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Evidence in Support of a Photoreceptoral Origin For the "Light-Peak Substance"

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The light peak of the DC ERG was recorded in in vitro preparations of chick neural retina-RPE-choroid. Either the pharmacological agents Co^{2+}, Mg^{2+}, and low Ca^{2+}, known to block calcium-dependent synaptic transmission or 2-amino-4-phosphonobutyric acid (APB), which blocks transmission in the ON pathway, were added to the solution perfusing the retina. These agents suppressed the b-wave of the ERG, thus indicating a severe suppression of synaptic transmission in the neural retina. The retina was also perfused with the OFF pathway inhibitor *cis*-2,3-piperidine dicarboxylic acid (PDA). None of these agents, however, significantly depressed the light peak, supporting the hypothesis that the light peak is generated by a photoreceptor-RPE interaction. Invest Ophthalmol Vis Sci 29:566–571, 1988

The light peak (or light rise) is a prominent slow positive potential recorded in both the DC electroretinogram (DC ERG) and the electrooculogram (EOG). It originates as a depolarization of the basal membrane of the retinal pigment epithelium (RPE).[1-3] The spectral properties and sensitivity of the light peak indicate that the initial event in its generation is the absorption of light by the photoreceptors,[1,2] but subsequent steps probably include a change in the concentration of a substance, a first messenger, in the subretinal space that then affects the RPE either directly or perhaps via a second messenger. However, the nature of this substance or the cells in the neural retina that generate it have not been determined.

Previous studies from this laboratory supported a photoreceptoral origin for a first messenger,[1] and were consistent with the hypothesis that the light peak is the result of a photoreceptor-RPE interaction. Other experimental and clinical studies, however, have indicated that the neural retina *proximal* to the photoreceptors may also contribute to the light-peak mechanism.[4-9] To address this possibility, we investigated whether light-peak generation requires synaptic transmission from photoreceptors to second order cells. We blocked synaptic transmission by either adding divalent cations (Co^{2+} or Mg^{2+}) or lowering the calcium concentration in the perfusate bathing the retina, thereby blocking the calcium-dependent release of transmitters from presynaptic terminals.[10,11] In addition, we blocked transmission in the ON pathway with the excitatory amino acid receptor agonist, 2-amino-4-phosphonobutyric acid (APB),[12,13] and in the OFF pathway with *cis*-2,3-piperidine dicarboxylic acid (PDA).[14] We found that blockage of transmission in the neural retina proximal to the photoreceptors with each of these agents did not reduce the amplitude of the light peak. These findings support the hypothesis that the light peak is generated by a photoreceptor-RPE interaction.

Materials and Methods

These experiments were performed on retina-RPE-choroid tissues isolated from 1–14-day-old white chicks (*Gallus domesticus*) as previously described.[15] All animals were treated in accordance with the ARVO Resolution on the Use of Animals in Research. The chicks were first light-adapted for at least 2 hr to facilitate adhesion of the neural retina to the RPE and then were dark-adapted for 10 min prior to decapitation. An eye was enucleated and mounted, cornea down, in a dissecting chamber filled with a control perfusate (solutes in mM: 120 NaCl, 25.0 $NaHCO_3$, 25.0 dextrose, 5.0 KCl, 3.0 $MgCl_2$, and 1.8 $CaCl_2$) bubbled with 95% O_2-5% CO_2, with a pH of 7.5 ± 0.1, and a temperature of 36.0 ± 1.0°C. Test solutions were modifications of this perfusate as follows: 2.0 mM and 3.0 mM $CoCl_2$, 10.0 mM $MgCl_2$, 0.18 mM $CaCl_2$ (a ten-fold reduction), 1.5 mM and 3.0 mM PDA, and 0.25 mM APB. For test solutions in which the agent altered the perfusate by more than

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2.0 mOsm, the solution was made iso-osmotic with normal Ringer by adding or removing NaCl. The measured osmolarity of the control solution was 308 mOsm ± 2% (Advanced Wide Range Osmometer 3W2, Advanced Instruments, Needham Heights, MA).

An incision was made through the sclera, with care taken not to penetrate the choroid, and the sclera posterior to the ora serrata was dissected. A 2–5 mm incision was made through the choroid, RPE, and retina, and a circular retina-RPE-choroid tissue, 4–8 mm in diameter was excised and placed with the choroidal surface down over a 3 mm hole in the center of a thin plastic film. The plastic film and the tissue were then mounted between two lucite plates in a perfusion chamber identical to those described previously for other preparations,[2,16] except that all solutions were preheated and that heater coils were inserted before the retinal and choroidal inlets of the perfusing chamber to maintain the temperature of the perfusate at 36.0 ± 1.0°C.

Chick retinas are prone to spreading depression—a condition readily produced by tissue damage, low temperature and light.[17] We reduced the frequency of spreading depression by using careful dissection technique, by controlling the temperature, by dark adapting the chicks for 10 min following the initial period of light adaptation, and by elevating the concentration of Mg^{2+} to 3.0 mM (three times the usual concentration used in similar preparations of frog[16] and lizard[2]). Because of this relatively high concentration of Mg^{2+} in the control solution, synaptic transmission was normally suppressed to some degree, as evidenced by the small b-wave in the control electroretinogram (Fig. 1).

A halogen lamp was used to deliver 6×10^{-5} W/cm^2 diffuse white light through a mirror, a neutral density filter and a pair of condenser lenses. To elicit a light peak, a 5 min stimulus was presented at intervals of approximately 45 min. To determine the normal variability of light-peak amplitude, at least three consecutive light peaks were evoked in control solution. We found the variability of light-peak amplitudes to be less than 20% in all cases (n = 4 tissues). Therefore, we consider changes in light-peak amplitude greater than 20% to be significant.

The DC ERG was recorded across the tissue as the light-evoked change in trans-tissue potential. The placement of electrodes and recording methods has been previously described.[2] In brief, the DC ERG was recorded between the extracellular electrodes that were placed in the solutions that separately perfused the retinal and choroidal sides of the tissue. Responses were displayed on both a storage oscilloscope (Tektronix 5111; Beaverton, OR) and a chart recorder (Brush 220; Gould Instrument Co., Cleveland, OH) and were also stored on magnetic tape (Racal 4 DS; Racal Recorders, Southampton, England). Recordings were subsequently sampled and digitized (DEC PDP 11/03; Digital Equipment Corp., Marlboro, MA) and plotted on a digital X-Y plotter (Tektronix 4662).

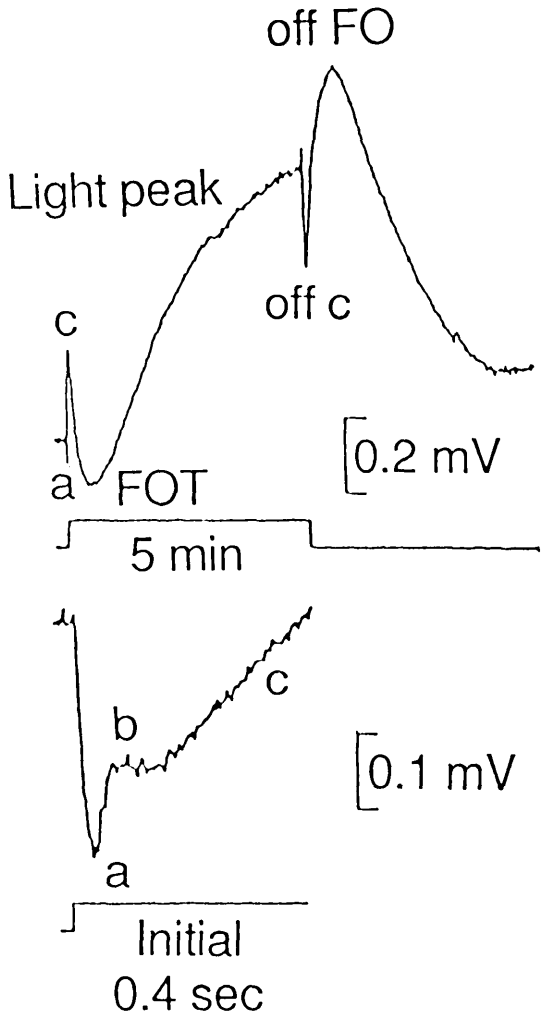

Fig. 1. Chick DC ERG. The responses of the chick DC ERG in temporal order are: a-wave, b-wave, c-wave, fast-oscillation trough (FOT) and light peak. The stimulus diffusely illuminated the retina with 6.0×10^{-5} W/cm^2; stimulus duration was 5 min, indicated (as in all Figures) by an upward deflection of the trace below the response. The initial components of the ERG are shown below on an expanded time scale.

Results

Normal Chick DC-ERG

Figure 1 shows an example of the normal DC ERG of the chick retina-RPE-choroid preparation in response to 5 min of diffuse illumination. The response begins with the negative a-wave, and continues with the positive b-wave and then the rising phase of the positive c-wave, which are shown below the complete

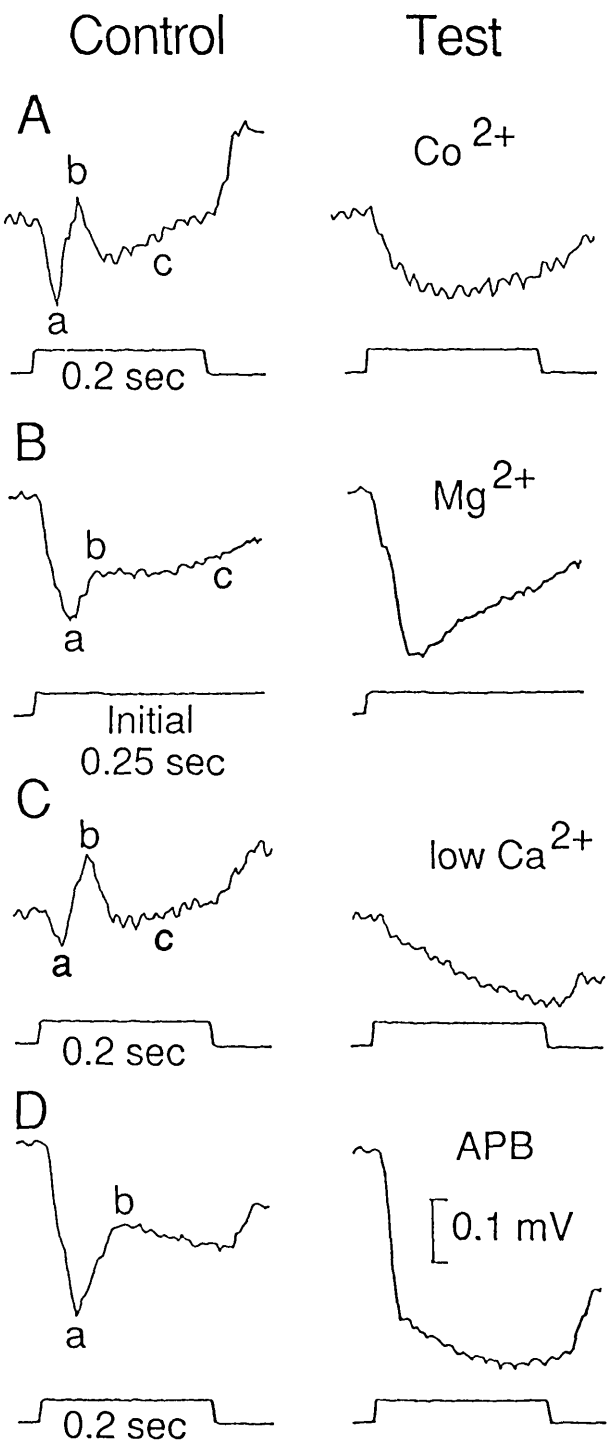

Fig. 2. Suppression of the b-wave during retinal perfusion with: (**A**) 3.0 mM Co^{2+}, (**B**) 10.0 mM Mg^{2+}, (**C**) retinal and choroidal low Ca^{2+} (0.18 mM, a ten-fold decrease) and (**D**) 0.25 mM APB.

response at a faster time scale. Regarding the amplitude of these early waves note that: (1) the b-wave is relatively small because of suppression by 3.0 mM Mg^{2+} in the control perfusate (see *Methods*); (2) the a-wave, at the slower time scale (top trace, Fig. 1) can be artifactually reduced or absent because of points dropped during data digitization; and finally (3) the initial positive response at the slower time scale (top, Fig. 1) is dominated by the c-wave, and not the b-wave. The c-wave is followed by a negative-going potential that drops to a level at or below the baseline. This negative response is termed the fast-oscillation trough (FOT) and depends on a hyperpolarization of the RPE basal membrane.[18-20] The fast-oscillation trough is followed by a slow rise of potential, the light peak, that reaches a maximum positivity about 5 min from the onset of illumination. (We define light-peak amplitude as the maximum positivity during illumination as measured from the minimum of the fast-oscillation trough.) At the offset of illumination, the light peak is followed by the "off c-wave"[21-23] and the "off" of the fast oscillation (off FO). As shown in Figure 1, the light peak is the largest and slowest component of the DC ERG. The light peak recorded from the in vitro chick preparation is similar in waveform and time-course to that recorded in the intact cat preparation.[1]

Effects of Inhibitors

Three modifications of the solution bathing the neural retina were used to suppress Ca^{2+}-dependent synaptic transmission: addition of cobalt, further elevation of magnesium levels, and reduction of calcium. Figure 2 shows the results of these modifications on the b-wave. 3.0 mM Co^{2+} always abolished the b-wave completely (n = 13) (Fig. 2A), and 2.0 mM Co^{2+} usually abolished it (n = 4), except in one case where it was 90% suppressed (not shown). Increasing retinal Mg^{2+} from 3.0 mM to 10.0 mM (Fig. 2B), also abolished the b-wave (n = 3). Decreasing the calcium concentration ten-fold, from 1.8 mM to 0.18 mM, in *only* the retinal bath diminished the b-wave (n = 3), or abolished it (n = 3). The b-wave always could be abolished, however, by lowering Ca^{2+} concentration in *both* the retinal and choroidal baths (n = 6) (Fig. 2C). All effects on the b-wave were reversible, and provided evidence for suppression of calcium-dependent synaptic transmission from photoreceptors to second order cells.

Since the light peak is a response to illumination of the retina, ie, an ON response, it could depend on transmission in the ON pathway from photoreceptors to second order cells. APB, a glutamate analogue, is thought to prevent transmission in the ON pathway at the level of the depolarizing bipolar cell.[12,13] Figure 2 shows that 0.25 mM APB in the retinal bath also eliminated the b-wave, consistent with its suppression of the ON pathway.[24,25] We also tested the possibility that light-peak generation involves a change in the OFF pathway during illumination. The OFF pathway

inhibitor PDA[14] did not significantly suppress the light peak at concentrations of 1.5 mM (n = 3) and 3.0 mM (n = 3) (not shown).

If the light peak does depend on synaptic transmission from photoreceptors to second order cells, then during synaptic blockade we would expect at least a partial reduction of light-peak amplitude and, more likely, its complete suppression. Co^{2+}, Mg^{2+}, low Ca^{2+} or APB, however, never diminished light-peak amplitude.* In contrast, the light peak was actually enhanced in some instances. It was increased in amplitude by 3.0 mM Co^{2+} (n = 13), and usually by 2.0 mM Co^{2+} (three out of four experiments). Figure 3A is taken from the experiment in which 2.0 mM Co^{2+} did not significantly increase the light peak. (The b-wave had been completely abolished in this experiment.) Elevation of retinal Mg^{2+} to 10.0 mM did not significantly alter light-peak amplitude (n = 3) (Fig. 3B). The effects of low Ca^{2+} depended on the technique of perfusion. Whenever both the retinal and choroidal sides of the tissue were perfused with low Ca^{2+} (n = 6), light peaks were significantly enhanced (Fig. 3C). When only the retinal side of the tissue was perfused with low Ca^{2+}, however, the light peak increased in some cases (n = 3) and remained unchanged in others (n = 2). In APB, light-peak amplitude either increased (n = 1) (Fig. 3D), or remained the same (n = 2) (not shown). To emphasize, in all these conditions, the light peak was unchanged in amplitude or enhanced under conditions that abolished the b-wave.

Other changes in the ERG were observed, in addition to suppression of the b-wave. Co^{2+} consistently enhanced the c-wave and fast-oscillation trough (Fig. 3A) and, as mentioned above, the light peak. While retinal and choroidal perfusion with low Ca^{2+} enhanced the light peak, effects on the c-wave and fast-oscillation were inconsistent. In contrast, elevating Mg^{2+} to 10 mM consistently suppressed the c-wave (Fig. 3B), without affecting light-peak amplitude. In addition, low calcium enhanced the background noise of the trans-tissue potential (Fig. 3C). These effects on the ERG will not be further considered in this paper, although their mechanisms are now under investigation.

Discussion

We have shown that perfusion of the neural retina with inhibitors of synaptic transmission, Co^{2+}, high Mg^{2+}, and low Ca^{2+} abolished the b-wave of the DC ERG without attenuating the amplitude of the light peak. In addition, suppression of either the ON pathway or the OFF pathway in the retina with APB and PDA, respectively, did not suppress the light peak. These results will be discussed with regard to their implications for the origin of the light peak.

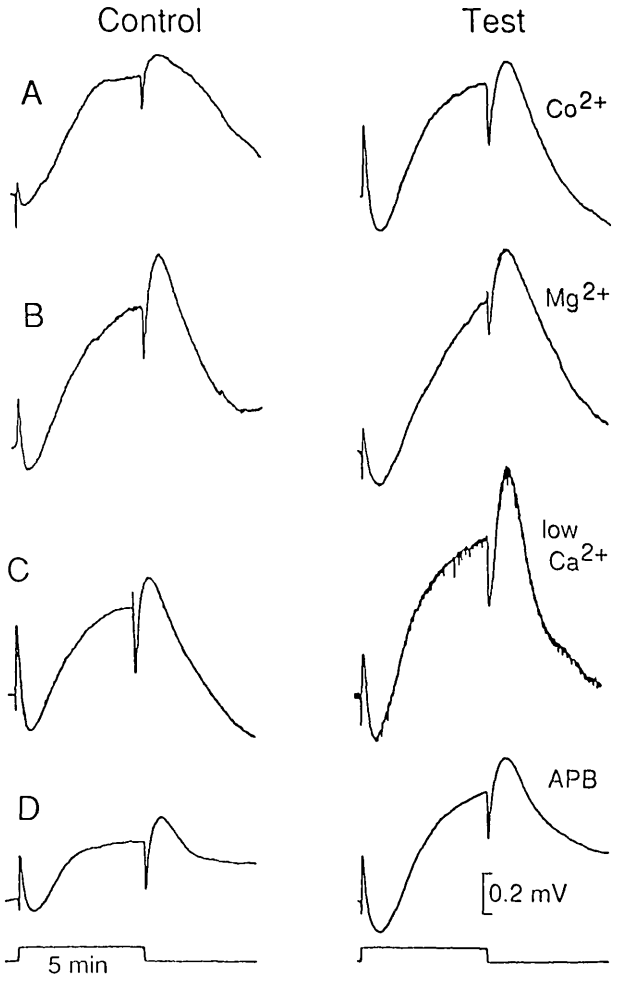

Fig. 3. Chick light peak is not suppressed by retinal perfusion with: (**A**) 2.0 mM Co^{2+}, (**B**) 10.0 mM Mg^{2+}, (**C**) retinal and choroidal low Ca^{2+} (0.18 mM, a ten-fold decrease), or (**D**) 0.25 mM APB.

Based on the stimulus-response properties of the light peak, the initial event must be the absorption of light by the photoreceptors. It was hypothesized that communication of this event to the RPE involved a diffusible substance generated somewhere in the neural retina.[1] Evidence to support a photoreceptoral origin for this substance was provided by studies of the effects of mild systemic hypoxia on the DC ERG of cat.[26-28] The photoreceptors were the primary site of the effects of mild systemic hypoxia and at the levels studied, P_aO_2 = 60–80 mm Hg, there were no observable effects on neural retinal responses proximal to the photoreceptors. (Linsenmeier's recent ox-

* Similar results were also obtained in the retina-RPE-choroid preparation of the lizard (*Gekko gekko*). Magnesium (40 mM) or cobalt (1 mM) completely suppressed the b-wave while the light peak persisted.

ygen depth profiles indicate that the inner segments of cat photoreceptors are exposed to much lower levels of oxygen than are both the remainder of the neural retina or the RPE.[29]) The suppression of the light peak by the same levels of hypoxia suggested that some aspect of photoreceptor metabolism may be involved in the light-peak mechanism.

Some experimental studies, however, implicate the neural retina proximal to the photoreceptors in light-peak generation. Gouras and Carr,[4] for example, showed that light peaks in monkey, along with the b-wave, disappeared following chronic interruption of the central retinal artery. Yet, such a catastrophic event might disturb the entire neural retina, especially its metabolism. There also could be nonspecific effects on the RPE or on photoreceptor-RPE interactions as indicated in another study by the decline in c-wave amplitude that followed central retinal artery occlusion.[30] Madachi-Yamamoto[7] injected sodium aspartate into the vitreous of monkeys at a high concentration (70 mM). The observed suppression of the light peak could have resulted from direct effects of aspartate on the RPE or the photoreceptors. (The observation that the c-wave was enhanced also could be due to a direct effect on the RPE.)

Dawis and Niemeyer recently observed that dopamine, as well as other monoamines depressed the light peak of the perfused cat eye.[8,9] They proposed that dopamine or a dopamine-like substance may be involved in light-peak generation and, assuming that dopamine is *released* on illumination from the inner retina,[31] that the inner retina may be involved in light-peak generation. Dopamine release in carp retina, which is related to synaptic transmission, is thought to be Ca^{2+}-dependent and can be eliminated by perfusion with cobalt concentrations within the range used in our experiments.[32,33] Since the light peak was not at all depressed by perfusion with Co^{2+}, our findings are inconsistent with the hypothesis that Ca^{2+}-dependent release of a neurotransmitter, such as dopamine, is required for light-peak generation.

Though our results support a photoreceptoral origin for the light peak's first messenger, they have not completely eliminated a role for other retinal cells. There is evidence from studies in cold-blooded vertebrates (salamander and toad) of a residual component of synaptic transmission from photoreceptors to bipolar cells and horizontal cells that survives blockade by cobalt and low calcium.[34] We have no knowledge, however, about a similar component of non-calcium-dependent synaptic transmission in the chick retina. There is the additional chance that light-peak generation requires a *non-synaptic* mechanism involving the photoreceptors and other retinal cells. A neuromodulator released by photoreceptors, for instance, could alter the release of a second modulator by other retinal neurons or glia that then diffuses to the RPE.

Overall, we can conclude that light-peak generation *does not* require: (1) Ca^{2+}-dependent synaptic transmission from photoreceptors to second order neural retinal cells or between other retinal cells; (2) transmission in the ON pathway that can be blocked by APB; or (3) transmission in the OFF pathway that can be blocked by PDA. These results are consistent with the hypothesis that the light peak results from a photoreceptor-RPE interaction mediated by a "light-peak" substance released from the photoreceptors.

Key words: DC electroretinogram, light peak, photoreceptors, retinal pigment epithelium, electrooculogram, chick

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