withdrawn back into this cylinder during a standard depth profile. Such a response degradation should be more severe at the thin OPL than at the relatively thick IPL.

To assess the extent to which either electrode positioning or tissue damage along the electrode track was responsible for the relatively low frequency with which the distal K increase was observed, the following experiments were carried out using "careful depth probing." Electrodes were carefully advanced through the proximal retina to depths just distal to those over which the proximal K increase was well developed. If a distinct distal K increase was seen during penetration, then this response was immediately studied. However, the distal K increase was more often not evident, and the electrode was then further advanced to the depth at which the negative b-wave was maximum. Since standard depth profiles showed that the distal K increase, when observed, was located just proximal to the depth at which the negative b-wave was maximum, the electrode was then withdrawn in steps of 2–4 μm until the distal K increase was observed. With this strategy of careful depth probing, evidence for a distal K increase could now be observed in almost all penetrations in mudpuppy (12 of 13 penetrations), and while only a few frogs were tried, evidence could now be found in half of these penetrations (three out of six).

The distal K increase was sufficiently large and reliably recorded in mudpuppy so that quantification of several of its aspects could be readily achieved. A summary of these analyses is presented in Table II. There was a tendency for all responses (both proximal and distal K increases) obtained with careful depth probing to have shorter latencies and larger amplitudes than responses in standard depth profiles. This, together with the observation that the distal K increase could be more reliably recorded with careful depth probing, suggests that electrode-induced tissue damage may be greater in standard depth profiles, thus leading to relatively degraded responses. Other aspects of the K increases, however, were similar regardless of the recording strategy employed. For example, as in standard depth profiles, results obtained with careful depth probing indicated that the distal K increase, compared with the proximal K increase, has a significantly longer latency and smaller amplitude. Also, surround antagonism was observed in both responses, and its magnitude was similar to that seen in standard depth profiles. Table II also shows that there was no significant difference in the half rise times of the proximal and distal responses. As with standard depth profiles, it should be clear that with careful depth probing, the distal K increase may still receive a contribution from K^+ diffusing from the proximal K increase. This contribution may be smaller than in standard depth profiles, but the values in Table II are probably somewhat distorted by this effect. Some light-evoked increases in V_K, which were recorded from the distal and proximal retina of a mudpuppy with the technique of careful depth probing, are illustrated along with simultaneously obtained ΔV_o in the "pre-drug" responses of Figs. 9 and 10, which are discussed below in greater detail.

Retinal Slice Preparation

The results described above show that evidence for a distal K increase can be recorded in an appreciable number of depth profiles. However, even with careful insertion and positioning of a fine K-ISM to only about the depth at which the distal K increase is expected, certain problems remain: (a) a distal K increase is

not always seen, and one contributing factor here might be the difficulty of accurately positioning the ISM tip in the outer plexiform layer; (*b*) the distal K increase may still be substantially degraded by tissue damage, either by the electrode tip in the outer plexiform layer or by the electrode barrel in the inner nuclear layer; (*c*) the proximal K increase, and possibly the distal K decrease, may interfere with recordings of the distal K increase because these large $\Delta[K^+]_o$'s can diffuse through the damaged tissue along the barrel of the electrode. In an attempt to minimize these problems, recordings were made in a retinal slice preparation.

A photomicrograph of the side-view of a live slice of frog retina is shown in Fig. 4*A*. The slice shown in this photograph appears as it did during an
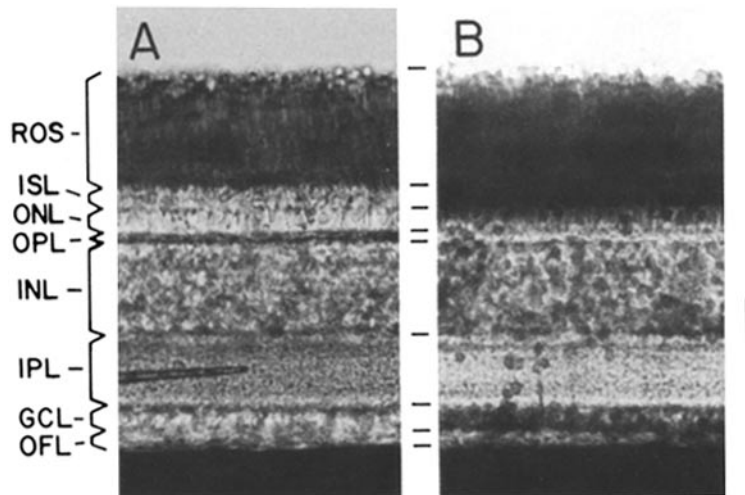

FIGURE 4. (*A*) Photograph of the cut side of an unstained retinal slice of the frog. The tip of a microelectrode can be seen in the IPL. (*B*) Photograph of the same slice, but after application of toluidine blue dye, which selectively stains nuclear layers and the inner segment layer. Abbreviations for this and subsequent figures: ROS, rod outer segments; ISL, inner segment layer; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; OFL, optic fiber layer. Scale bar, 100 μm.

experiment, when it was viewed on a video monitor via an infrared image-converting system. The various nuclear and plexiform layers, along with the row of photoreceptor inner and outer segments, can be readily discriminated, although at times there was some ambiguity regarding the exact location of the OPL. To confirm the location of each of the retinal layers, the same slice was superfused with the dye toluidine blue, which preferentially stains cell nuclei and the inner segment layer, and another photograph was made (Fig. 4*B*). Since it is apparent that the retinal layers are reasonably demarcated even without staining, it proved feasible to insert an electrode directly into any desired layer and record ΔV_K and field potentials (ΔV_o). The procedure has several virtues: (*a*) ambiguity regarding depth of recording is eliminated, since the position of the electrode

tip is continuously under visual control; (b) the possibility is excluded that a cylinder of damaged tissue created by the electrode might serve as a diffusion channel that permits $\Delta[K^+]_o$ occurring at one depth to influence ΔV_K recorded at other depths; (c) insertion of the electrode into any retinal layer usually occurs with minimal dimpling (<5 μm) of the surface, compared with dimpling that, in standard penetrations through the inner limiting membrane, can be estimated from physiological criteria to reach 50–100 μm. Electrodes were inserted deep (at least 40 μm below the cut surface of the slice) to minimize the possibility of recording responses that were decreased due either to diffusion of $\Delta[K^+]_o$ into the bath or to cellular damage along the cut edge of the slice. It should also be noted that since the slice is ≤300 μm thick, stimulation with a large-diameter (3.0 mm) spot is not possible. Thus, if all other neural connectivity in the slice is
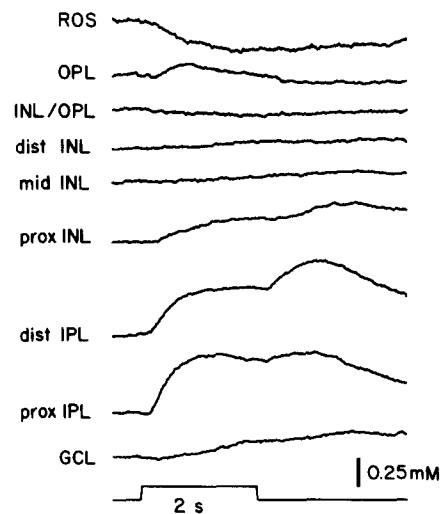

FIGURE 5.   Depth profile of light-evoked ΔV_K from the retinal slice preparation. The baseline V_K was the same in the slice as in the superfusate, 2.5 mM.

relatively normal, light stimulation of the slice would be most analogous to some form of slit illumination in the eyecup.

One series of ΔV_K recordings from the retinal slice is shown in Fig. 5. Here, only a small, slowly developing K increase could be discerned in the ganglion cell layer at light onset. This increase reached maximum amplitude in the proximal half of the IPL, and it was smaller, but still of substantial amplitude, in the distal half of the IPL, the depth at which the K increase at light offset was maximum (Karwoski et al., 1978). Unlike the eyecup results, however, K increases at all levels in the inner nuclear layer were extremely small in amplitude. A distinct K increase was observed in the OPL, and, although depths just proximal and distal to the OPL were repeatedly probed, K increases to either side of the OPL were either absent or extremely small and of long latency. There was no consistent evidence for a separate K increase at light offset in the OPL.

Finally, in the region of the rod outer segments, only the light-evoked decrease in V_K was observed, and its amplitude was small, presumably because the lack of pigment epithelial cell processes around the outer segments resulted in a larger volume of extracellular space.

A depth profile of ΔV_K from another retina is shown in Fig. 6 (left column). This profile of ΔV_K has certain similarities to the one shown in Fig. 5 in that prominent light-evoked K increases can be found in both plexiform layers, and only small, slow responses are seen in adjacent nuclear layers. However, unlike in Fig. 5, the K increase at light offset in the IPL of this retina is relatively weak.

Field potentials were not well developed within the retinal slice, presumably because of shunting to the bath via low-resistance pathways (unlike the eyecup, the slice possesses tangential shunting paths and also a low-resistance path
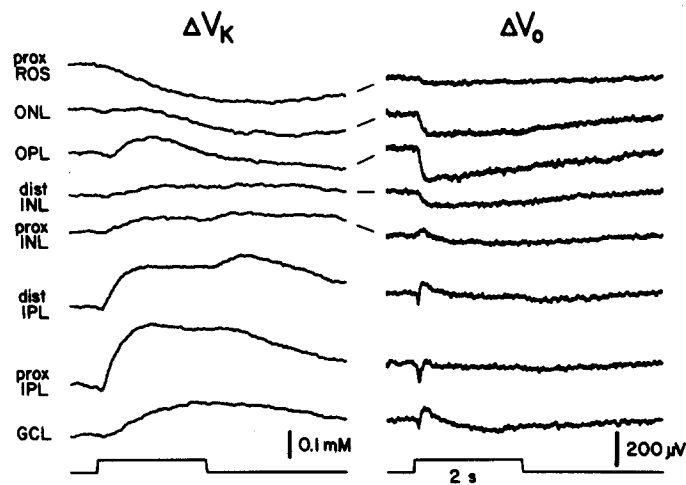

FIGURE 6.   Depth profile of simultaneously obtained light-evoked ΔV_K and ΔV_o from a frog retinal slice. At the OPL, $\Delta[K^+]_o$ is 0.07 mM. Since the ΔV_K of this same response is 400 μV, compared with the 200-μV ΔV_o at that depth, ΔV_K cannot be a differencing artifact.

through the rod outer segments). The small amplitude of ΔV_o in the slice can be viewed as something of an advantage, since it eliminated the possibility that ΔV_K might contain a substantial differencing artifact (Dick, 1979; Vogel, 1980): specifically, in four retinas exhibiting a clear distal K increase, the magnitude of the ΔV_o ranged from only 25 to 50% of that of ΔV_K (measured in millivolts). A profile in which ΔV_o was relatively large is shown in Fig. 6, together with simultaneous recordings of ΔV_K. An initial, sharp negativity (probably the proximal negative response [PNR] of Burkhardt, 1970) can be seen at light onset throughout the IPL and the ganglion cell layer. A slower, "distal negativity" can be observed near the OPL, but the origin of this field potential is uncertain because both the a-wave and b-wave should be negative-going at this depth. Supporting an a-wave origin for the distal negativity is the fact that it had a short

latency (<50 ms at the highest intensities), similar to that of the a-wave recorded in eyecups. On the other hand, one observation that supports a b-wave origin is that, although all slices showed a well-developed K increase within the IPL, the distal K increase was seen only in those slices (four out of six) in which the distal negativity was detected. Thus, the distal negativity may consist of components of both the a- and b-waves.

For each series of ΔV_K recordings, light-evoked ΔV_K values were measured at various times after light onset, converted to $\Delta[K^+]_o$, and plotted as a function of depth. A graph of normalized amplitudes of the light-evoked increases in $[K^+]_o$ vs. depth for all profiles ($N = 6$) is shown in Fig. 7. Here, K responses were measured at 0.5 s, since at this time the b-wave amplitude is large (although it decreases from its peak at 0.2–0.4 s), and the proximal and distal K increases have developed substantial amplitude. The data points at the OPL include two
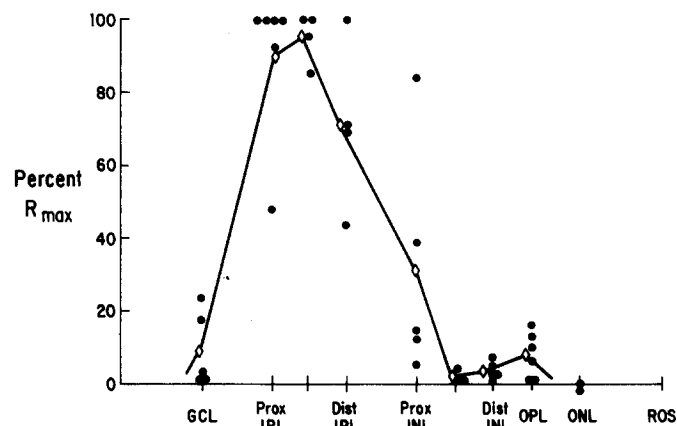

FIGURE 7. Amplitude of light-evoked K increases recorded at various depths from frog retinal slices. In all cases, ΔV_K values at 0.5 s were converted to $\Delta[K^+]_o$, and then, for each depth profile, the maximum amplitude response was normalized to 100%. Circles: individual data points; diamonds: means.

profiles in which virtually no K increase was seen, and four in which the K increase was up to 15% of the maximum K increase seen in the IPL. At 0.5 s, the absolute amplitudes of the K increases ranged up to 0.34 mM in the IPL and 0.05 mM in the OPL. The maximum amplitudes of these K increases were usually reached before 1 s, and ranged up to 0.6 mM in the IPL and 0.13 mM in the OPL.

The important features of these depth profiles of ΔV_K in retinal slices (Figs. 5–7) include: (a) a substantial K increase at all depths within the IPL, with the off increase reaching a maximal amplitude more distally than the on increase; (b) weak K increases within the inner nuclear layer, which supports the possibility that the substantial responses found in this layer in standard depth profiles in eyecups are artifacts of diffusion through damaged tissue along the electrode track; (c) a distinct, although relatively small, light-evoked K increase in the OPL.

The electrode had to be positioned within or immediately adjacent to the OPL to record this response. For example, in Fig. 5, a clear response is seen when the microelectrode was positioned in the OPL, but not when it was moved ~3 μm into the inner nuclear layer. In all retinas, depths just distal to the OPL were also repeatedly probed, but no clear K increases were ever observed. The overall impression gained from the slice experiments was that the distal K increase was well recorded only *within* the OPL.

In one slice with a particularly large distal K increase, responses were obtained in both the IPL and OPL at intensities spanning 6 log units. The latencies of these responses are plotted in Fig. 8, along with the latencies of the PNR, which
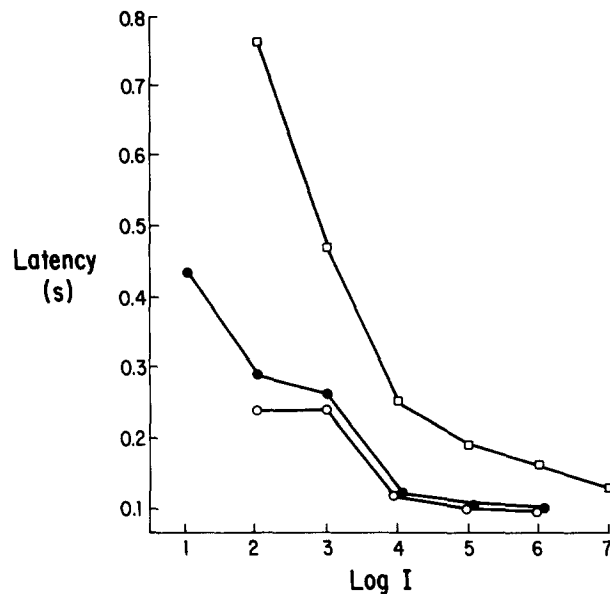

FIGURE 8.   Latencies of responses evoked by flashes of increasing intensity from a frog retinal slice. Squares: distal K increase; filled circles: proximal K increase; open circles: ΔV_o, the PNR recorded simultaneously with the distal K increase. The abscissa is an arbitrary logarithmic scale in which the stimulus intensity of 1 is equivalent to 5×10^{-4} lm/m².

was recorded simultaneously with the proximal K increase. As previously shown in Fig. 2 and in Karwoski and Proenza (1980), the latency of the proximal K increase approaches that of the field potential. (In Fig. 8, the PNR, rather than the b-wave, was the measured field potential, since the b-wave could not be unambiguously identified in the slice, and since the latencies of the PNR and b-wave in eyecups are comparable.) At all intensities, the latency of the distal K increase was substantially longer than that of the proximal K increase. The latencies of the distal and proximal K increases were measured at several stimulus intensities in the other slices, and in all cases the latency of the proximal K increase was shorter. The longer latency of the distal K increase was predicted

by current source-density analysis of the b-wave (Newman, 1980) and is not surprising, given that responses of depolarizing bipolar cells have a gradual rise time compared with that of the depolarizing neurons of the proximal retina (i.e., amacrine and ganglion cells).

Pharmacological Dissociation of Distal and Proximal K Increases

In addition to the isolation of the distal K increase in depth recordings, we have been able to confirm, in five mudpuppy eyecups, the findings of Dick and Miller (1978) and Dick (1979) that application of ethanol (EtOH) and GABA enhances the distal K increase and the b-wave, while depressing the proximal K increase. Figs. 9 and 10 present these basic findings, but with some important extensions. (a) In Dick and Miller's study, a prominent light-evoked decrease in V_K is observed along with the distal K increase, so that it is conceivable that EtOH/
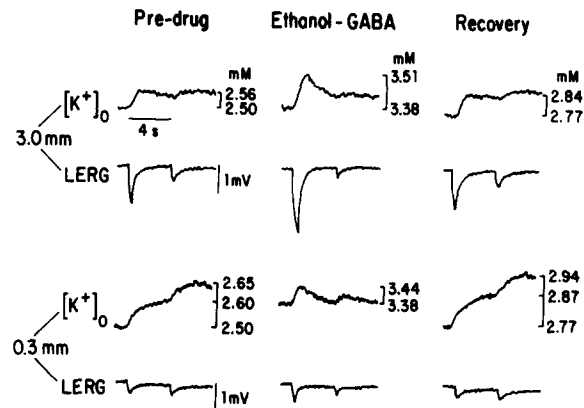
FIGURE 9. Simultaneous recordings of the distal K increase and the local ERG obtained with 3.0- and 0.3-mm spots in mudpuppy retina. Left column: pre-drug responses; middle column: responses after 5 min superfusion with EtOH/GABA; right column: recovered responses after 5 min of drug-free solution.

GABA, rather than enhancing the K increase, depressed the K decrease. This alternative interpretation is excluded by the present results, which were obtained in the presence of light-adapting background illumination that suppressed the distal K decrease. (b) The responses to both 3.0- and 0.3-mm spots are presented and reveal that, for both the distal and proximal K increases, surround antagonism is greatly reduced or abolished after addition of EtOH/GABA. This result was seen in all five preparations, and further supports the idea that the distal K increase arises from depolarizing bipolar cells, since EtOH/GABA selectively weakens surround responses in these neurons (Dick, 1979). However, it should be noted that part of the decrease in surround antagonism in the distal K increase may be due simply to the action of EtOH/GABA on the proximal K increase, from which K^+ had diffused to "contaminate" the distal K increase (Fig. 3). (c) The application of aspartate also depresses the proximal K increase while en-

hancing the distal K increase and b-wave (prior to its eventual isolation of the a-wave), but interpretation of these results, particularly with regard to the ERG, is confounded by a simultaneously observed large increase in retinal resistance (Shimazaki, 1983; Shimazaki et al., 1984). During the EtOH/GABA applications of the present experiments, transretinal resistance was measured in three preparations (along with ΔV_K and ΔV_o) and was found to increase only minimally (to 109 ± 3% of the pre-drug amplitude). Therefore, drug-induced resistance changes would not seem to play a role in b-wave enhancement by EtOH/GABA.

Valinomycin vs. Corning Exchanger

Before a quantitative comparison between distal and proximal K increases can be attempted, it is necessary to determine whether some portion of the ΔV_K recorded with ISMs containing the Corning exchanger results from ions other
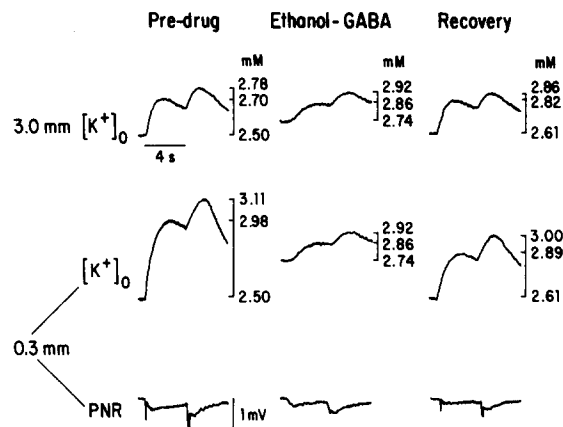

FIGURE 10. Recordings of the proximal K increase to 3.0- and 0.3-mm spots, and of the simultaneously recorded PNR to 0.3-mm spots. Other details are the same as in Fig. 9.

than K$^+$. For example, since the Corning exchanger has a high selectivity for ACh over K$^+$ (Oehme and Simon, 1976; Wuhrmann et al., 1979), a light-evoked increase in ACh in the IPL could lead to a large ΔV_K, and hence an overestimation of the true $\Delta[K^+]_o$. This possibility was tested by recording responses with K-ISMs containing a liquid membrane based on valinomycin (see Methods) and comparing the resultant $\Delta[K^+]_o$ with that obtained with the Corning exchanger.

Experiments were initially performed on 13 nonsuperfused eyecups of the frog, *Rana ridibunda*. The resting $[K^+]_o$ in the vitreous humor and in the proximal retina was initially between 2.0 and 3.0 mM, but averaged ~0.5 mM lower when measured with valinomycin electrodes, presumably because of the presence of some ion(s) to which the Corning exchanger is highly responsive. Such a difference in the resting K$^+$ activity has also been reported in *Chironomus* salivary gland nuclei (Palmer and Civan, 1977; Wuhrmann et al., 1979) and in leech neuropile glial cells (Schlue and Wuttke, 1983).

In these nonsuperfused frog eyecup preparations, the resting $[K^+]_o$ gradually increased to 5.0–7.0 mM over the course of the next 2–5 h (presumably because of evaporation; see below), and this changing level of resting $[K^+]_o$, in conjunction with the apparently different resting levels of $[K^+]_o$ recorded with the two types of ISMs, made quantification of the light-evoked $\Delta[K^+]_o$ difficult. Nevertheless, our best estimate in these preparations showed that the proximal K increase (in millimolar) recorded with valinomycin electrodes was on the average ~20% smaller than when recorded with the Corning exchanger. However, when the ΔV_K's of the K-ISMs containing the Corning exchanger were filtered to correct for the electrode rise time (see Methods), the remaining difference in $[K^+]_o$ between the two types of K-ISM (~10%) was no longer statistically significant. No conclusions could be made regarding the distal K increase, since this response could not be identified in experiments in eyecups with sufficient confidence to permit quantitative comparisons between the two K-ISM types. Finally, the amplitude of the light-evoked decrease in $[K^+]_o$ in the subretinal space was not

TABLE III

Comparison of Proximal K Increase Obtained in Superfused Mudpuppy Eyecups with Corning and Valinomycin Electrodes

	Corning (unfiltered)	Corning (filtered)	Valinomycin
Amplitude (mM) to	0.28±0.03	0.24±0.03	0.18±0.04*
3.0-mm spot	($N = 6$)	($N = 6$)	($N = 7$)
0.3-mm spot	0.61±0.08	0.52±0.07	0.39±0.07*
	($N = 6$)	($N = 6$)	($N = 7$)
Peak time (s) to	1.1±0.3	1.9±0.6	1.7±0.3
3.0-mm spot	($N = 6$)	($N = 6$)	($N = 7$)
0.3-mm spot	2.0±0.5	2.6±0.6	2.5±0.5
	($N = 6$)	($N = 6$)	($N = 7$)

* t tests showed significant differences ($P < 0.05$).

significantly different with the two types of K-ISMs, which is in agreement with Shimazaki and Oakley (1984).

To avoid the difficulty of specifying resting $[K^+]_o$ in nonsuperfused eyecups, additional experiments were performed in superfused eyecups of mudpuppy. Here, the resting $[K^+]_o$ in the superfusate and proximal retina was measured at ~2.5 mM with both types of K-ISM, and this value did not change over time. Only the proximal K increase was successfully studied in detail, and the results, for both 0.3- and 3.0-mm spots, are presented in Table III. The uncorrected values for $\Delta[K^+]_o$ obtained with K-ISMs containing Corning exchanger were significantly larger and faster-peaking than the $\Delta[K^+]_o$'s obtained with valinomycin electrodes. After the ΔV_K values recorded by the Corning exchanger were low-pass filtered, the differences in rise time essentially disappeared and the amplitude differences narrowed. However, the proximal K increase measured in response to both the 0.3- and 3.0-mm spots with the valinomycin electrodes were still, on the average, ~25% smaller than the Corning exchanger.

In general, it would thus appear that the responses recorded with the Corning exchanger are primarily the result of increases in $[K^+]_o$, but they may be slightly overestimated. Such an overestimation may arise because light stimulation increases the extracellular concentration of some ion, other than K^+, to which the electrode is highly responsive. It is not known whether the distal K increase itself is also overestimated, but in any case, compensating for the presumed overestimation in the proximal recordings does little to alter the ratio of the amplitudes of distal to proximal K increases—specifically, the distal K increase remains severalfold smaller than the proximal K increase.

Comparison of the Magnitudes of Distal vs. Proximal K Increases

Recent modeling of the relationship of the light-evoked $\Delta[K^+]_o$ to b-wave generation in the retina predicts that, at any one time, the total amount of K^+ that makes up the distal K increase should be about half that of the proximal K increase. For example, Newman and Odette (1984) proposed distal and proximal K increases of the same spatial extent, and then calculated that the amplitude of the distal response should be 0.62 that of the proximal. In the present study, estimates of the total amount of K^+ that makes up the distal and proximal K increases were computed by calculating spatial integrals of $\Delta[K^+]_o$ at various times after light onset. These integrals were computed for responses obtained in eyecups of frog and mudpuppy and in slices of frog retina, and the results were summarized as ratios of distal to proximal responses. For example, for the standard depth profile shown in Fig. 1, the amplitudes of the light-evoked ΔV_K were measured at 0.5 s, converted to $\Delta[K^+]_o$, and plotted as a function of percent retinal depth (Fig. 2, bottom graph). In Fig. 2, the distal K increase is considered to consist of the response at 67% and half of the response at 61.5%, and to drop to zero amplitude at adjacent depths. The proximal K increase is considered to consist of the responses at 56% and at all depths proximal, and half the response at 61.5%. Computing the areas of these curves describing the distal and proximal K increases results in a magnitude ratio (distal/proximal response) of only 0.06. Although the b-wave peaked at ~0.3 s in this preparation, the largest ratio (0.083) in this depth profile was found at 0.75 s. The values of these ratios for depth profiles in other eyecups were typically much smaller than 0.08, but in one profile (which had a weak proximal K increase), the ratio reached 0.12. These ratios would be even smaller if the distal K increase could be measured accurately in the absence of interference by K^+ diffusing from the proximal K increase.

In retinal slices, the procedure for calculating these magnitude ratios was somewhat different. Since a distal K increase could only be found in the OPL, the amplitude of the response was measured and assumed to extend 6 μm, the thickness of the OPL. The proximal K increase was particularly large at all depths within the IPL, although it tended to be largest in the proximal half of the IPL. Since we usually recorded responses at only two depths (mid-distal and mid-proximal) within the IPL, we estimated the amplitude of the K increase in the IPL by taking the mean of these two measures and then assuming this value extended across the whole IPL, which was observed to be 40 μm. In Figs. 5 and

6, a stimulus intensity was selected to generate maximum ratios of distal/proximal K increases, and, within these responses, peak ratios were obtained at times close to the peak amplitude of the distal K increase, i.e., 0.75–1.0 s. In both of these profiles, the magnitude ratios were ~0.035–0.04; ratios were even lower in other profiles in retinal slices. Since surround antagonism may be weaker in the distal K increase than in the proximal K increase, it can be argued that it is unfair to compare the ratio of 0.04, obtained in slices with illumination perhaps analogous to small spots, to the 0.62 required by a model of generation of the b-wave evoked with diffuse illumination. However, since surround antagonism in the proximal K increase had a value in eyecups of ~0.5 (Tables I and II: the response to 3.0-mm spots averaged about half that of the response to 0.3-mm spots), even a complete absence of this phenomenon in the distal K increase would only increase the ratio to 0.08.

The results from both eyecups and slices indicate that, at any one time, the distal K increase has at most ~8% the total amplitude of the proximal K increase. In large part, this can be attributed to the fact that the distal K increase always has a longer latency, a smaller amplitude, and a more limited spatial extent than the proximal K increase. Even if it is assumed that a full 25% of the proximal response, but none of the distal K increase, is an artifact due to an interfering ion, then the distal K increase would still have only 11% of the amplitude of the proximal K increase.

DISCUSSION

Distal K Increase

The principal goals of this study were to determine the magnitude and spatial distribution of the distal light-evoked increase in $[K^+]_o$, to examine some of the factors that may influence its recording, and to assess the relationship of this response to the ERG b-wave. The magnitude of the distal K increase is small, its relationship to the b-wave is uncertain (see below), and its spatial distribution is quite narrow. The site of generation of this response is the OPL, a locus precisely specified in the present study by direct visual observation in the retinal slice. Hence, the distal K increase can be aptly termed the "OPL K increase." Independent evidence for the existence of a distal K increase separate from the more prominent K increase observed in the IPL has previously been provided by pharmacological manipulations of the two responses (Dick and Miller, 1978; Shimazaki et al., 1984), and these results are supported in the present experiments by observations with ethanol/GABA. The relationship of the distal K increase to the b-wave is discussed below.

As shown in the Results, the distal K increase was seen in some of the standard depth profiles in both frog and mudpuppy eyecups, but was more reliably recorded, and with somewhat larger amplitude, with careful depth probing in eyecups. The difficulty in recording the distal K increase during standard depth profiles probably arose from improper positioning of the K-ISM and from electrode-induced tissue damage—factors that probably played less of a role with careful depth probing. These factors should have exerted the least influence in

the retinal slice, and the distal K increase was in fact more distinct, and sometimes of larger amplitude, in this preparation. However, the distal K increase in the slice still had a longer latency than the proximal K increase, and it was not necessarily seen more reliably than it was in eyecups. Perhaps its unreliability was caused in some slices by the procedure of creating the slice, which damaged the cells that generate this response. This possibility was supported by our observation that the distal negativity (a field potential in the retinal slice that may be in large part the b-wave; see Results) was recorded only in those preparations exhibiting the distal K increase.

Proximal K Increase

A large increase in $[K^+]_o$ in the proximal portion of the IPL is evoked by light onset. This location was determined in eyecup preparations by depth profiles and in retinal slices by direct visual observation. These results confirm a previous study (Karwoski et al., 1979) in which staining techniques were used. In the IPL, a separate K increase is generated at light offset and has maximum amplitude in the distal half of this layer (Karwoski et al., 1979)—a finding again verified by recordings in the retinal slice. In analogy with our suggestion concerning the distal K increase (OPL K increase), this proximal K increase can be referred to as the "IPL K increase."

Valinomycin electrodes were used to determine whether any of the ΔV_K recorded in the IPL was due to light-evoked changes in some other ion to which the Corning exchanger is sensitive, rather than to K^+. Comparisons of computed $\Delta[K^+]_o$ with the two electrode types showed that only a small percentage (~25%) of this response may be due to such interfering ions. For various reasons, however, this finding is somewhat tentative, and 25% should be considered an upper limit for the contribution of ions other than K^+. Comparisons of $\Delta[K^+]_o$ recorded by electrodes containing valinomycin or Corning exchanger have also been made elsewhere: our finding that the light-evoked decrease in $[K^+]_o$ in the subretinal space was comparable to both electrode types is in agreement with that of Shimazaki and Oakley (1984) in toad retina. Similarly, Coles and Tsacopoulos (1979) found a comparable light-evoked increase in $[K^+]_o$ with both electrode types in drone retina. However, Schlue and Wuttke (1983) found that in leech neuropile glial cells, the Corning exchanger seriously overestimated the changes in intracellular $[K^+]$ measured with valinomycin electrodes. Since resting $[K^+]_o$ may also be overestimated with Corning exchanger (see Results), it would appear that measurements of both resting and $\Delta[K^+]_o$ made with Corning exchanger should also be verified with valinomycin electrodes.

K Increases in the Inner Nuclear Layer

The light-evoked $\Delta[K^+]_o$ was relatively small in the inner nuclear layer of all preparations. Some of the more plausible hypotheses of why this might be include: (a) less K^+ is released per unit area of cell membrane in nuclear layers than in plexiform layers; (b) the same amount of K^+ is released per unit area of cell membrane in nuclear and plexiform layers, but a constant volume of damaged tissue about the electrode tip will more greatly dilute responses in the nuclear

layers, because they have a lower extracellular space volume fraction (Karwoski et al., 1984); (c) the volume of electrode-induced tissue damage may be greater in the nuclear layers, because destroyed somas should occupy a greater volume than destroyed dendrites.

It should be noted that all these hypotheses would apply to both eyecup and slice preparations. The differences between these preparations in the amplitude of the relatively weak K increases in the inner nuclear layer must be due to some other factor. We favor the possibility that electrode-induced tissue damage through the various retinal layers in eyecups creates a pathway through which K increases in the IPL may diffuse more readily to the inner nuclear layer recording sites. However, we cannot rule out differences between the experiments such as stimulus intensity, level of background illumination, superfusates, and the geometry of the preparation (regarding lateral diffusion of K^+ and the effective shape and diameter of the stimulating spot). Regardless of the reason, however, the retinal slice provides a preparation in which the separation of the distal and proximal K increases is clear, and therefore the study of the distal K increase becomes less ambiguous.

If electrode-induced tissue damage does explain the relatively greater K increases in the inner nuclear layer of eyecup retinas than in slices, this phenomenon would be of importance wherever an accurate determination of ΔV_K is required at nearby locations and where ΔV_K is changing appreciably as a function of depth. It is less clear to what extent tissue damage would affect profiles of tissue resistance, but the effects should again be greatest where accurate determination of resistance is required across adjacent depths of small separation, or where resistance changes rapidly with depth. These issues are relevant with regard to computation of ion source-density (Karwoski et al., 1982) and current source-density (Freeman and Nicholson, 1975) profiles in general, and especially in the retina (Faber, 1969; Newman, 1980; Vogel, 1980; Newman and Odette, 1984).

Comparison of Magnitudes of Distal vs. Proximal K Increases

Our calculations suggested that the distal K increase has, at the very most, only ~10% the total magnitude of the proximal K increase, whereas a recent model of b-wave generation (Newman and Odette, 1984) predicts a value closer to 60%. Although our results do not obviously support the Müller cell hypothesis of b-wave generation, they do not directly contradict the hypothesis, either. There are a number of possible factors, some not previously incorporated into models of b-wave generation, whose influence is uncertain and could possibly affect the magnitude of the distal K-increase needed to generate the b-wave. These include the following.

(a) The model of Newman and Odette (1984) assumed similar K^+ conductance in the Müller cell in both the OPL and the IPL. A K increase in the OPL would generate a b-wave response proportionately larger than that generated by a K increase in the IPL if (i) Müller cell K^+ conductance were found to be higher in the OPL than in the IPL or if (ii) other conductances (such as voltage-sensitive Ca^{2+} or K^+ channels, which have been observed in other glial cells [Chiu et al.,

1984; MacVicar, 1984]) were found in disproportionate numbers in the OPL. The Müller cell is known to possess at least one region (the endfoot) of high K^+ conductance (Newman, 1984), and although Newman's study did not demonstrate a relatively high conductance region of the Müller cell membrane in the OPL, such a membrane specialization cannot be ruled out from the data currently available.

(*b*) The small size of the distal K increase may still be due, at least in part, to electrode-induced tissue damage. For example, Newman and Odette (1984) calculated that a 12.5-μm-diam cylinder of damaged tissue (with an extracellular volume fraction of 1.0) centered on the electrode tip would result in an ~40% response reduction in recorded $\Delta[K^+]_o$. Since their model assumed a relatively thick (25 μm) layer acting as a K^+ source, response degradation due to electrode-induced tissue damage should be even more severe in the thin (6 μm) OPL. On the other hand, it is reasonable to suppose that tissue damage will induce less distortion in recordings of K increases from the retinal slice, particularly in plexiform layers, which are composed of small-diameter neural processes. It is additionally confounding, however, that the precise shape and diameter (and hence effects) of electrode-induced damage in a tissue that exhibits radial inhomogeneity in the volume fraction of extracellular space (Karwoski et al., 1984) is uncertain. It is also not clear whether the volume fraction approaches 1.0 in damaged tissue—it is possible that the presence of an electrode decreases the responsivity of adjacent tissue, but has little effect on the geometry of extracellular space.

(*c*) The differences in such factors as the species, the stimulus (intensity, diameter, background level), the preparation (eyecup vs. slice), and the superfusate (its composition, and whether superfusion was even used) between the present study and previous studies make any comparisons imperfect. However, we doubt that any of these differences are critical, since similarly low ratios of distal/proximal K increases were obtained in the present study with a variety of stimulus intensities, preparations, and superfusates. Also, the present results, along with those of Shimazaki (1983) and Shimazaki et al. (1984), are in general agreement with those of Dick and Miller (1978) and Dick (1979) in that all find a distinct distal K increase that is substantially smaller than the proximal K increase. In contrast, though, a relatively large distal K increase has been reported in at least one recording in skate retina (Kline et al., 1978).

In summary, the results of the present study demonstrate unequivocally the existence of a light-evoked K increase arising from the OPL. This increase, as measured with fine K-ISMs, is significantly smaller than that predicted by the Newman and Odette (1984) model of b-wave generation. While our results do not rule out the Müller cell hypothesis of b-wave generation, they do suggest that either the recorded distal K increase is still greatly degraded in time course and amplitude by electrode-induced tissue damage, or that some aspect of the Newman-Odette model may need modification. Additional quantitative studies both on Müller cell physiology and on the various factors that influence measurements of $\Delta[K^+]_o$ in neural tissue are needed to ultimately understand the mechanism of b-wave generation.

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