A Proximal Retinal Component in the Primate Photopic ERG a-Wave

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Purpose. The monkey photopic ERG was studied during administration of glutamate analogs to determine whether the photopic a-wave derives exclusively from photoreceptors.

Methods. Monkey photopic ERGs were elicited using 200-msec flashes or 30-μsec xenon photostrobe flashes on a steady light-adapting background of 40 cd/m² (3.3 log scotopic troland). Intravitreal injections of APB, PDA, or both were given to block transmission to depolarizing and hyperpolarizing second-order retinal neurons, respectively.

Results. After injecting PDA to block light responses of horizontal cells and hyperpolarizing bipolar cells, part of the photopic a-wave was eliminated. The PDA-sensitive component, presumed to be due to activity postsynaptic to cones, was responsible for the photopic a-wave threshold and dominated the response over the initial 1 to 1.5 log units of intensity. For brighter stimuli, this component made a constant contribution to the photopic a-wave. A non-PDA-sensitive contribution to the a-wave, presumed to originate directly from cones, was first evident 1 to 1.5 log units above photopic a-wave threshold. It progressively dominated the a-wave at higher intensities, particularly at early time points after the flash. Injecting PDA almost eliminated the photopic a-wave elicited with bright xenon photostrobe flashes that are commonly used for human clinical ERG diagnostic testing, indicating that this a-wave may contain significant postreceptoral activity.

Conclusion. The primate photopic ERG a-wave derives, in part, from retinal activity postsynaptic to cone photoreceptors, particularly for stimuli near the photopic ERG threshold that are typically used for human clinical studies. Invest Ophthalmol Vis Sci. 1994; 35:635-645.

The vertebrate ERG a-wave has been thought to originate primarily, if not exclusively, from photoreceptors. Granit1 considered the a-wave to be the leading edge of Process III (PIII) and, due to the short latency of the a-wave, to be associated with photoreceptor activity. Penn and Hagins2 used microelectrodes to map the light-evoked extracellular current flow around rat photoreceptors. They found that the rod photocurrent was similar in sign and waveform to the ERG a-wave and could account for the major part of it. However, they could not rule out a contribution from cells more proximal in the retina. Recently, it has been demonstrated that the leading edge of the human rod a-wave can be described by a quantitative model that derives from single unit responses of rod receptors3,4 and the biochemical stages of transduction.5 Thus, several lines of evidence support the idea that the scotopic ERG a-wave reflects rod photoreceptor activity.

The notion that the light-adapted (photopic) a-wave also derives from cones originated with studies of the local ERG recorded in the monkey fovea by Brown and Watanabe.6 Later work7-11 confirmed that the extracellularly recorded late receptor potential resembled the photopic a-wave. These studies were primarily attempts to isolate the cone receptor potential and were not necessarily designed to prove or disprove that cones were exclusively responsible for the photopic a-wave. Most of these recordings were made using intraretinal microelectrodes that recorded from a localized region of the retina. Therefore, a contribution to the corneal photopic ERG a-wave from massed responses of cells more proximal in the retina could not be ruled out. Furthermore, in most studies, the a-wave was not studied over a full range of intensities to learn...
whether a contribution from the proximal retina might be significant at some intensities.

Not all intraretinal studies confirmed that the a-wave derives from photoreceptors alone. The technique of fractional recording, first employed by Murakami and Kaneko on the duplex (mixed rod and cone) frog and turtle retinas in vitro, showed that part of the dark-adapted a-wave originated postsynaptically to the photoreceptor layer. Although this proximal component had a longer latency than the receptor component, it still occurred before the b-wave and was called proximal PIII. Tomita also observed a proximal PIII in the amphibian retina and suggested that the a-wave is a composite of potentials from different retinal layers, with the relative contributions varying with species. A proximal PIII component in mammals has been a topic of speculation. Murakami and Kaneko and Whitten and Brown commented that experiments by Brown et al., in which the retinal circulation of monkeys was clamped, did not rule out a postsynaptic contribution to the a-wave. The idea that elements postsynaptic to photoreceptors might contribute to PIII, particularly for latencies longer than a photoreceptor component, led Armington to recommend measuring only the earliest part of the a-wave to obtain the best measure of the photoreceptor response.

Recently, new doubt has been cast on the idea that photoreceptors are the exclusive source of the photopic a-wave, based on studies of the light-adapted monkey retina in vivo and of the isolated dogfish retina. Both studies used 2-amino-4-phosphonobutyric acid (APB) to eliminate the b-wave. APB uncovered a large negative ERG response to stimulus onset. APB is one of a relatively new set of pharmacologic agents that block transmission at the photoreceptor synapse. These substances are analogs of the likely neurotransmitter glutamate and act at pharmacologically distinct receptors on different types of second-order neurons. Although the drugs themselves are not absolutely specific, they are increasingly well characterized as to their site of action. APB blocks synaptic transmission at the sign-inverting synapse between photoreceptors and depolarizing ON-center bipolar cells (DBC). GABA-2,3-piperidine-dicarboxylic acid (PDA) strongly suppresses transmission at the sign-conserving synapse between photoreceptors and hyperpolarizing OFF-center bipolar cells (HBC) and horizontal cells, as well as between bipolar cells and third-order neurons. Neither intracellular nor extracellular measures of photoreceptor activity have been found to be affected by these drugs in the animals tested (primarily anthropids). When given together or sequentially, they serve to isolate the contributions to the ERG from various cell types.

Knapp and Schiller observed that after they injected APB into the vitreous of the monkey eye to eliminate the b-wave, the negative wave that remained was larger than the a-wave and lasted for the duration of the stimulus. Although the evidence was indirect, Evers and Gouras proposed that both the larger negative response after APB and the enhanced OFF-response resulted from a contribution from HBCs. Further, they proposed that the corneal negative potential generated by HBC activity at stimulus onset might normally contribute to the photopic a-wave. Falk and Shillers reached a similar conclusion after simultaneous ERG and intracellular recordings from the dogfish retina in the presence of APB. However, they attributed the more negative ERG after APB to horizontal cells.

We have now used PDA to explore more directly the possible contribution from HBCs and horizontal cells to the primate photopic a-wave. Our results support the hypothesis that the primate photopic a-wave over the first log unit of intensity above photopic threshold originates primarily from neuronal activity postsynaptic to the cones. They suggest that only for brighter stimuli does cone activity contribute significantly to the photopic a-wave. Our evidence implies a situation analogous to the scotopic rod ERG in which a potential generated in the inner retina, the scotopic threshold response, dominates the negative response near threshold. One ramification of this is that, for clinical studies using the photopic ERG, one must be cautious when tracking cone photoreceptor activity with threshold a-wave responses.

METHODS

Animals and ERG Procedure

Adult cynomolgus (Macaca fascicularis) and rhesus (Macaca mulatta) monkeys were studied in accord with the guidelines of the ARVO resolution on animal experimentation. Nine animals were studied with drugs, and the quantitative results from six eyes of four animals are included here. Animals were pretreated subcutaneously with atropine sulphate (0.04 mg/kg) and anesthetized intramuscularly with 7 mg/kg ketamine and 0.6 mg/kg xylazine. Supplemental oxygen was given by external nasal cannula. Hydration was maintained by intravenous lactated Ringer’s solution. Pupils were dilated fully with topical 1% atropine sulfate and 10% phenylephrine.

Burian-Allen bipolar contact lens ERG electrodes (Hansen Ophthalmic Development Labs, Iowa City, IA) were used with tetracaine HCl as a corneal anesthetic. A subcutaneous needle on the back served as the indifferent electrode. ERGs were recorded at 8000 gain at either 0.1 to 1000 or 0.1 to 100 Hz and were filtered at 60 Hz. Responses were averaged by a microcomputer and displayed on a digital plotter.
Flashes of 200 msec were presented on a rod-saturating background either in a Ganzfeld bowl for full-field stimulation or by a rear-illuminated screen that subtended about 110°. Both systems produced the same response amplitudes and waveforms when matched for photopic intensity. Responses were averaged 10 to 30 times. The 200 msec flashes gave a clear separation of ON- and OFF-responses. Flash durations from 10 to 200 msec gave the same a-wave amplitudes, indicating that the integration time of the a-wave was less than 10 msec.

For fully dilated pupils, the Ganzfeld bowl provided "white" (2800°K color temperature) stimuli up to 3.8 log photopic trolands (td) on a continuous background of 3.3 log scotopic td (40 cd/m²) that suppressed rod responses. Stimulus duration was controlled by a mechanical shutter (Uniblitz, Vincent Associates, Rochester, NY) with 90% opening and closing times of less than 3 msec. Stimulus intensity was set by neutral density filters, and wavelength was set by broad-band Wratten filters. The rear projection stimulus provided "white" (approximately 3700°K) light of up to 5.7 log photopic td on a 3.3 log scotopic td continuous background. Light from a 300-watt projector was focused by custom optics onto a Uniblitz shutter and then projected onto a 110° diffusing screen placed 4 cm in front of the eye. Intensity was set by calibrated glass neutral density filters (Melles Griot, Irvine, CA).

Because many diagnostic clinics use brief xenon flashes from a Grass PS-22 photostimulator (Grass Instrument, Quincy, MA) to elicit the ERG from human patients, we also tested the effects of APB and APB + PDA on the monkey ERG using this xenon stimulus. Maximum intensity of the 30 μsec xenon flash (at 0 neutral density) was 2.1 cd-sec/m² (2.1 log td/sec for a fully dilated pupil) and gave the same a-wave as the 200 msec flash at 4.0 log td. The integrated intensity of the xenon stimulus was mathematically equivalent to the first 10 msec of the 4.0 log td 200 msec flash, again indicating that only this portion was integrated by the retina to produce the a-wave.

To learn whether the light-adapted a-wave near threshold might result from rod activity, we used scotopically matched red (Wratten #29) and blue (Wratten #47), filters to show that responses near photopic threshold were cone driven.

**Drugs and Injections**

Drugs (Sigma, St. Louis, MO) were dissolved in sterile saline and passed through a 0.2 μm filter. The concentrations used in this paper were determined from dose response studies. The concentrations were 40 mM APB, 200 mM PDA, 200 mM NMA (N-methyl-DL-aspartic acid), 2 M ASP (sodium aspartate), and 40 mM Co++ (cobalt chloride). Injections of 0.05 ml were given through a 30-gauge needle inserted through the pars plana approximately 6 mm posterior to the limbus. The 2.1 ml volume of the vitreous would yield a 40X dilution of these drug concentrations assuming full mixing. However, the actual concentration at the retina may be different. Drug effects on the ERG were stable for several hours.

Recordings were made before and after intravitreal injection of drugs in the same session and subsequently in follow-up sessions to show unassisted physiologic clearance of the drugs from the eye. Repeat recordings from 3 days to 3 weeks after the injection of APB and PDA showed complete recovery of ERG responses to predrug amplitudes and waveforms. As an additional control, 0.05 ml of 0.9% saline was injected and found to have no effect on the a-wave. Retinal histologic sections from an eye removed within several hours of the injection of these drugs and viewed under the light microscope showed no disruptions of cone inner and outer segment morphology. Aspartate and Co++ both caused nonreversible ERG changes and histology confirmed disruption of photoreceptors. Consequently, Asp and Co++ thereafter were used only in terminal experiments.

**Curve Fitting**

The Michaelis-Menten equation, 
\[
\frac{V}{V_{max}} = \frac{I^n}{I^n + \sigma^n}
\]

was fitted to the a-wave intensity-response data before and after drugs. Best fit values for \(V_{max}\), \(\alpha\), and the exponent (n) were obtained as simultaneous unconstrained parameters using a least squares algorithm (Easy Plot II, Spiral Software, Brookline, MA). The Michaelis-Menten equation has been used to describe rod and cone intensity-response functions as well as the rod a- and b-waves, extracellularly recorded monkey cone photoreceptor responses, and light-adapted psychophysical increment threshold responses.

**RESULTS**

**Drug Effects on the Photopic ERG Near Threshold**

Control responses for each eye in Figure 1. show the general nature of the primate photopic ERG to a 200 msec flash at approximately 1 log unit above threshold intensity. Flash onset produced the initial a-wave deflection that was negative-going until terminated by the positive b-wave deflection. Stimulus cessation caused the positive d-wave OFF-effect. The negative region between the b- and d-waves is the cone negative plateau and reflects photoreceptor activity.

Injecting APB (Fig. 1, Eye #1) to block synaptic transmission between the cones and DBCs but not HBCs or horizontal cells substantially reduced the b-
wave, as reported previously.\textsuperscript{15,24} APB also made the plateau more negative and enhanced the d-wave. The a-wave after APB continued longer and more negatively than the control a-wave maximum (dashed line), consistent with the supposition that the b-wave ordinarily masks the full extent of the a-wave.

PDA was injected into this same eye 2 hours later (Fig. 1, Eye \#1). PDA made the a-wave considerably smaller than either the control or the response after APB alone (at time of dashed line). PDA blocks transmission between cones and HBCs and cones and horizontal cells but not between cones and DBCs. Because the combination of APB and PDA blocks transmission from photoreceptors to both post synaptic ON- and OFF-pathways, it appeared that the a-wave had a component that derived postsynaptic to the photoreceptors (for this stimulus intensity). PDA also suppressed the d-wave, consistent with the supposition that the d-wave derives partially from OFF-pathway activity,\textsuperscript{15,20} in addition to reflecting the termination of the photoreceptor cone response.\textsuperscript{1,7}

Figure 1 also shows the effect of injecting PDA and APB into a different eye in reverse order. For Eye \#2, intravitreal injection of PDA alone virtually eliminated the a-wave response and revealed a large and positive ERG response after PDA as deriving from DBC activity in the absence of hyperpolarizing cell activity postsynaptic to the cones (i.e., by elimination of horizontal cell and HBC activity). Because PDA also blocks glutamate receptors on third-order neurons in amphibians\textsuperscript{19,20} and mammals,\textsuperscript{38} the possibility that the ERG effects we noted were due to its action at this level was tested on one monkey using NMA. This drug, which suppresses primarily third-order neuron responses in amphibians,\textsuperscript{20} had only subtle effects on the b-wave and only partially suppressed the a- and d-waves (data not shown).

Of specific interest for the photopic a-wave origin, note that the ERG response after PDA had positive polarity at the same time that the control a-wave was maximally negative (Fig. 1, Eye \#2, dashed line). Because the extracellular photoreceptor currents have a corneal-negative dipole moment,\textsuperscript{2} cone hyperpolarization cannot explain this positive wave after PDA. The latency of this positive-going response was longer than the control a-wave latency, and it was eliminated by the subsequent injection of APB into Eye \#2. As seen previously for Eye \#1, PDA + APB revealed what appeared to be PI in as identified by Brown et al\textsuperscript{7} after clamping the retinal circulation, but it greatly reduced the response during the time course of the normal photopic a-wave. The kinetics of the residual negative wave were too slow to account for the observed control a-wave. These results support the idea that activity postsynaptic to cones can contribute to the normal photopic a-wave.

**Cobalt and Aspartate**

Aspartate and cobalt were also tested on the primate photopic ERG (Fig. 2), and both diminished the a-wave amplitude similar to the combination of APB + PDA. Aspartate is a glutamate analog that blocks retinal light-evoked responses postsynaptic to photoreceptors nonselectively\textsuperscript{39} without suppressing either rod\textsuperscript{23,25} or cone\textsuperscript{40} responses. Cobalt eliminates Ca\textsuperscript{2+}-dependent synaptic vesicular release and thereby eliminates light-evoked activity postsynaptic to the photoreceptors by a mechanism different from aspartate or APB + PDA.\textsuperscript{41} Neither cobalt nor aspartate were used further for these monkey studies because the ERG effects were poorly reversible and because histology showed photoreceptor damage.

**Intensity-Response Function**

Figure 3 shows photopic a-waves recorded over a 3-log unit intensity range beginning at the photopic a-wave threshold. Elimination of the b-wave by applying APB had little effect on the leading edge of the a-wave, except at the higher intensities above 5.06 log td where the a-wave amplitude was greater after APB. As previous investigators have pointed out for the scotopic ERG, the rod b-wave intrudes on the rod a-wave, and, at certain intensities, the a-wave amplitude is only part of the underlying PI potential.\textsuperscript{5,42} Our data indicate that this is true for the cone a- and b-waves also.

When PDA was injected after APB, the a-wave amplitude was reduced at all intensities. The a-wave de-
FIGURE 2. Comparison of effects of aspartate and cobalt to the effect of APB and APB + PDA on the photopic ERG a-wave of three different eyes of two monkeys. Drugs were given by serial intravitreal injection. Inset (top) shows 400 msec traces for eye #1, to 200 msec flashes. Bottom three traces show the first 40 msec after flash onset (indicated by arrow). Stimulus was 3.76 log td (2.01 log cd/m²) on steady background of 3.3 log td (1.55 cd/m²).

crease after APB + PDA was proportionally greatest for dimmer intensities, and no appreciable a-wave could even be recorded until 4.06 log td, which was more than 1 log unit above the control threshold. For intensities above 4.06 log td, the a-wave amplitude after APB + PDA grew parallel to that after APB alone, and the absolute interval between the post-APB response and the post-APB + PDA response became relatively constant. PDA did not alter the latency of the a-wave. However, the a-wave suppression by PDA was evident at early time points on the a-wave leading edge. By comparison, removing the b-wave by APB did not substantially affect the a-wave amplitude until later times near the time of the a-wave peak in the control response.

Intensity-response curves of the a-wave after APB alone and after the further addition of PDA (Eye #1) are shown in Figure 4. The amplitudes were measured at the time corresponding to the control a-wave peak. The post-APB curve follows the control curve closely until the highest intensities where the a-wave is partially truncated by the b-wave in control responses. After blocking hyperpolarizing cells postsynaptic to

FIGURE 3. Effects of APB and APB + PDA on the photopic ERG a-wave of monkey, recorded for intensities over a 3 log unit range. Data are from eye #1 in Figure 1. Inset (top) shows entire response to the 200 msec flash; dashed box (inset) indicates the 40 msec window used for the separate intensity traces. Responses at intensities of 2.76 to 5.76 log td show only the a-wave over the first 40 msec, beginning at flash onset. Drugs were given by serial injection with APB first, followed by PDA. Steady background = 3.3 log td (1.55 cd/m²).

FIGURE 4. V-log I plots of the monkey photopic a-wave, measured at times corresponding to a-wave peak in control responses (●—●). A-wave responses after APB (○—○) and after APB + PDA (△—△) were measured at the same latencies as for the control a-wave peak at each intensity. Data are from responses in Figure 3. Solid lines were drawn between the points for clarity.
cones with PDA, essentially no a-wave was present for stimuli below 3.8 log td. Above 3.8 log td, the APB and APB + PDA a-wave curves in Figure 4 rise roughly in parallel. It appears that the PDA-sensitive component makes up a large proportion of the photopic a-wave near threshold but becomes relatively less significant to the total a-wave for brighter stimuli.

The data of Figure 3 indicated that the PDA-sensitive potential influenced the a-wave early in its time course. Consequently, we also plotted intensity-response curves measured at various times after flash onset (Fig. 5). After APB has eliminated most of the b-wave, the a-wave should then be attributable to activity of the cone photoreceptors plus any activity contributed from postsynaptic cells without interference from the b-wave. These post-APB curves show a relative plateau between 3.5 to 3.8 log td for amplitudes measured at fixed times of 25 msec and 30 msec and at the time of the a-wave peak, but not at times of 20 msec or earlier. Because these curves are after APB, this plateau cannot be the result of b-wave intrusion. Rather, the biphasic a-wave curve indicates at least two underlying components, with a threshold segment for dimmer stimuli saturating near 3.5 log td and a second segment produced by higher intensities above 3.8 log td.

The addition of PDA simplified the curves at later time points by eliminating the threshold component between 2.8 to 3.8 log td (Fig. 5). After APB + PDA, the response consisted of only the higher intensity process. Above that intensity, the response can be presumed to result directly from cone activity. A "PDA-sensitive component" was isolated mathematically by subtracting the curves in Figure 5 after APB + PDA from those after APB alone and is shown in the log-log plot in Figure 6. It was apparent that the PDA-sensitive component reached a plateau about 1 to 1.5 log units above photopic a-wave threshold and thereafter made a fairly constant contribution to the a-wave. The mean maximum amplitude of the PDA-sensitive component at 4.0 log td was 14.1 ± 6.0 μV (n = 6 eyes). These curves also show that there is only a small contribution to the photopic a-wave from the PDA-sensitive component as early as 10 msec after stimulus onset for the brightest stimuli.

To show the effect of PDA alone on the photopic a- and b-waves, the intensity-response data for Eye #2 in Figure 1 before and after the injection of PDA are plotted in Figure 7. Note that the control a-wave curve shows a plateau, as did the APB data in Figures 4 and 5, about 1 to 1.5 log unit above threshold. Although PDA eliminated the a-wave at intensities below this plateau and reduced the a-wave over the entire intensity range, PDA markedly increased the b-wave amplitudes and shifted the threshold for the appearance of the b-wave to lower intensities. The enhanced b-wave amplitude after PDA indicates that it is unlikely that PDA caused a depression of cone photoreceptor sensitivity.

Michaelis-Menten Curve Fitting

The Michaelis-Menten equation was fit to the intensity-response data for the photopic a-wave after APB.
alone and after APB + PDA. The fits were made with $V_{\text{max}}$, the half-saturation constant $\sigma$, and exponent (n) as simultaneous unconstrained parameters. The amplitude data measured at each time point after APB + PDA were well fit by single curves with an average exponent $= 1.02 \pm 0.02$ (N = 5). The curve fit for the 25 msec data is shown in Figure 8. Fitting the equation to the data after APB alone gave values for (n) and $\sigma$ that were close to those after APB + PDA if only data for intensities above 4 log td (Fig. 8, dark circles) were included. The curve fit to the data at high intensities diverged from the data at low intensities. $V_{\text{max}}$ after PDA was lower than that before PDA by an amount approximately equal to the amplitude decrease measured 1 log unit above a-wave threshold. Fitting the equation to the APB data over the whole range of intensities gave values of (n) that were close to 1 only for the 10 and 15 msec time points. For 20, 25, and 30 msec and the time of the a-wave peak, the values were 0.89, 0.80, 0.73, and 0.69, respectively. These results supported the hypothesis that activity of postsynaptic neurons remaining after APB made an increasingly significant contribution to the photopic a-wave at later time points. The fact that above 4.0 log td, both the post-PDA and APB data could be fit using similar parameters except for a reduction in $V_{\text{max}}$ indicates that response sensitivity was not altered by PDA and that the data after PDA may represent primarily photoreceptor responses.

**Brief Xenon Flash Results**

APB + PDA caused essentially the same suppression of the a-wave from xenon flashes (Fig. 9) as in the previous results with the 200 msec stimulus. After APB + PDA, the a-wave for the Grass xenon flash was reduced substantially for all intensities, and the threshold was elevated by 1 log unit. Some “b-wave” remains in Figure 9 after APB because the duration of the flash was so short that this is actually the d-wave in response to stimulus off. The results for APB + PDA suggest that almost the entire photopic a-wave recorded clinically using the maximum intensity of the Grass PS-22 stimulator originates from proximal retinal activity.
postsynaptic to the cone photoreceptors and not from the cones directly.

DISCUSSION

This study demonstrated that PDA substantially reduced the monkey photopic a-wave for flashes near photopic threshold. Because PDA blocks synaptic transmission from photoreceptors to hyperpolarizing second-order neurons, this suggests that activity of either HBCs, horizontal cells, or both contribute to the primate photopic a-wave. Though no one has yet recorded from monkey cones and shown that PDA does not affect the photoreceptor light responses, evidence from this study indicates that the effect of PDA on the photopic a-wave was not a result of suppressing cone activity. The Michaelis-Menten fitting of the a-wave V-log I function after APB + PDA indicated that the cone photoreceptor sensitivity was not affected by PDA. In addition, PDA enhanced the photopic b-wave, which is indirectly dependent on photoreceptor responses, even at those lower intensities where the a-wave was eliminated (Fig. 7). Any reduction in photoreceptor response would also tend to reduce the b-wave unless the effect were specific for the a-wave generating mechanism. However, the current flow generating the a-wave is an integral part of the response transmitted to the synapse. 2 In addition, previous studies in the amphibian 21-25 and chick retina 22 in vitro have shown that PDA does not affect photoreceptor-dependent ion fluxes. Based on this evidence, the most likely explanation of our results is that PDA removes a corneal negative potential generated by cells postsynaptic to photoreceptors that sums with the ERG a-wave.

When PDA was applied alone, it produced a striking enhancement of the corneal positive b-wave, which resulted in the photopic ERG actually being positive at a time when the a-wave is normally negative (Fig. 1, Eye #2). The polarity reversal at the outset of the response has implications for understanding the normal photopic a-wave. It cannot be due to an effect on the photoreceptor current flow generating the a-wave because the source/sink locations are fixed along the photoreceptor length. This implies that, although the b-wave is presumed to mask the underlying PIII response beyond the a-wave peak, activity postsynaptic to the cone photoreceptors can contribute even earlier in the course of the a-wave. Hence, the short latency of the control a-wave may not guarantee that it derives exclusively from cone activity.

The large positive response after PDA alone would appear to be due to DBCs because it was eliminated by APB. The size of the response may seem larger than expected from the removal of a competing negative ERG potential. The large size of the response may be, in part, the result of an enhancement of DBC activity due to the removal of HBC, horizontal cell responses, or both. One possibility is that PDA suppresses the DBC antagonistic surround mechanism. 19 A detailed discussion of the possible origins of this response is in preparation. The results of NMA on a single animal suggested that none of the effects of PDA could be explained by the suppression of third-order neurons.

Michaelis-Menten Function

If APB and PDA had no effect on photoreceptors in this study, as has been shown in other systems, then the V-log I data for the a-wave after APB + PDA should represent photoreceptor responses without interference from postsynaptic activity. The Michaelis-Menten fit of the post-APB + PDA curves in Figure 5 gave an exponent that is in good agreement with the exponent of 1.0 obtained by Hood et al., 36 who fit light-adapted psychophysical increment thresholds in humans, and by Normann and Perlman, 39 who fit the equation to intracellular responses of turtle cones. Hood and Birch, 35 found an exponent of 1.0 when they fit the function to photopic ERG a-wave amplitudes from humans. They used data from early time points on the a-wave (<15 msec) recorded in response to high intensities. Our finding that the intensity-response data measured at 10 and 15 msec both before and after PDA could be fit by the Michaelis-Menten equation with an exponent of 1 is consistent with their results and suggests that there is little input to the photopic a-wave from cells other than cones at these early time points. Current responses recorded from monkey cones using a suction pipette electrode were best fit by a weighted average of the Michaelis-Menten equation and a saturating exponential. 43 The weighting was about the same for both red and green cones, indicating that including both cone types in our data does not affect the fit. Hood and Birch 35 discussed possible reasons why the intensity-response relationship from single cell recordings may differ from that of the ERG.

A lower value of n = 0.74 was found in extracellular studies of the late receptor potential recorded using intraretinal microelectrodes in the monkey fovea. 8,34 These amplitudes were measured on the PIll plateau, which corresponds to times later than the a-wave peak. In our experiments, measuring amplitudes at later time points (e.g., greater than approximately 25 msec) across the entire range of intensities was only possible after APB had removed the b-wave. Our data after APB alone gave a value of n = 0.73 at 30 msec, the latest time point measured, but 1.03 after APB + PDA. This raises the possibility that the extracellular data of Boynton and Whitten 8 and Valeton and van Norren 34 may be contaminated by a contribution from the proximal retina. Valeton and van Norren 34 have
discussed other possible reasons why they obtained an exponent lower than 1. The lowest value of \( n \) in our study (0.69) was calculated for the APB data measured at the time of the control a-wave peak, suggesting that measurements of the a-wave peak amplitude have the greatest contribution from postsynaptic elements.

**Cone Contribution in the Control a-Wave**

The V-log I curves indicated that above about 4 log td, at least for later time points, the PDA-sensitive contribution had saturated but remained present (Fig. 6) and further growth in the response was due solely to a potential not sensitive to PDA, presumably the result of cone activity. This can also be seen in the traces in Figure 3 where, at higher intensities, the interval between the responses after APB alone versus after APB + PDA remained relatively constant. However, at the highest flash intensities, the cone a-wave after APB + PDA converged toward the amplitude of the control a-wave. This probably reflected the intrusion of the b-wave into the control a-wave because the amplitude of the curve after APB alone, at least at later time points, was larger than the control. Thus, by 5.8 log td, the a-wave augmentation from post-photorceptor activity was nearly canceled by the b-wave intrusion from the ON-pathway. It remains to be learned, for still higher intensities, how similar the true cone a-wave amplitude is to the control a-wave when input from the proximal retina is included.

**Comparison to Scotopic a-Wave**

It has been previously suggested that horizontal cells may contribute a corneal negative potential to the ERG a-wave by acting as a distal potassium sink. It has also been suggested that, by analogy with DBCs that underlie the conic positive b-wave, HBCs enhance the photopic a-wave by contributing a corneal negative potential. Our results cannot distinguish between these two possibilities. However, some insight may be gained by comparing the primate photopic to the scotopic ERG because the rod pathway does not appear to involve HBCs.

By analogy with the a- and b-wave relationship of the scotopic ERG, it is not surprising that dim photopic stimuli could favor a post-photorceptor contribution to the ERG at lower intensities than a photoreceptor component. Under dark-adapted conditions, the scotopic b-wave, reflecting rod-driven post-receptoral DBC activity, has a threshold nearly 3.5 log units lower than the rod a-wave. The difference between rod a- and b-wave thresholds has been attributed to a 100-fold gain at the rod to DBC synapse, and to the longer extracellular b-wave current path compared with the shorter a-wave current loop alongside the rod photoreceptors. In the case of cones, a 10- to 15-fold synaptic gain between cones and HBCs in turtle has been reported. If this can be extrapolated to monkey, it could explain a 1 log unit lower threshold for an HBC contribution to the photopic ERG than for a contribution from cones. Blocking cone transmission to hyperpolarizing second-order neurons, leaving only DBCs and photoreceptors as the main sources of the ERG, uncovered a 3 log unit difference in threshold between the photopic a- and b-waves (Fig. 7), whereas the control a- and b-wave thresholds were the same. This is similar to the difference in threshold between the scotopic a- and b-waves of the monkey.

Could an HBC-generated ERG potential have a latency short enough to contribute to the photopic a-wave? In the turtle retina, cone synaptic transfer to HBCs is 5 to 10 times faster than to DBCs, and intracellular light responses peak 35 to 40 msec before those in DBCs. These results suggest that HBC-light responses can occur earlier than the b-wave from DBCs and can sum with the photoreceptor a-wave. Most of the HBC-generated ERG signal, however, would probably occur during the b-wave.

Other examples of corneal-negative ERG waves arising postsynaptically to photoreceptors include the proximal PI1, the photopic m-wave, the scotopic threshold response (STR), and the slow negative response. The human STR is the initial negative ERG deflection under very dark-adapted conditions and thereby mimics the rod a-wave. However, the STR threshold is nearly 4.0 log units lower than the rod a-wave and originates from the proximal retina. Thus, the STR is a further example of postsynaptic neurons contributing a pseudo-a-wave, not of photoreceptor origin.

**Importance for the Clinical Human Photopic a-Wave**

These results could be important for interpreting human clinical ERG recordings, especially when attempting to gauge cone photoreceptor function based on photopic a-wave amplitudes and even the slope. Our results show that nearly the entire a-wave that is elicited by the Grass photostrobe at its highest intensity in a Ganzfeld bowl originates postsynaptic to the cones. This flash intensity lies squarely within the 1.5 to 3.0 cd · s/m² range that is recommended by the International ERG Standardization Committee when recording human photopic ERGs for diagnostic studies. Our results show that the photopic a-wave recorded at these intensities is not from the cones directly. Therefore, care is warranted in interpreting the cellular origins of clinical photopic a-wave recordings.

**Key Words**

electroretinography, photopic ERG, a-wave, primate, cones, hyperpolarizing bipolar cells, Cis-2,3-piperidine-dicarboxy-
lic acid, depolarizing bipolar cells, 2-amino-4-phosphonobutyric acid

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References


