Fast and Slow Components in the Electroretinogram of the Gastropod

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— The electroretinogram of the isolated eye of the gastropod (Halinstis discus) was studied with penetrating microelectrodes. The response recorded from the retinal surface with respect to the back of the eye was a slow negative potential change, but the response from the deeper layer was positive in polarity, indicating a depolarization of the distal part of the receptor cells. The electroretinogram of the dark adapted retina was a monophasic potential which had a smooth rising phase, but that of the light adapted retina was more complex in form, having an inflection which suggests the existence of the two responses in this simple retina of the abalone. Experiment of strong light adaptation and application of hypertonic KCl or ether vapor revealed that the electroretinogram consists of fast and slow components, and that the slow component is more susceptible to light adaptation and chemicals. Since one of the two responses originates undoubtedly from the receptor cells, the other origin was concluded to be the supporting cells, the response of which may be generated by some metabolic processes between the receptor and supporting cells. — gastropod; abalone; retina; electroretinogram

Since the earlier studies on the cephalopod electroretinogram (Beck 1899; Piper 1904; Fröhlich 1914), the cephalopod retina has received considerable attention because of the simplicity of its structure. In the retina of these animals the initial event of photoreception can be studied more easily than in the complex retina of the vertebrate. The gastropod eye, on the other hand, has not been studied until recently, although its structure is basically the same as that of the cephalopod. From the eye of the land snail, Gillary and Wolbarsht (1967) have recorded a monophasic, cornea negative electroretinogram concomitantly with spike activity of the optic nerve.

In the present study, another species of the gastropod mollusc, the abalone, was used to investigate the nature of the photoreceptor potential of this simple retina. The eye of the abalone is situated at the end of the eye stalk and the retina contains only sensory and supporting cells. The axons of the sensory cells pass out the retina and form a bundle of the optic nerve which leads to the brain. Much of the anatomical studies on the abalone retina have been made by a light

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microscope (Fraisse 1881; Hilger 1885) and also by an electron microscope (Tonosaki 1967), but no electrophysiological investigation has ever been attempted.

**METHODS**

The experimental animals were abalones (*Haliotis discus*) which had been obtained commercially. In the laboratory they were maintained in aerated sea-water aquaria. Before use the animals were dark adapted for at least one hour. The eyes were isolated by dissecting the eye stalks and the anterior half of the eye was removed by cutting with a razor blade under a dim red light. The retinal preparation was then transferred into a small cup of Ag-AgCl which served as an indifferent electrode.

The recording electrodes were glass pipettes filled with 3 mol. KCl; the tip diameter was less than 1 μ. The signals were amplified by a d.c. and an a.c. amplifiers with cathode follower input stages and displayed on a cathode ray oscilloscope for photography.

The light source was a Xenon arc lamp and the stimulating light was focused on the retina with a simple optical system. Spectral lights were obtained by interposing a series of interference filters in the optical path and the light intensity was adjusted by neutral density filters to provide equal energy for all wavelengths.

**RESULTS**

The electroretinogram (ERG) recorded from the surface of the retina with respect to the back of the eye was a slow negative potential change, which increased gradually after a long latency, reached a peak, and returned more slowly to the initial potential level. This basic pattern was independent of the stimulus duration, showing no plateau and off response. The ERG of the dark adapted retina was a simple monophasic potential with a smooth rising phase (Fig. 1, A), but that of the light adapted retina appeared somewhat complex in form, having an inflection on the rising limb of the response (Fig. 1, B).

![Fig. 1. ERG of the isolated abalone eye under dark (A) and light (B) adapted state. The third trace from top indicates stimulus mark. Upward deflection of the ERG indicates negativity of the retinal surface with respect to the back of the eye, in this and all the following figures.](image)

When the microelectrode was inserted deeper into the retina, the surface negative response remained almost constant during the first 30-50 μ, but further advancement of the electrode caused marked attenuation of the response amplitude and reversal of the response polarity. The sequence of potential change during the retinal penetration with a microelectrode is essentially the same as
observed in the octopus retina (Tasaki et al. 1963), although in the abalone retina the potential reversal is less marked and positive response is much reduced. This is probably due to the anatomical characteristics of this retina: A spherical eye of the abalone is very small (about 0.5 mm in diameter) and surrounded by tough connective and muscular tissues which do not allow the retina to be flattened. However, that the response reversed in polarity from negative to positive during the retinal penetration indicates the existence of an electric dipole layer in the retina. The structural basis for this double layer could well be an array of the receptor cells of which distal portion is depolarized by light as in the case of the other mulluscan retinas (Hagins et al. 1962; Tasaki et al. 1963; Gillary and Wolbarsht 1967).

As described already, when the retina was light adapted there appeared, in addition to reduction of the response amplitude, a small hump on the rising limb of the ERG (Fig. 1, B). It should be noted, however, that the hump was not necessarily to appear on the rising limb but it could appear either on the falling phase or on the peak. This observation may suggest that at least two different processes exist in the abalone retina. Thus, the following experiments were focused to separate these two components.

Effect of light adaptation. Fig. 2, A is a record obtained from a dark adapted retina. In this particular example, the response had a sharp rising phase, pointed peak, and much slower falling phase. When the retina was light adapted, the rising limb remained unaltered but the response fell rapidly, the slow response being selectively suppressed. From this and the preceding results (Fig. 1), it may be suggested that the abalone ERG consists of fast and slow responses and that the slow component is much more susceptible to light adaptation. Provided that the relative amplitude and latency of these two responses are different from one preparation to another, different results between Fig. 1 and Fig. 2 may be explained as follows: In Fig. 1 the fast response is much smaller than the slow, and in Fig. 2 the latency, rise time and amplitude are same in both the responses.

Effect of hypertonic KCl and ether. The previous results show that the slow component is highly susceptible to light adaptation. The next experiment further
studied the different behavior between the two responses, demonstrating the higher susceptibility of the slow component to various chemicals. For example, application of hypertonic KCl or ether vapor caused diminution of the two responses, but the slow component is much more markedly affected by these chemicals, thus separation of the two responses being manifested. An example is shown in Fig. 3. In this experiment the effect of ether was investigated under moderately light adapted state so that the two responses could be partially separated even before application of the vapor (top trace in Fig. 3). The second trace is the record taken immediately after application, and the third and fourth were taken 200 and 400 sec afterward.

![Fig. 3. Effect of ether vapor.](image)

*Effect of stimulus intensity and duration upon the two responses.* The effect of light intensity upon separation of the two responses was investigated under moderately light adapted state, so that a point of separation of the two components could be barely noticeable on the rising phase of the ERG. With increasing stimulus

![Fig. 4. Effect of increasing stimulus intensity.](image)
Two Components in the Gastropod ERG

Fig. 5. Effect of increasing stimulus duration of constant intensity.

intensity a relative height of inflection point (marked by a in Fig. 4) to the peak of the ERG (b in Fig. 4) appeared gradually lower, thus the ratio a/b becoming smaller. Although it is difficult to find from this result a definite relation between the stimulus intensity and each of the two responses, the result shown in Fig. 4 may at least suggest that the intensity-amplitude relation is different between these two responses. Essentially the same result could be obtained by increasing the stimulus duration of the constant intensity (Fig. 5).

DISCUSSION

The abalone ERG investigated in the present experiment is characterized by an extremely long latency, a slow rising and falling phase, and the absence of a plateau phase. This is probably due to the poor development of the receptor cells in this animal (A in Fig. 6). Recent electron microscopic study of the abalone retina (Tonosaki 1967) has shown, in the very tip of the receptor cell, some membrane infoldings which consist of the quipple-layered compound membranes. Since the basic structure of this membrane infoldings is essentially

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Fig. 6. Schematic drawing of the abalone retina. A: visual cell. B: supporting cell. a: axon of the visual cell. b: basement membrane. Based on Tonosaki (1967).
the same as that found in the primary photoreceptive region of all animals (Moody and Robertson 1960), it was concluded that, also in the abalone retina, photoreception would take place in this structure which is located in the restricted area at the apical tip of the visual cells (Tonosaki 1967). In the octopus retina, on the contrary, the photoreceptive site (outer segment) occupies the major part of the long visual cell and is characterized by the horizontal and vertical array of highly developed microvilli (Moody and Robertson 1960; Yamamoto et al. 1965). The ERG of this animal has a much shorter latency, rapid rising and falling phase, and a plateau which sustains as long as the stimulus continues (Tasaki et al. 1963).

The present study has also shown that the abalone ERG recorded from the retinal surface was a slow negative response with respect to the back of the eye, and that the surface response changed its polarity from negative to positive as a microelectrode was advanced deeper into the retina. In agreement with the previous studies in the cephalopod retina (Hagins et al. 1962; Tasaki et al. 1963) the present finding obtained with a penetrating microelectrode could be explained by assuming that the distal segments of the receptor cells would be depolarized by light. Such depolarization of the receptor cell is expected to act as a generator potential for the optic nerve fiber as demonstrated in the isolated retina of the land snail (Gillary and Wolbarsht 1967). The preliminary experiment with an intracellular microelectrode has indeed revealed that the receptor potential of the abalone retina is a slow depolarizing response (Tasaki and Tsukahara 1971).

The existence of the two responses in the simple retina of the abalone may not be unexpected, since similar findings were made in other molluscan retinas. Fujimoto et al. (1966) noted the separation of two components in Onchidium ERG, one of the two being speculated to arise from non-sensory neural elements in the vicinity of the visual cells. In the abalone retina there is no neural structure other than sensory cells and their axons, and therefore the possibility that the receptor potential is contaminated by other neural activity could be excluded.

Concerning the origins of the two responses in the cephalopod ERG, two diverse opinions have been proposed: Byzov and Orlov (1962) assume two loci along visual cell, and Tasaki et al. (1963) postulate the second potential origin in the pigment bearing supporting cell. Either of these two alternatives is difficult to determine in the present study of the abalone ERG. However, the possibility of two separate origins, one being receptor and the other supporting cell, may be excluded for the following reason. If each of the two kinds of the retinal cells, receptor and supporting, absorbs light independently and produces its own response, the wavelength-amplitude relation should be different each other, since the spectral absorption characteristics of the visual pigment differ from those of the screening pigment (Strother and Superdock 1972). Although the wavelength-amplitude relation or spectral sensitivity of these two responses was not able to investigate separately, the experiment with colored lights of equal energy seems to suggest that both the responses peak at the same spectral region near 500 nm (Fig. 7). It follows that the result of this experiment demands rejection of the
possibility of two independent cellular origins, but suggests that the two responses are generated sequentially. A reasonable explanation may be that light absorbed by receptor cells initiates the receptor potential, which in turn acts to trigger the second response, probably in the supporting cells. The mechanism for generation of the supporting cells' activity is not known at present, but it may be related to the metabolic processes between the receptor and supporting cells.

References


