Growth in Amplitude of the Human Cone Electroretinogram with Light Adaptation

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The human cone electroretinogram gradually increases in amplitude an average of 75% (range 23 to 157%) during light adaptation, over a period of approximately 20 min. This increase involves both the a- and b-wave components of this response, and both waves follow a similar time course, implying that the photoreceptors themselves are responsible for the effect. The phenomenon occurs with suprathreshold, but not with threshold, levels of stimulation, and the stronger the test light, the greater the effect. An increase in the intensity of the adapting light shortens the time course of the ERG response, measured as b-wave implicit time, but this occurs almost immediately, and the implicit time then remains constant during the slow increase in response amplitude. The stronger the background adapting light, the smaller is the ERG amplitude, but the percentage growth (or rate of recovery) is unchanged. This slow increase in amplitude is thought to reflect the redepolarization of the cones, after their initial hyperpolarization to an adapting field. It does not reflect the d.c. potential of the eye (the EOG). It is essential to control this phenomenon in any studies of the human cone ERG, in order to minimize variability.

Herman Burian was the first person to notice that the human electroretinogram (ERG) can increase in amplitude during light adaptation, a somewhat paradoxical phenomenon since light adaptation in general tends to reduce the sensitivity of vision. This curious effect was confirmed by Armington and Bielsdorf, who provided quantitative data to support Burian’s anecdotal observations. They concluded that the increase was greatest when large areas of the retina were stimulated and when relatively high levels of illumination were used. They also thought that it only involved the b-wave and not the a-wave of the response. They also did not expose the magnitude of the effect. They hypothesized that it might be related to the light-evoked increase of the d.c. potential of the eye, ie, the electro-oculogram. They also performed psychophysical experiments paralleling their electrophysiological ones and found that the sensitivity for light detection (threshold) did not increase but in fact decreased slightly during the same time that the b-wave of the ERG was increasing, thereby demonstrating a difference between subjective threshold experience and suprathreshold electrophysiology. This confirmed earlier measurements of subjective threshold changes during light adaptation.

Independent of these observations on the human cone ERG, have been earlier demonstrations that the amplitude of the ERG to flicker also increased in the light-adapted state. Hood confirmed the increase of the cone flicker ERG in isolated frog retina and demonstrated two important points, one that this adaptation effect was due to light absorbed by cones and not rods and two, that it involved the a-wave as well as the b-wave of the ERG.

We have been studying this interesting phenomenon over a number of years but have only briefly reported some of our results. We present here a more complete and up-to-date account of this research. Our results show that this phenomenon has a major influence on the cone ERG, and that it also involves both the a- as well as the b-wave components of this response. The involvement of the a-wave provides an important clue to the possible cellular basis of this event.

Adequate control of this phenomenon is essential for reproducible clinical electroretinography.

Materials and Methods

The data presented here were obtained from 14 normal subjects between 14 and 76 years of age. Informed consent was obtained from all subjects, after the nature of the procedures had been explained fully. The methods we use have for the most part been
Fig. 1. ERG responses to a strongly suprathreshold white flash (3 Hz) at 5, 10, 15 and 20 min intervals after a change in retinal illumination from dim ambient levels (0.2-0.4 photopic trolands) to a brighter illumination: 3096/yellow (above), 860/green (middle) and 34/blue (below) photopic trolands. Each row of responses is obtained from a different subject. The calibration lower right indicates 10 /mV vertically for the upper and middle rows and 5 /mV vertically for the lower row of responses; it indicates 23 msec horizontally for all traces.

Results

Figure 1 illustrates ERGs taken at 5 min intervals after subjects had been exposed to a constant adapting light. The test stimulus was identical in all three cases. The upper row of responses were obtained on a yellow adapting field of 3096 photopic trolands, the middle on a green of 860 photopic trolands and the lower on a blue of 34 photopic trolands. In all three cases, there is a progressive increase with time of both the a- and the b-wave of the ERG. The magnitude of this increase is similar, regardless of the amount of background retinal illumination. The responses from the more light-adapted retina (top) have a shorter implicit time to the peak of the b-wave and are less oscillatory than those obtained from the less light-adapted retinas (middle and bottom). The a-wave was
measured in the usual way, from the baseline before the response to the negative peak just before the onset of the b-wave.

In all three states of retinal adaptation, the ERG response reflects cone activity predominantly, despite the relatively low photopic effectiveness of the blue and green background adapting lights. This is true because the adapting fields that are photopically weak (green and blue) are scotopically very strong, ie, they have a much stronger effect on rods than cones. The rods are saturated therefore, by the green and blue lights, and are not contributing to the ERG.

Figures 2 and 3 show how the normalized amplitudes of the a- and b-waves of four normal subjects increase with time during exposure to a yellow adapting light of 3096 photopic trolands. Both the a- and b-waves increase with time at approximately the same rate. This process takes at least 20 min before it reaches what appears to be an asymptotic maximum value.

Figure 4 illustrates one of the most interesting aspects of this phenomenon, the relationship between the strength of the light stimulus and the amplitude of the b-wave as this slow adaptation process evolves. Three different levels of stimulus intensity were used: a Wratten 21 (yellow) stimulus, marked 0.0, the same stimulus with a 0.6 neutral density filter added, and the same stimulus with a 1.1 neutral density filter added. Two different backgrounds were used, a yellow Wratten 21 (photopic effectiveness 3096 trolands) and a green Wratten 61 (photopic effectiveness 860 trolands).

Two facts are evident in Figure 4. First, the stronger the stimulus intensity, the greater the growth in amplitude of the ERG. Second, the stronger the photopic effectiveness of the adapting light, the lower the amplitude of the ERG, although the percent growth is little affected. The yellow (3096 photopic trolands) is much more effective than the green (860 photopic trolands) adapting light at reducing the amplitude of the ERG. Since the yellow is only one-quarter as effective for the rods as the green, this amplitude reduction must be predominantly a cone-mediated phenomenon.
Fig. 4. The absolute increase in b-wave amplitude with time of the same subject using three different intensities of a yellow stimulus flash: 0.0 = maximum; 0.6 and 1.1 indicate neutral density filtering used to reduce the flash intensity. Two different levels of retinal adaptation, 3096 (yellow) and 860 (green) photopic trolands were used. Numbers over or adjacent to each curve indicate the appropriate conditions.

Figure 5 illustrates the growth of cone b-wave amplitude to the same strongly suprathreshold test stimulus, but at two different levels of background adaptation covering approximately a ten-fold range in retinal illumination. Under both background adapting conditions there is a relatively similar rate of growth for this test stimulus.

Figure 6 illustrates how extraordinarily stable the implicit time of the b-wave response remains during the same time that its amplitude is increasing. Interestingly, there is an inverse relationship between implicit time and the strength of adapting light in photopic trolands. The greater the effectiveness of the adapting light for stimulating the cones, the shorter is the implicit time of the cone b-wave. Obviously, the amount of light that is being absorbed by the cones is determining the implicit time of the b-wave, at least at these light levels. We have also observed that for any one state of retinal adapting light for stimulating the cones, there is a characteristic implicit time of the cone b-wave. We have observed that at any one state of strong retinal adaptation the implicit time of the cone b-wave is virtually independent of the strength of the stimulus used to elicit the response. It is, therefore, the state of cone adaptation, rather than the strength of stimulation, that has the most influence on b-wave implicit time.

Discussion

Our results build on the earlier anecdotal observations of Burian1 and the more quantitative work of Armington and Biersdorf.2-3 These earlier investigations revealed that the light-adapted ERG could increase gradually in amplitude. They did not separate cone from rod activity, however, and they did not take advantage of ganzfeld stimulation.10 Therefore, they never appreciated the enormous magnitude of this effect on the cone ERG. Their much lower sensitivity for detecting this effect undoubtedly prevented them from noticing that it involved the a-wave as well as the b-wave component, thus putting the origin of this phenomenon at an even more distal site in the retina.
We have already suggested that the most reasonable explanation of this phenomenon is that it reflects the redepolarization of the cones following their hyperpolarization by the adapting light.7,8 A similar phenomenon was observed by Dowling and Ripps11 in intracellular studies of horizontal cells in fish retina. Horizontal cells also redepolarize slowly after first being hyperpolarized by light. This horizontal cell hyperpolarization and redepolarization is mediated by cones. They thought that the redepolarization of the light-adapting cones was leading to a corresponding redepolarization of the horizontal cells. As cones redepolarize they become able to generate larger hyperpolarizing responses to incremental light stimuli. This could explain why this effect is not strongly dependent upon the intensity of the adapting light (Fig. 5) because at each level of retinal illumination, the cone response would adapt to approximately the same operating range, i.e., membrane potential.

Short-wave-sensitive cones saturate at lower levels of background illumination than the longer wavelength cones. Indeed, the longer wavelength cones may never saturate. This may prevent short-wave cones from adapting to light as to great a degree as the other two cone mechanisms. Therefore this phenomenon may be due to the two longer wavelength-sensitive cone mechanisms alone. We already know it is mainly due to these mechanisms, because they dominate the human10 and primate12 cone ERG.

It is interesting that although the amplitude of the light-adapting cone ERG increases with time, the speed of the response, as measured by b-wave implicit time, remains quite constant. Response speed appears to be determined by a somewhat independent mechanism and this occurs almost immediately as the adapting light is turned on. Our measurements of the action spectrum of the mechanism responsible for shortening the implicit time of the cone ERG indicate that it is light absorbed by cones, not rods, that is responsible. This is consonant with Hood’s conclusions in the frog6 and with the current idea that adaptation is mainly a photoreceptor phenomenon.13

The fact that the increase in cone ERG amplitude occurs at supra- but not at threshold levels of stimulation may explain why there is no counterpart of this phenomenon in psychophysical studies of light adaptation. Psychophysical methods, in general, are constrained to threshold rather than suprathreshold visual phenomena.

Armington and Biersdorf1 hypothesized that the ERG growth during light adaptation was caused by the light-evoked increase of the d.c. potential of the eye (the EOG). Our data indicate this is not correct. The percent rise in the cone ERG with light adaptation is relatively independent of the strength of the adapting light, unlike the d.c. potential, where the stronger the adapting light, the greater the light rise. Also, the d.c. potential reaches a peak in the first 10 min and then recedes, while the amplitude increase of the cone ERG continues for 20 min or more and appears to be maintained.

This phenomenon is extremely important to recognize and control, because it could lead to considerable variations in responsiveness from the same or different individuals or different laboratories. We have observed the phenomenon at frequencies of 1, 3 and 5 Hz, and presume that it occurs at all frequencies at which the cones respond. This is in agreement with Hood’s study of the cone flicker ERG in the frog retina.6 Recently Miyake et al14 have detected this phenomenon in humans using relatively high rates of flicker, 35 Hz. Both techniques for isolating the cone ERG (i.e., low-frequency flashes on a rod-saturating background, or high-frequency flicker alone) must therefore be modified to control for cone growth with light adaptation. We currently wait at least 10 min after the background light is turned on, before definit-
ing cone b-wave amplitude, but clearly 15 to 20 min may be necessary for optimum accuracy.

Miyake et al have reported that this growth with light adaptation is abnormally exaggerated in patients with incomplete congenital stationary night blindness. We have not yet detected any other disease entities that show abnormalities in this growth, but it is likely that they exist.

**Note added at proof:** After this paper had been prepared, we discovered a relatively recent publication by Burkhardt DA and Gottesman J (Vision Res 27: 1409, 1987) showing that the membrane potential of fish cones takes 10-20 min to repolarize.

**Key words:** cone electroretinogram, light adaptation

**References**