B-Wave of the Electroretinogram

A Reflection of ON Bipolar Cell Activity

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ABSTRACT Light-evoked intraretinal field potentials (electroretinogram, ERG) have been measured simultaneously with extracellular potassium fluxes in the amphibian retina. The application of highly selective pharmacologic agents permitted us to functionally isolate various classes of retinal neurons. It was found that: (*a*) application of APB (2-amino-4-phosphonobutyrate), which has previously been shown to selectively abolish the light responsiveness of ON bipolar cells, causes a concomitant loss of the ERG b-wave and ON potassium flux. (*b*) Conversely, PDA (*cis* 2,3-piperidine-dicarboxylic acid) or KYN (kynurenic acid), which have been reported to suppress the light responses of OFF bipolar, horizontal, and third-order retinal neurons, causes a loss of the ERG d-wave as well as OFF potassium fluxes. The b-wave and ON potassium fluxes, however, remain undiminished. (*c*) NMA (*N*-methyl-DL-aspartate) or GLY (glycine), which have been reported to suppress the responses of third-order neurons, do not diminish the b- or d-waves, nor the potassium fluxes at ON or OFF. This leads to the conclusion that the b-wave of the ERG is a result of the light-evoked depolarization of the ON bipolar neurons. This experimental approach has resulted in two further conclusions: (*a*) that the d-wave is an expression of OFF bipolar and/or horizontal cell depolarization at the termination of illumination and (*b*) that light-induced increases in extracellular potassium concentration in both the inner (proximal) and outer (distal) retina are the result of ON bipolar cell depolarization.

INTRODUCTION

The electroretinogram (ERG) is a complex field potential produced by the retina. It is generated through the summation of currents contributed by different groups of retinal cells, including neurons, glia, and epithelia. This phenomenon has been known since 1865 (Holmgren) and has been the object of intense scientific investigation since the 1940s. In early studies the ERG was used as an indicator of generalized retinal and brain functions in such conditions as anoxia, toxicosis, or ischemia (Granit, 1933; Noell, 1953, 1954). More recently, cellular methods have led to a mechanistic analysis of the contributions made by the various types of retinal cells in

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Volume 93 January 1989 101–122

ultimately producing the ERG (cf. reviews by Brown, 1968; Rodieck, 1972, 1973; Tomita and Yanagida, 1981). Such knowledge provides the potential of a clinically sensitive technique for the detection of subtle disruptions in retinal function. The ERG component most amenable to clinical study is the large amplitude, corneapositive b-wave. Understanding the specific cellular mechanisms leading to its generation has been the goal of numerous investigations and is also the objective of the work that we report below.

Müller Cell/Potassium Hypothesis

The Müller cell is a retinal glia that probably serves as the direct generator of the b-wave. Faber (1969), based on current-source density analysis, proposed that the sources and sinks most closely related to the b-wave were probably due to ionic fluxes across the membrane of the Müller cell. Miller and Dowling (1970) demonstrated by intracellular recording that the Müller cell light response has a latency, time course, and a dynamic range very similar to the b-wave. The Müller cell toxin DL-alpha amino adipate causes a loss of the b-wave, which provides another link between the b-wave and the Müller cells (Bonaventure et al., 1981; Szamier et al., 1981; Zimmerman and Corfman, 1984), although there is evidence that acute application affects retinal neurons (Stockton, unpublished).

The Müller cell potential changes are not directly light induced and therefore it was hypothesized that the currents across the Müller cell membrane were the result of extracellular potassium increases produced by the depolarizations of retinal neurons. Newman (1980, 1984, 1985, 1986) has shown that the Müller cell membrane has a selective and high permeability to potassium. He also found a Nernstian relationship between extracellular potassium and Müller cell membrane potential. This correlates with Miller's (1973) finding that the b-wave has a Nernstian relationship with extracellular potassium. In addition, Fujimoto and Tomita (1981) and Yanagida and Tomita (1982) found that when potassium is injected intraretinally, negativities (sinks) are produced at the depth of the injection within the retina, whereas positivities (sources) are always seen at the retinal surface and in the vitreous irrespective of the depth of injection. These studies provide strong support for the hypothesis that local increases in extracellular potassium produce currents along the Müller cell that result in the induction of a field potential. The b-wave occurs at light onset and is presumably a reflection of ON cell activity. Three cell types depolarize at light onset: the ON bipolar cells in the distal retina, and the ON amacrine cells and ON ganglion cells in the proximal retina. Therefore, some or all of these neurons are the likely sources of the potassium flux that leads to the generation of the b-wave.

Actual measurements of light-evoked potassium fluxes in the various retinal layers do not clearly indicate which neurons play a primary role in generating the b-wave. The Müller cell hypothesis presumes that an extracellular increase in potassium occurs at light onset near neurons that are linked to b-wave generation. Using ion-selective microelectrodes, extracellular increases in potassium are found in both the outer and inner plexiform layers at light ON; whereas a decrease is found in the region of the photoreceptor cells (Oakley, 1975; Dick, 1979; Steinberg et al., 1980; Dick and Miller, 1985; Karwoski et al., 1985; Kline et al., 1985). Dick and Miller

(1978, 1985) have found that when they use GABA or alpha amino pimelic acid they can suppress the proximal potassium flux without diminishing the distal potassium flux nor the b-wave. This suggests that the distal potassium flux generates the b-wave. Newman and Odette (1984) using an electrical model to analyze currents also suggested that the relatively short duration distal ON potassium increase is the dominant factor in the generation of the b-wave and that experimental procedures tend to underestimate the magnitude of this distal flux. But the magnitude of the ON potassium flux in the proximal retina is greater than in the distal retina. Dick and Miller (1985) reported a calculated distal ON potassium increase that is 56% of the proximal ON increase, while Karwoski et al. (1985) found the distal increase to be 10% of the proximal ON increase. The latter concluded that the potassium flux in the outer retina may be insufficient to generate the b-wave.

Recent findings that specific neurotransmitter agonists and antagonists have highly selective effects on specific classes of retinal neurons permits a new approach for determining the neuronal generators of the various ERG components. In particular, we have taken advantage of the complementary actions of several glutamate analogues that differentially block the action of glutamate, the putative photoreceptor transmitter. APB (2-amino-4-phosphonobutyrate) selectively blocks the light responsiveness of ON bipolar cells while leaving horizontal and OFF bipolar cells undiminished. Therefore, all depolarizing (ON) responses to an increase in illumination are eliminated in the retinal network (Slaughter and Miller, 1981).

PDA (*cis* 2,3-piperidine-dicarboxylic acid; Slaughter and Miller, 1983*b*; 1985*a*) and KYN (kynurenic acid; Coleman et al., 1986) are glutamate antagonists that strongly suppress synaptic transmission from photoreceptors to horizontal and OFF bipolar cells although they do not diminish the response of the ON bipolars. Both antagonists also greatly reduce the transmission between bipolar cells and third-order neurons. The net result of these actions is to leave the ON bipolar cell as the only fully functional postphotoreceptor neuron.

Other neurotransmitter analogues predominately affect only third-order neurons. GLY (glycine) suppresses the light response of amacrine and ganglion cells through a chloride-dependent mechanism that usually produces a pronounced hyperpolarization (Miller et al., 1981*b*). NMA (*N*-methyl-DL-aspartate) strongly suppresses the light responses of these third-order neurons through a depolarizing block (Slaughter and Miller, 1983*a*).

Use of these drugs serially or in combination permits a differential analysis of the neuronal basis of b-wave generation (Stockton and Slaughter, 1986, 1987). Our results confirm the hypothesis that ON bipolar activity closely correlates with the b-wave and suggests a similar relationship between OFF bipolars and/or horizontal cells with the d-wave. These results also indicate that the ON potassium flux in both the distal and the proximal retina is primarily a result of ON bipolar activity.

METHODS

Preparation and Stimulation

Experiments were conducted using a superfused retina-eyecup preparation (Miller and Dacheux, 1976) in mudpuppy (*Necturus maculosus*) and tiger salamander (*Ambystoma tigrin-*

ium). No differences were found between these closely related amphibians. In brief, the animal was decapitated and the head was transected sagitally. The globe and some surrounding tissue were dissected free and placed upon Ringer's-soaked cotton. The cornea, iris, and lens were excised and the vitreous was removed by dissection with a fine pledget and iris scissors. A piece of absorbant tissue, with a small hole exposing the retina, was centered over the eyecup to draw off the superfusate. The eyecup preparation was placed upon Ringer's-soaked cork in contact with a Ag/AgCl reference electrode. Solutions were delivered through a pipette connected to a manifold system having a dead time of ~30 s. Control perfusate consisted of 111 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl, 1.0 mM MgCl, 10 mM glucose, and 5 mM HEPES buffer. The pH was adjusted to 7.8 with NaOH and the perfusate was continuously bubbled with 100% O_2. Pharmacologic agents were added to this Ringer's to form the following experimental solutions: 100 μM APB (Calbiochem Behring Corp., La Jolla, CA), 5 mM KYN (Sigma Chemical Co., St. Louis, MO), 5 mM PDA (Cambridge Research Biochemicals, Harston, Cambridgeshire, England), 4 mM Co^{++} (cobaltous chloride; Sigma), 500 μM NMA (Sigma), 500 μM GLY (Sigma).

Light stimulation, produced by a red Stanley LED (Nygaard and Frumkes, 1982), was a diffuse step illumination of variable duration and interstimulus interval and of sufficient intensity (13 $\mu W/cm^2$) to evoke a b-wave of near-maximal amplitude. The use of the red LED preferentially stimulated cones and there was no attempt to dark adapt the animals before the experiments.

Electrodes and Recording Procedures

Potassium ion-selective microelectrodes (Coles and Tasacopolos, 1977, 1979; Dick, 1979; Deyhimi and Coles, 1982) were fabricated from double-barreled triangular glass (1 mm/side, Glass Company of America, Bargaintown, NJ) and pulled in a horizontal puller (Narashige Scientific Laboratory, Tokyo, Japan). The tips were broken under a microscope. One barrel was filled with Ringer's for voltage recordings. The other barrel was silanized (4% dimethyldichlorosilane in carbon tetrachloride) by immersion, its tip filled with exchange resin (477317; Corning Glass Works, Corning, NY) and then back-filled with control Ringer's (2.5 mM KCl). The electrode tips were then beveled to a diameter of ~3–5 μM. Electrodes were calibrated before and after recording by placement in a series of Ringer's solutions (as above) that had varying concentrations of KCl (typically 3, 4, 5, and 25 mM), and then by computing an average response slope. A slope of >52 mV/tenfold concentration change (Nernstian = 58 mV/10X), was considered acceptable. Intracellular recordings were obtained using microelectrodes fabricated from borosilicate "omega dot" glass (0.6-mm i.d., 1.2-mm o.d.; Glass Company of America) back-filled with 2 M potassium acetate.

Simultaneous measurements of potassium fluxes ($[K^+]_o$; Lux and Neher, 1973; Fisher et al., 1976; Nicholson et al., 1979) and field potentials (V_o, ERG) were made using a dual channel, high impedance (10*15Ω) preamplifier that permitted cross-capacitance neutralization (modified from Dick and Miller, 1985). Signals were then fed to second-stage amplifiers for additional gain, filtering, and DC offset. Intracellular recordings were obtained using a preamplifier (707A; World Precision Instruments, Inc., New Haven, CT). Data were recorded by means of an oscilloscope and penwriter.

Electrode Localization

Previous workers (Brown and Wiesel, 1959; Faber, 1969; Dick and Miller, 1985; Karwoski et al., 1985) have established a consistent relationship between electrode position (retinal depth) and both field potential waveform and potassium flux pattern. We have used these criteria along with readings from our micrometer drive to estimate the location of the electrode tip. Typical responses at selected retinal depths are shown in Fig. 1.

RESULTS

The selective glutamate agonist APB eliminates the light responses of ON (depolarizing) bipolar cells in the distal retina, but does not suppress the responses of photoreceptors, horizontal cells, or OFF bipolars. Since the ON bipolar cell is the source of

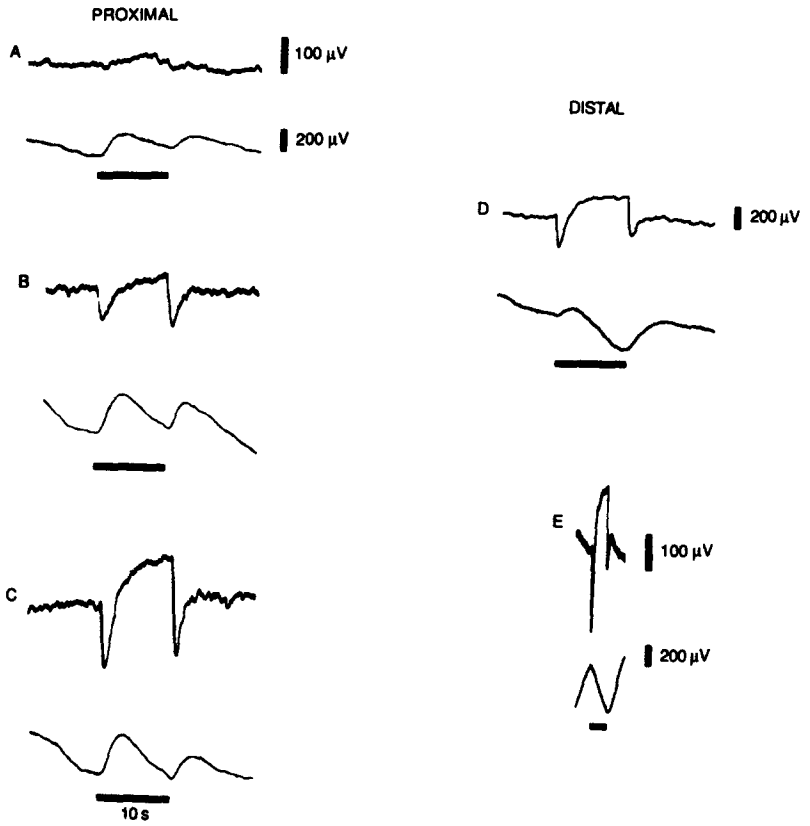

FIGURE 1. Simultaneous extracellular potassium and field potential recordings using a double-barreled micropipette. The top trace of each pair is the intraretinal ERG while the bottom trace shows the concomitant changes in potassium ion activity in response to a diffuse illumination (bar). Shown are typical waveforms encountered as the pipette is advanced from the ganglion cell layer (*A*) to the photoreceptor layer (*E*). The responses shown in *B* were recorded near the polarity reversal of the b- and d-waves in the inner plexiform layer (IPL) and those in *C* are from the inner nuclear layer (INL) before the ON potassium flux begins to diminish. *D* shows the transition from the proximal to distal retina at the outer plexifiom layer (OPL), which is characterized by an increase (53%) in the amplitude of the b-wave (note gain change) and a decrease (80%) in the ON potassium flux. Near the photoreceptors (*E*) there is only a decrease in extracellular potassium during illumination following an increase at light OFF.

the ON pathway, APB effectively eliminates all responses to light onset throughout the retina. Fig. 2 shows the effect of APB on the ERG. In this eyecup, in which perfusate exchange was unusually slow, it is possible to clearly see the progressive loss of the negative going b-wave in the intraretinally recorded ERG (arrow), which

eventually results in a light response waveform that is a monotonic positive potential. The effect is fully reversible, as shown in the far right of the figure. This implies that the b-wave of the ERG is associated with the ON system in the retina. Similar findings have been reported by Slaughter and Miller (mudpuppy, 1981), Massey et al. (rabbit, 1983) and Knapp and Schiller (primate, 1984). However, APB eliminates the ON responses in both the inner and outer retina and therefore this drug cannot be used alone to determine which cell type leads to the generation of the b-wave. The a-wave and the d-wave are not diminished by APB and therefore it appears that

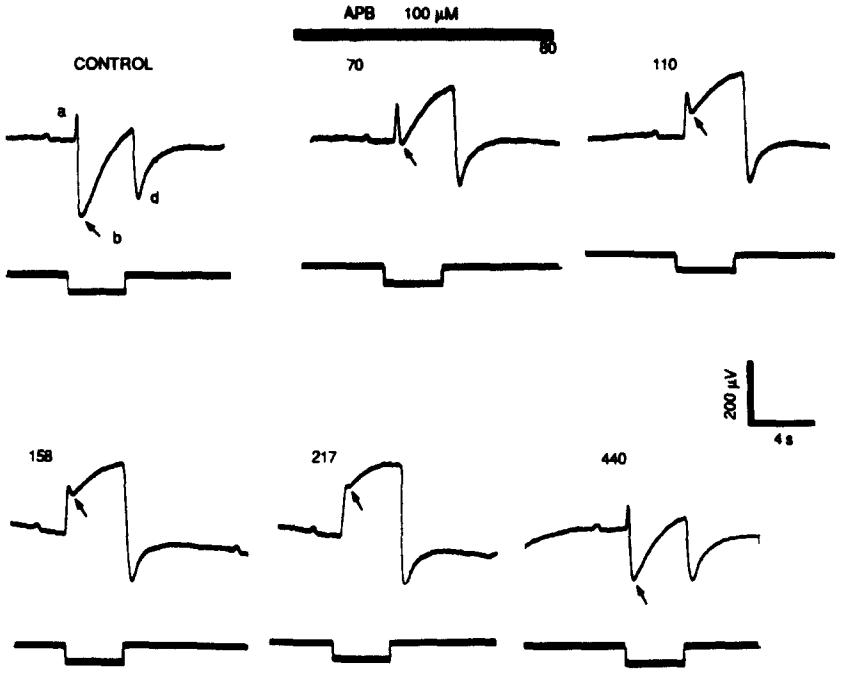

FIGURE 2. Intraretinal ERG recorded with a low-resistance single-barreled pipette in response to a diffuse illumination of a duration indicated by the square wave in the lower trace. The a-, b-, and d-waves of the ERG are identified in the control response. The numbers indicate the time in seconds after the change from control solution to 100 μM APB. At 70 s the b-wave amplitude (arrow) has been reduced to about one-third control. By 217 s the B-wave had been virtually abolished but this effect is fully reversible (440 s). Note that d-wave (OFF response) is not diminished by APB.

these components of the ERG represent activity in cellular elements that are not related to the ON system.

The results using APB indicate that ON bipolars, or the ON components of amacrine and ganglion cells, or some combination thereof, are indirect generators of the b-wave. To distinguish between these distal and proximal elements, PDA or KYN were used. These glutamate antagonists block OFF bipolar and horizontal cell light responses, but do not diminish photoreceptor nor ON bipolar light responses. Therefore, in the distal retina, PDA or KYN act in a complementary manner to APB

by the selective suppression of the OFF channel. However, in the inner retina, they suppress both ON and OFF responses in all third-order neurons. The only neurons that remain fully functional in the presence of PDA or KYN are the photoreceptors and the ON bipolars. In the context of the present experiments, these antagonists help to distinguish the effects attributable to light-induced ON bipolar cell activity, which are not suppressed by these antagonists, from those effects dependent upon ON responses of amacrine and ganglion cells, which are suppressed. Fig. 3 shows the effects of PDA and KYN on the intraretinal ERG. The top set of figures show an ERG recording that contains very prominent b- and d-waves. Application of 5 mM

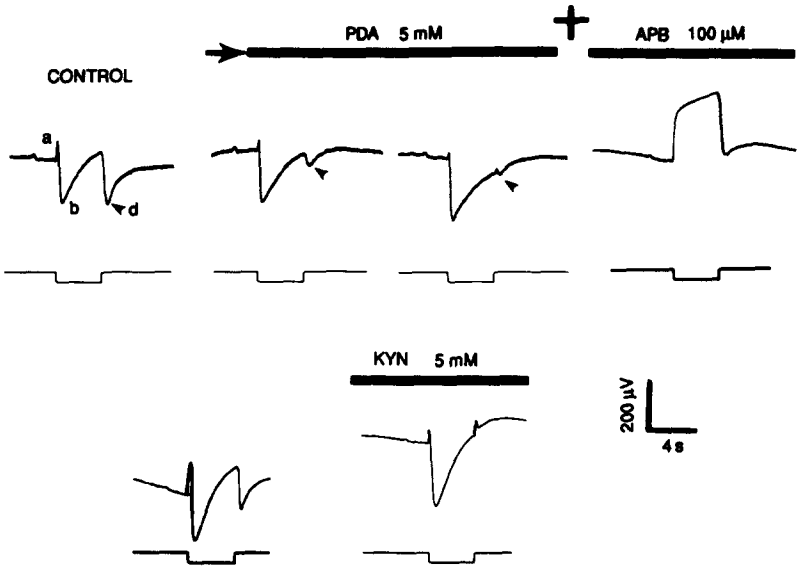

FIGURE 3. Intraretinal ERG recorded as in Fig. 2 which shows that applications of PDA (5 mM) or KYN (2 mM) result in the virtual elimination of the d-wave (arrows) while leaving the b-wave undiminished or even augmented. The combination of PDA and APB blocks the light responsiveness of all postphotoreceptor neurons, thereby eliminating both the b- and d-waves. What remains is the photoreceptor-dependent a-wave/slow PIII complex. These effects are reversible as shown by the lower left trace, which also serves as a control before KYN application.

PDA initially produces a prolongation of the time course of the b-wave. The full effect of PDA is seen 30 s later, at which point the light response consists of a negative potential throughout the duration of the light stimulus. At OFF, there is a positive deflection in the ERG. This waveform looks like an inverted ON bipolar response and, based on the known cellular actions of PDA, it would suggest that the ERG ON response is dominated by the ON bipolar cell under these conditions. Support for this is shown in the last trace on the top row; when APB is applied in the presence of PDA, the ERG waveform flips over and the response is a monotonic positive wave. There is no negative undershoot at light off, as was seen during application of APB alone (Fig. 2), because of the suppression of the d-wave by PDA. The

ERG, in the presence of both APB and PDA, represents a loss of all postphotoreceptor synaptic activity. The loss of the negative-going potentials at ON and OFF (b- and d-waves, respectively) is similar to that seen when cobalt was applied (see below), which also blocks all postphotoreceptor light-driven neuronal activity. The remnant positive-going potential is the photoreceptor-dependent combination of a-wave and slow PIII (Granit, 1933; Witkovsky et al., 1975; Oakley et al., 1979; Winkler and Gum, 1981; Oakley and Schimazaki, 1984), although a component of this remaining response may be a c-wave (Matsuura et al., 1978). The lower record shows that KYN

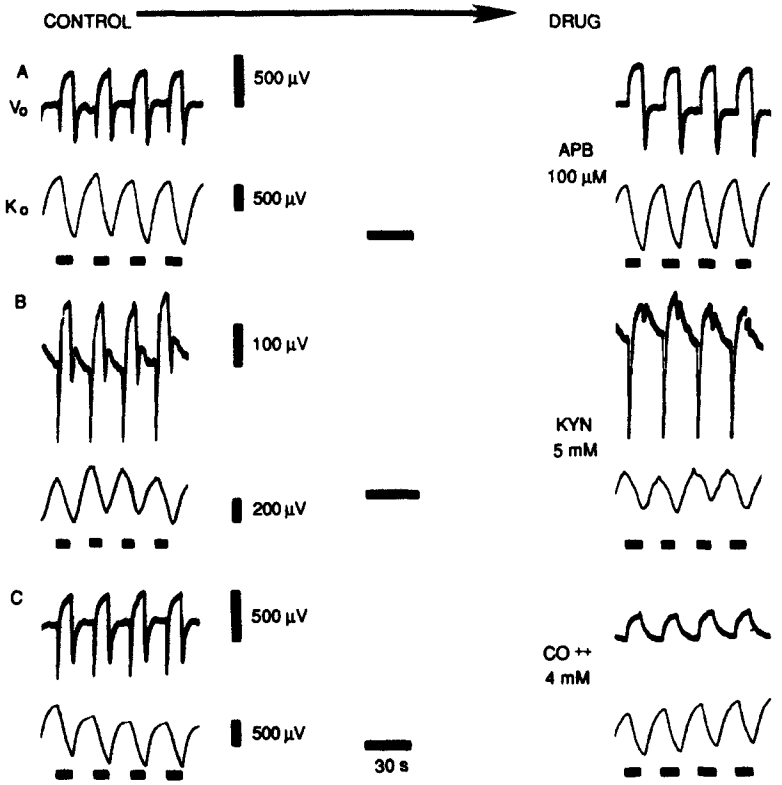

FIGURE 4. Photoreceptor layer. Simultaneous extracellular recordings of the ERG (V_o) and potassium activity $[K^+]_o$ in response to diffuse illumination (bar) showing the control response and and maximal drug effects after applications of the indicated agents for a minimum of 3 min. Note that the potassium fluxes are virtually unaltered under all conditions, indicating that in the far distal retina they are associated with photoreceptor activity.

has a similar effect. 2 mM KYN also prolonged the timecourse of the negative ON response and the d-wave was completely blocked. Thus, PDA and KYN, agents that suppress most second- and third-order neurons but spare the ON bipolars, enhance the b-wave. The enhancement of the b-wave matches the effects of these antagonists on the ON bipolars, which show larger and more sustained responses to full-field illumination in the presence of PDA or KYN, due to the loss of the horizontal cell–mediated antagonistic surround (Slaughter and Miller, 1983*b*). In contrast, the d-

wave is virtually eliminated. This presumably represents suppression of the OFF system, either OFF bipolars or OFF responses of third-order neurons, or both.

These results indicate that the b-wave of the ERG is dependent upon ON bipolar cell activity. In view of the Müller cell hypothesis, we wanted to determine the effect of these drugs on potassium fluxes at the different retinal layers. Fig. 4 shows the effect of APB, KYN, and cobalt on the ERG (V_o, upper traces) and potassium activity ($[K^+]_o$, lower traces) in the far distal retina at the level of the photoreceptors. The ERG contains the transient ON and OFF negative potentials (b- and d-waves, respectively) and the prominent sustained positive potential (slow PIII) characteristic of recordings taken near the photoreceptor layer. APB produced a loss of the b-wave of the ERG but very little change in the potassium record. KYN produced a marked diminution in the d-wave but also had little effect on potassium fluxes. At light offset, the ERG potential decays slowly to baseline. In trace *C*, cobalt is applied, which blocks both the b- and d-waves leaving a simple monotonic positive response (a-wave and slow PIII) during illumination. The potassium record again remains essentially unaltered. Overall, recordings made in the photoreceptor layer indicate that antagonists of the b-wave, the d-wave, or both, have little effect on the local extracellular potassium activity around the photoreceptors (Immel and Steinberg, 1986).

In a similar series of experiments, which were performed while recording slightly more proximally, near the outer plexiform layer, effects on local potassium fluxes as well as the ERG were detected (Fig. 5). The ERG record showed transient negative b- and d-waves and a positive-going wave that is less prominent than in the previous Fig. 4. The potassium recordings showed an initial small transient increase at light onset followed by a potassium decrease for the remainder of the light stimulus. This small potassium increase has been described previously by Dick and Miller (1978) who proposed that it represents a distal potassium efflux resulting from ON bipolar cell depolarization and that it indirectly produces the ERG b-wave. Application of APB eliminated the b-wave with a concurrent loss of this small potassium increase at light onset. KYN blocked the d-wave of the ERG and slightly diminished the sustained positivity, but the b-wave remained. The potassium recordings indicate that the transient potassium increase at light onset persisted but the fast potassium increase at light offset was attenuated.

In Fig. 5 *C*, cobalt was used to block all postphotoreceptor light-driven activity. The resultant changes in the ERG and potassium response waveforms appear to result from a combination of the upper two records. Before cobalt application, the ERG exhibits negative b- and d-waves and a sustained positivity, but cobalt produces a loss of both b- and d-waves, and a slight decrease in the amplitude of the positivity. In the potassium record, cobalt eliminated both the transient increases at light ON and light OFF, leaving only a small potassium decrease at light onset which was similar to our findings near the photoreceptors. Thus, at the level of the outer plexiform layer (OPL), agents that block the b-wave, such as APB and cobalt, suppress the ON transient potassium increase, while agents that block only the d-wave have no effect on this potassium flux. Our finding is consistent with the hypothesis that the ON transient potassium increase is linked to the generation of the b-wave.

Recordings from the inner retina are illustrated in Fig. 6. As in the outer retina, APB blocked the b-wave of the ERG and blocked the large potassium increase at

light onset. Conversely, both PDA and KYN (Fig. 6, *B* and *C*) did not significantly decrease the ON potassium flux. In the example shown, the ON potassium flux, measured at the maximal effect of PDA (6 mM) application, was not appreciably different from the control. KYN (5 mM) resulted in a slight (16%) increase in the amplitude of the potassium flux at light ON. In the recordings from the inner plexiform layer (IPL), PDA and KYN produced the same general effects as were seen in the distal retina. There was no diminution in the amplitude of either the ERG or the potassium record to the onset of illumination, whereas both responses at light OFF were suppressed.

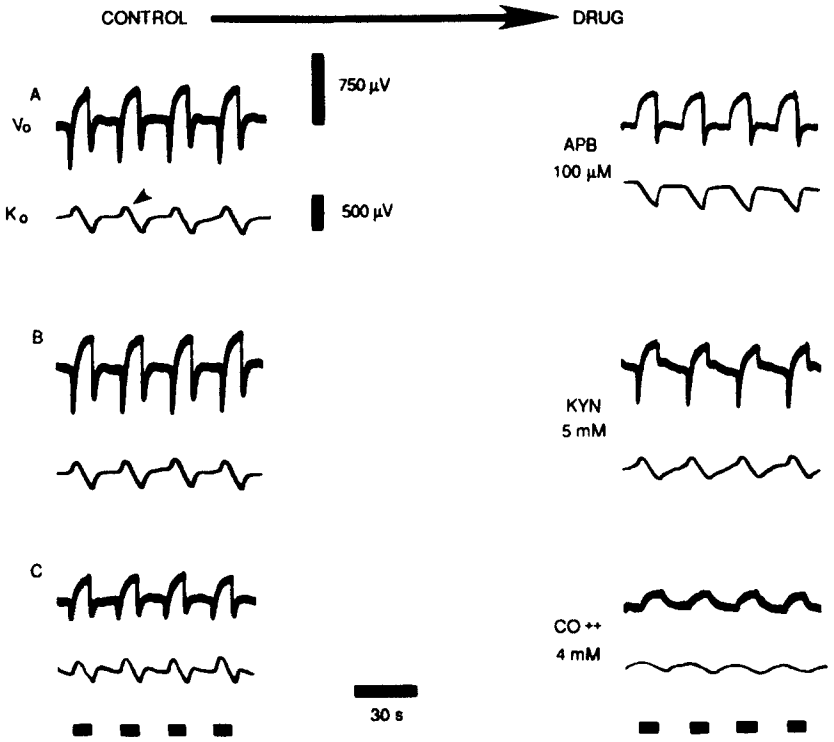

FIGURE 5. Outer retina. Recordings from the OPL under conditions similar to those of Fig. 4. There is a small potassium flux at light ON (distal ON), which is eliminated by APB application but not by KYN. Cobalt blocks all postphotoreceptor synaptic potentials and the remnant activity may be ascribed to the photoreceptors.

These results suggest that the potassium flux in the IPL, as measured in our experimental protocol, is primarily a product of ON bipolar cell depolarization. To further test this hypothesis we used neurotransmitter agonists NMA and GLY, which have dramatic effects on the light-evoked responses of third-order neurons but have comparatively little effect on the light responses of second-order cells. The intracellularly recorded responses of a representative sample of third-order neurons are shown in Fig. 7. GLY (Miller et al., 1981*a*) is an inhibitory amino acid that

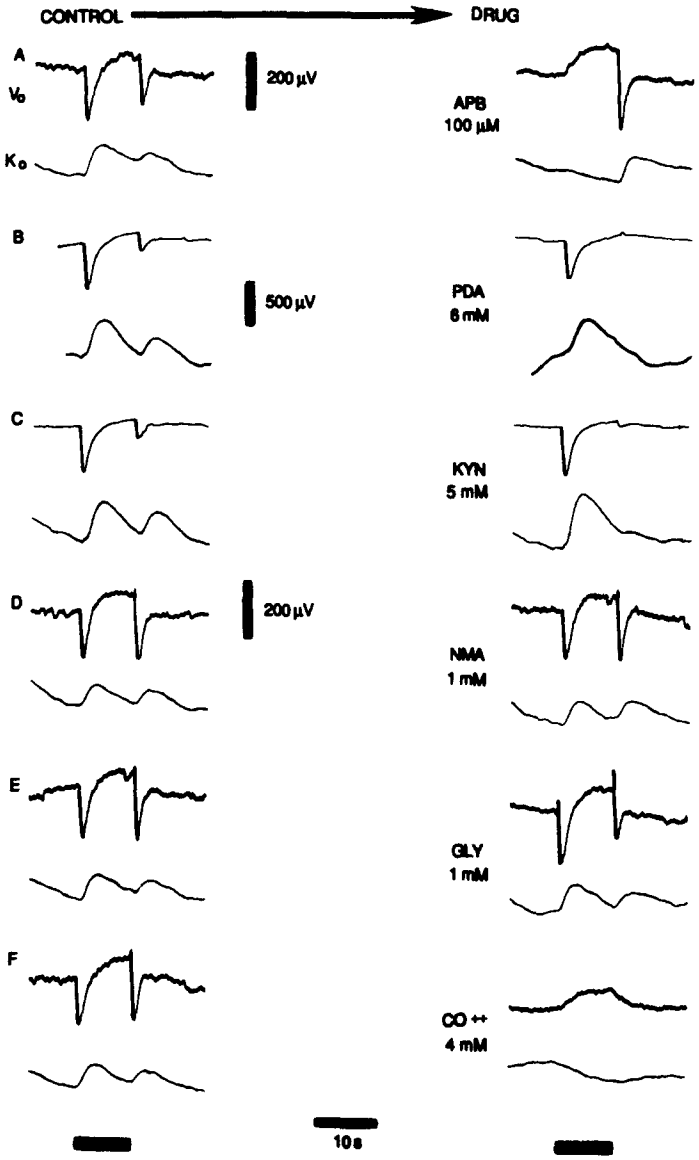

FIGURE 6. Inner retina. Recordings at ~20–30% retinal depth (IPL). Each trace is the response to the ON set and OFF set of a single diffuse light stimulus (bar). APB eliminates the b-wave and the concomitant potassium flux at light ON. PDA and KYN, conversely, have no discernable effect upon the ON channel but eliminate the d-wave and OFF potassium flux. NMA and GLY primarily affect inner retinal cells and do not substantially reduce the b- or d-waves or the potassium fluxes at ON or OFF. Cobalt produces a field potential similar to that seen in the outer retina, consisting of an a-wave and slow PIII. The potassium record was variable and this record does not represent a light response.

hyperpolarizes almost all third-order neurons and shunts their light responses (Fig. 7 *B*). It has a lesser effect on OFF bipolars and its effect on ON bipolars is small. NMA (Slaughter and Miller, 1983*a*) is a selective excitatory amino acid agonist that depolarizes amacrine and ganglion cells and eliminates their light responses (Fig. 7 *C*). KYN, like PDA, blocks synaptic input to the inner retina, presumably by antagoniz-

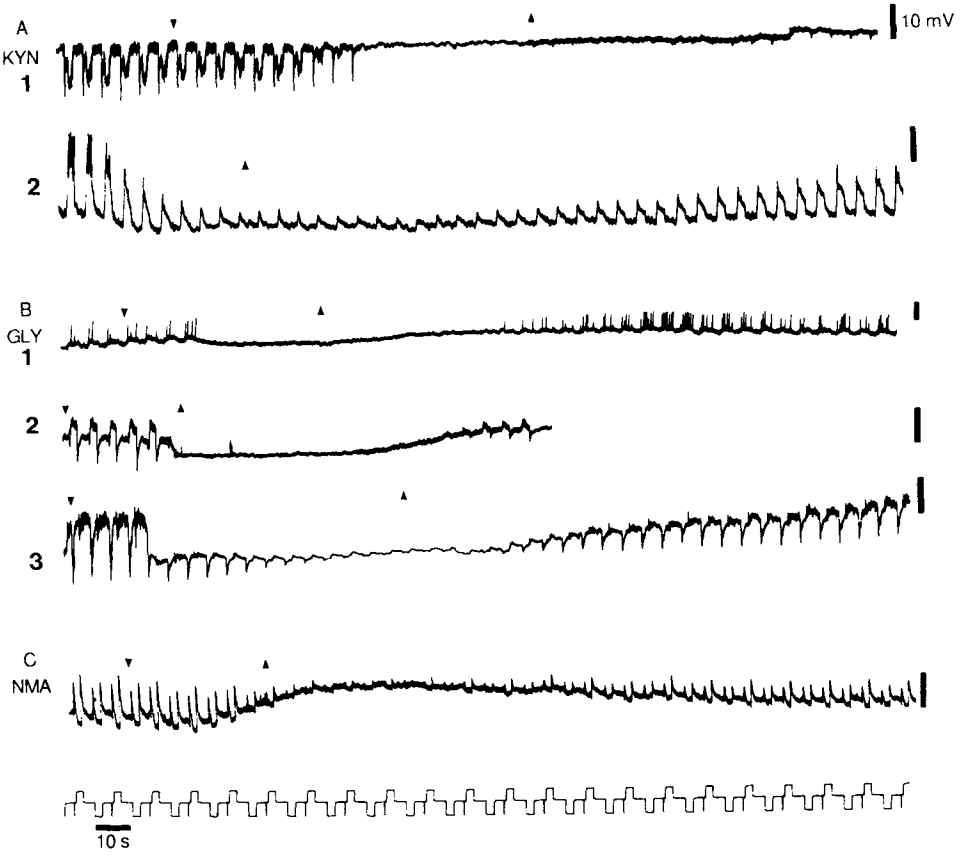

FIGURE 7. Intracellular recordings from a representative sample of third-order neurons: ON ganglion cells (*A2*, *B1*, *B2*), OFF ganglion cells (*A1*, *B3*), and an ON-OFF amacrine cell (*C*), demonstrating complete or near block of the light response after short applications (indicated by the arrow points) of 5mM KYN, 1 mM GLY, or 500 μM NMA, each of which acts by a different mechanism as described in the text. The square pulses at the bottom of the figure indicate the timing of light stimuli. Upward deflections represent a red stimulus and downward deflections a green. The calibration bars to the right of each trace are a 10 mV demarcation.

ing the bipolar cell transmitter (Slaughter and Miller, 1983*c*; Coleman et al., 1986).

Fig. 6 shows that neither GLY (Fig. 6 *E*) nor NMA (Fig. 6 *D*) diminish either the voltage or potassium responses as recorded in the proximal retina. Cobalt (Fig. 6 *F*) is used as a control and abolishes both the b- and d-waves of the ERG as well as the

ON and OFF potassium fluxes. All that remains is the sustained positivity which corresponds to the photoreceptor-related a-wave/slow PIII complex. It is interesting to note that NMA did not produce a potassium efflux due to depolarization of third-order neurons. This contrasts with our results using kainic acid, which depolarizes

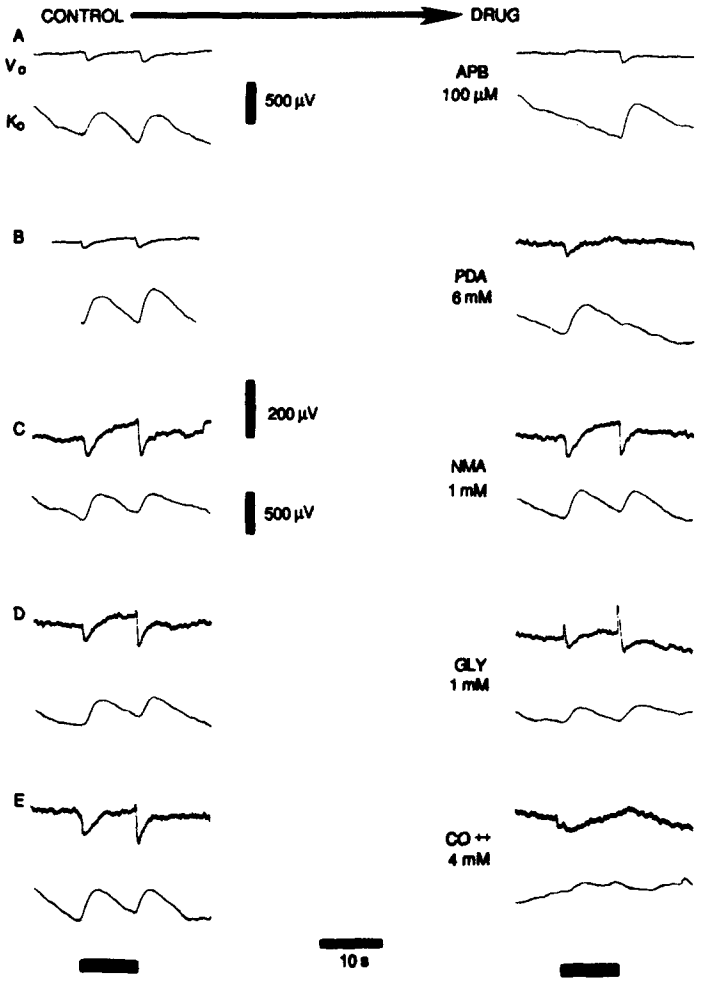

FIGURE 8. Ganglion cell layer (5% retinal depth). These results are substantially the same as seen in the IPL (Fig. 6). Even in the very superficial retina, the potassium fluxes remain after the application of drugs (NMA, GLY) known to strongly suppress light responsiveness in third-order retinal cells.

second- and third-order neurons, and produces a very large increase in extracellular potassium in both the OPL and IPL.

The recordings in Fig. 8 are from the ganglion cell layer. In this experiment the recording pipettes have not penetrated deeper into the retina to avoid possible contamination by potassium flowing through a transretinal electrode track. The results

of the pharmacologic manipulations are essentially the same as in Fig. 6. Although there was some trial to trial variability during GLY administration, the mean amplitude of the potassium flux was not appreciably reduced. These data indicate that agents that block light-evoked activity in third-order neurons, either by inhibition or depolarizing block, do not block the b-wave nor the potassium flux in the inner retina.

DISCUSSION

The pharmacological studies of Dick and Miller (1978, 1985), Shimazaki et al. (1984), and Karwoski et al., (1985) all demonstrated that when the proximal ON potassium increase was blocked, but the distal ON potassium increase remained, the b-wave persisted. This provides strong evidence to suggest that the distal ON potassium increase is linked to the b-wave. The use of more specific pharmacological agents in the present study helps to clarify the role of the ON bipolar in the generation of potassium fluxes in the retina. The main conclusion of this study is that the b-wave of the ERG is a result of the light-evoked depolarization of the ON bipolar neurons. Pharmacological agents that selectively block the activity of the ON bipolar also block the b-wave of the ERG. Agents that affect other cell types, but which do not block ON bipolar activity, do not block the b-wave. A variety of agents were used that blocked the light activity of (*a*) all postphotoreceptor neurons (cobalt or PDA + APB), (*b*) ON bipolar neurons selectively (APB), (*c*) all postphotoreceptor cells except the ON bipolars (PDA and KYN), or (*d*) all third-order neurons (*N*-methyl-aspartate and GLY). Each of these paradigms demonstrates a positive correlation between ON bipolar activity and the b-wave. This experimental approach has resulted in two further conclusions: (*a*) that the d-wave is an expression of OFF bipolar and/or horizontal cell depolarization at the termination of illumination and (*b*) that light-induced increases in extracellular potassium concentration in both the inner (proximal) and outer (distal) retina are mainly the result of ON bipolar cell depolarization.

Pharmacological Separation of Retinal Elements and Effects on the ERG

Our conclusions depend upon the use of several well-characterized pharmacologic agents as summarized in Fig. 9. Slaughter and Miller (1981) have shown that the glutamate analogue APB blocks synaptic transmission between the photoreceptors and ON (depolarizing) bipolar cells by mimicking the action of the endogenous transmitter. The net result is a suppression of the ON information channel. Light responses of the other classes of second-order neurons (horizontal cell, OFF bipolar) remain undiminished by doses tenfold greater than the concentrations used in these experiments (100 μM). Third-order neurons are apparently not directly affected by APB but ON responsiveness is lost due to the removal of ON bipolar synaptic input. OFF responsiveness remains intact. Our finding that the application of APB results in the loss of the b-wave confirms previous observations by Slaughter and Miller (1981).

We have also done the complementary experiment of suppressing the OFF bipolar horizontal cells and third-order neurons by the use of PDA or KYN (Slaughter and Miller, 1983*b*, *c*; Coleman et al., 1986). We found that when the ON bipolar is the

only fully functional postphotoreceptor neuron, the b-wave remains intact. We therefore have pharmacologically isolated the light-induced depolarization of the ON bipolar cell and shown it to be necessary for the generation of the ERG b-wave.

Agents that block the light responsiveness of only third-order neurons (NMA,

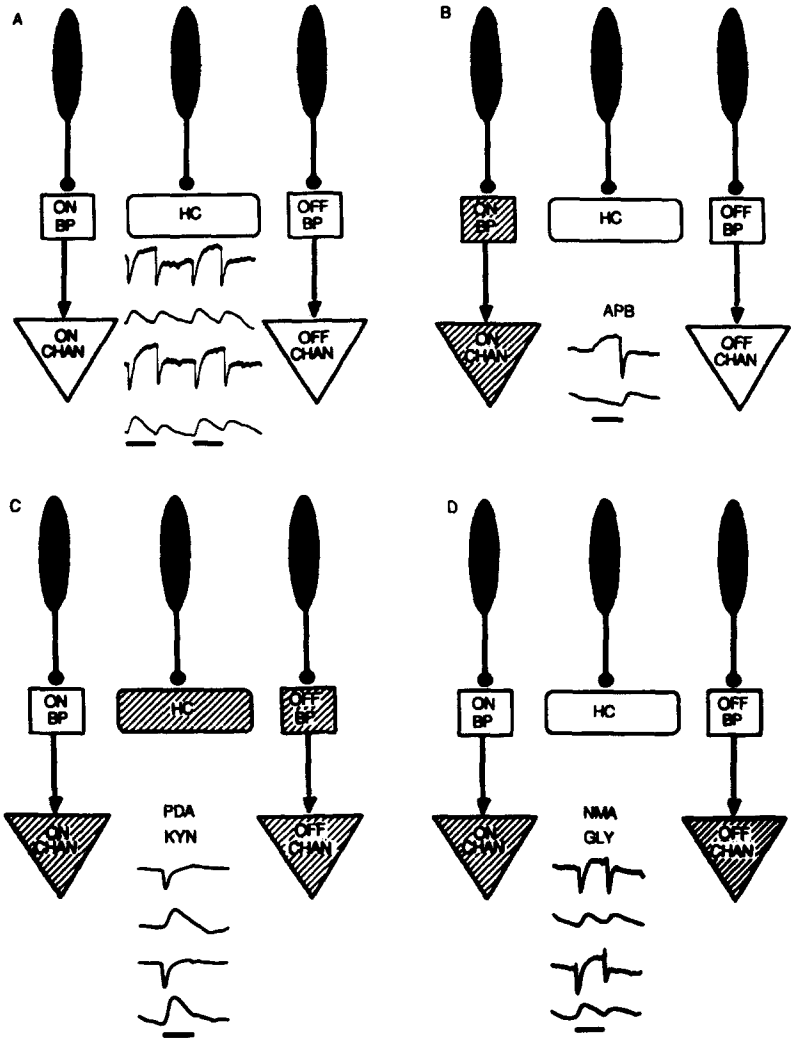

FIGURE 9. Summary diagram in which the elimination of cellular light responsiveness by a drug is portrayed by shading elements of the retinal network. Typical responses from the IPL are also shown in the same manner as Fig. 6. Four successive control responses (*A*) are shown to illustrate the slight variability.

Slaughter and Miller, 1983; GLY, Miller et al., 1981*b*) do not appreciably alter the amplitude or time course of either the b- or d-waves, indicating that both waves primarily reflect neuronal activity in second-order neurons. Cobalt, which abolishes all chemical synaptic transmission in the retina, abolishes both b- and d-waves leav-

ing only photoreceptor-related phenomena (a-wave, slow PIII). When APB and PDA are applied concurrently, the effect is very similar to cobalt.

The finding that PDA and KYN suppress the d-wave, while NMA or GLY have no significant effect, suggests that the d-wave is dependent upon the OFF bipolar and/or horizontal cell. At present there are no potent agents that permit us to definitively isolate these two classes of cells in order to determine their relative contributions to the ERG.

Light-evoked Extracellular Potassium Fluxes Associated with Specific Classes of Retinal Neurons

The link between the neuronal light responses and the ERG is presumably the effect of potassium fluxes. Several groups have found light-correlated potassium fluxes in both the proximal and distal retina (Dick and Miller, 1985; Karwoski et al., 1985), and Newman and Odette (1984) have modeled these fluxes in combination with current-source density data to derive the generator current for the ERG b-wave. One remaining issue is which retinal neurons are responsible for this increase in extracellular potassium at light onset. Using potassium ion–selective microelectrodes in combination with selective pharmacologic agents, we were able to demonstrate that both distal and proximal extracellular potassium increases are primarily due to ON bipolar cell depolarization.

It was anticipated that the distal $[K^+]_o$ increase would be associated with the ON bipolar cell which is the only second-order neuron to depolarize at light onset. In the proximal retina, however, many of the amacrine and ganglion cells also are depolarized by light and the potassium fluxes are of greater magnitude than in the OPL (Miller et al., 1977; Karwoski and Proenza, 1980). Our results indicate that most of the inner retinal potassium flux at light onset is also due to the depolarization of the ON bipolar cells. Application of agents such as NMA and GLY, which block the light responsiveness of third-order neurons did not eliminate the potassium fluxes at either light ON or OFF (Fig. 9). In the 12 experiments in which either or both drugs were tested, there was no discernable decrease in the average magnitude of the light-associated potassium fluxes (Fig. 6 *D*). This comparatively large contribution by bipolar cells may be explicable on the basis of their tonic response properties and the conductances at their synaptic endings. Many amacrine and ganglion cells respond only briefly at the onset of light. In our protocol, where the light is held on for 10 s, the transmembrane currents associated with the maintained depolarization of the ON bipolars may be far more significant than the brief ON currents of transient amacrine and ganglion cells. In addition, Kaneko and Tachibana (1985) have reported that the synaptic terminals of bipolars have large potassium conductances, so that much of the outward potassium current may be localized to this region.

Although PDA and KYN do not completely suppress ON activity in third-order neurons, intracellular recordings from both amacrine and ganglion cells show a >80% reduction in response amplitude using the same concentrations as in these experiments (Fig. 7). If potassium efflux is proportional to the integral of the depolarization (amplitude × duration), then there should be a large reduction in extra-

cellular potassium at light onset in the presence of KYN or PDA, but this was not seen.

Furthermore, even though NMA and GLY both abolish light responses in third-order neurons, they act by very different mechanisms. GLY acts to hyperpolarize these cells through an increase in chloride conductance (Miller et al., 1981*b*). NMA, in contrast, depolarizes amacrine and ganglion cells, thereby strongly suppressing their light responses, while having little effect upon outer retinal cells (Slaughter and Miller, 1983*a*). Both agents open large numbers of ionic channels and essentially voltage clamp third-order neurons. Therefore, synaptic currents do not produce any voltage change across the cell membrane nor a concomitant shift in the driving force for potassium. Under these conditions, we would not expect to see light-evoked potassium fluxes due to third-order neurons. We conclude that the preponderance of the light-associated extracellular potassium in the IPL is also produced by the ON bipolar cell.

Our results, which indicate that potassium fluxes in the inner retina arise from the ON bipolars, seems at odds with the conclusions of Dick and Miller (1978, 1985) and Karwoski et al. (1985). They reported that GABA, or GABA plus ethanol, diminished (~50%) the proximal ON potassium flux but not the distal flux nor the b-wave. They concluded that GABA was acting to suppress third-order neurons while not affecting the ON bipolars. But it is known that GABA also effects ON bipolar cells (Miller et al., 1981*a*; Attwell et al., 1987) and that it acts principally at the synaptic terminal region of the ON bipolars, in the inner plexiform layer (Kondo and Toyoda, 1983; Tachibana and Kaneko, 1987). Thus, another interpretation of their GABA experiments is that GABA is opening chloride channels at the synaptic terminal region of the ON bipolar cells, and therefore chloride may now carry some of the current formerly carried by potassium. This would result in a decrease in the ON potassium flux seen in the proximal retina, provided that the bipolar terminals are not strictly isopotential with the distal regions of the cell. In our experiments we used GLY because it is known that ON bipolars are more sensitive to GABA than to GLY, while third-order neurons are equally sensitive to both transmitter substances (Miller et al., 1981*b*; Stockton et al., 1988).

Shimazaki et al. (1984) report that after prolonged exposure (30 min) to 10–30 mM aspartate, the light-driven proximal potassium flux disappears completely, while the b-wave and the distal potassium flux remain relatively intact. Intracellular recordings indicated that second-order neuronal responses also persisted, although the light responses of third-order neurons are totally suppressed. In addition, the steady state levels of potassium in the proximal retina increased appreciably more than in the distal retina. They concluded that the b-wave is related to the distal ON potassium flux and that the proximal potassium flux is derived from amacrine and ganglion cells. However, another interpretation is that the aspartate and the elevated levels of potassium in the proximal retina depolarize the ON bipolar cells, turning off the voltage-dependent potassium current in the bipolar cell terminals. Kaneko and Tachibana (1985) have found that the primary potassium current carrier in fish bipolar cells is a voltage-dependent potassium channel that inactivates upon depolarization. For example, they report that this conductance inactivates with a time constant of 8 s when the cell is depolarized to −30 mV. This potassium

conductance of the bipolar cells is concentrated at the terminal region. A similar potassium current with faster kinetics has recently been reported in axolotl by Tessier-Lavigne et al. (1988). Thus, it is possible that the elevated potassium levels in the inner retina cause the bipolar cell terminal to depolarize, inactivating the potassium current in this region. This might also explain Shimazaki et al.'s (1984) observation that the ON potassium flux in the outer retina actually increases. If the potassium currents in the terminal region of the bipolars were reduced, then more of the outward current would be carried by the remaining potassium conductance located in the distal retina.

Our analysis of the effects of the pharmacological manipulations of neuronal activity upon the ERG are all predicated on the integrity of the glial system. For example, we expect APB to block the ON pathway and thus the ON potassium flux, without interfering with the Müller cell's potassium conductance mechanisms. One line of evidence supporting this assumption is that except for cobalt treatment, all of the agents we used left either the b- or the d-wave intact, which suggests that Müller cell function remained active and unaffected by drug application. We have no direct evidence that the drugs we used did not have a local or selective effect on portions of the Müller cell. However, this seems unlikely for several reasons. Previous studies have shown that APB acts as a glutamate agonist, whereas PDA is an antagonist, yet no differential effect upon Müller cell function has been observed. Additionally, the drug concentrations used in our experiments are those previously shown to optimally effect particular synaptic receptors.

Recently, Brew and Attwell (1987) have demonstrated that Müller cells have a powerful electrogenic glutamate uptake system. They found that this sodium-dependent mechanism results in a depolarization of Müller cells. Since photoreceptors and bipolar cells probably use glutamate as a neurotransmitter, they suggest that the release of glutamate at light onset may depolarize the Müller cells, contributing to the ERG. If this mechanism, described in isolated Müller cells from the tiger salamander retina, is applicable to the intact retina, our results suggest that this effect would be limited to glutamate uptake in the IPL. When we block the OFF bipolars, horizontal cells, and third-order neurons with PDA or KYN, the d-wave of the ERG is blocked. That is, photoreceptors continue to release glutamate at light offset, but this does not seem to be reflected in the ERG. The combined action of PDA and APB, in which photoreceptors continue to release transmitter but postphotoreceptor responses are blocked, is very similar to the effect of cobalt, where photoreceptor transmitter release is blocked, thereby eliminating all postphotoreceptor activity. This suggests that if the photoreceptors are releasing glutamate at light offset, glutamate is not acting on Müller cells to produce an ERG component. However, both APB block of the ON bipolars and PDA/KYN block of the OFF bipolars could presumably decrease glutamate release in the IPL and, therefore, our experiments cannot distinguish between glutamate and potassium effects on Müller cells in the proximal retina.

This work has been supported by National Eye Institute grant EY-05725 to Malcolm M. Slaughter and a Fight for Sight, Inc. fellowship to Richard A. Stockton.

Original version received 30 November 1987 and accepted version received 25 July 1988.

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