A Hyperpolarizing Component of the Receptor Potential in the Median Ocellus of Limulus

Abstract. *There are two classes of photoreceptor cells in the median ocellus of* Limulus. *One class of cells respond to long wavelength (visible) stimuli with a depolarizing receptor potential and to near ultraviolet light with a biphasic, initially hyperpolarizing, receptor potential. The other class of receptors respond with a depolarization to near ultraviolet and with a biphasic response to visible light. In the latter type of cell, visible light can counteract the depolarization elicited by near ultraviolet light. The evidence suggests that there are two photopigments in each cell and that both are involved in the generation of receptor potential.*

Unlike the lateral and ventral eyes of *Limulus*, the median ocellus has been shown to be sensitive to near ultraviolet light (*1*). After studying the electroretinogram of the ocellus, previous authors (*1*) concluded that two visual pigments were present, with their absorbance maxima at about 360 nm and 530 nm. We have recorded intracellularly from the photoreceptor cells of the ocellus to determine if the two pigments are present in the same cell or are segregated into separate cells.

Intracellular potentials were recorded between a fluid-filled micropipette electrode (3*M* KCl or 0.5*M* K_2SO_4) introduced into the cell and an agar-salt bridge placed in the seawater bath, by using a solid-state capacitance-compensated electrometer and conventional electronics. In order to pass current through the microelectrode into the cell, the amplifier was placed in a standard bridge configuration (*2*). The stimulating light (from a 150-watt xenon arc) was transmitted through a monochromator and/or bandpass and neutral density filters and focused onto the preparation via quartz and mirror optics. When a microelectrode is introduced into a photoreceptor cell, the measured resting potential lies between 35 and 65 mv, inside negative; the membrane time constant, determined by recording the voltage change to a constant current pulse passed through the electrode, is 100 msec or longer.

As shown in Fig. 1A, the response of one type of photoreceptor cell to a short pulse of ultraviolet light ($\lambda = 375$ nm; approximately 50 msec duration) is a depolarizing receptor potential. Longer pulses of light elicit a receptor potential which, as has been reported in all other photoreceptors in *Limulus* (*3*), has a spike-like component, a "transient" component which may overshoot zero potential, and a steady-state component which lasts as long as the light remains on. The "notch" occurring between the wave and steady-state components may be exceptionally large in these receptors (Fig. 1E). In addition, however, a short pulse of visible light ($\lambda > 500$ nm) elicits the biphasic response shown in Fig. 1B. The receptor potential has an initial hyperpolarizing phase, followed by a late depolarizing phase. The depolarizing and biphasic responses can be elicited by alternating ultraviolet and visible stimuli and do not differ markedly in latency. About 70 percent of the cells from which we have recorded respond as shown in Fig. 1. For convenience, we call this an ultraviolet-type cell.

The remaining 30 percent of the cells respond with similar waveforms, but to opposite wavelengths. That is, as shown in Fig. 2, visible light ($\lambda > 500$ nm) elicited a depolarizing receptor potential, whereas an ultraviolet stimulus ($\lambda = 375$ nm) elicited a biphasic receptor potential. Such cells we call visible-type (*4*).

The action spectra of the depolarizing receptor potentials of the visible-type and ultraviolet-type cells peak near 530 and 360 nm, respectively. The action spectra of the hyperpolarizing receptor potentials have similar maxima. With one exception (see below), the characteristics of the two types of photore-

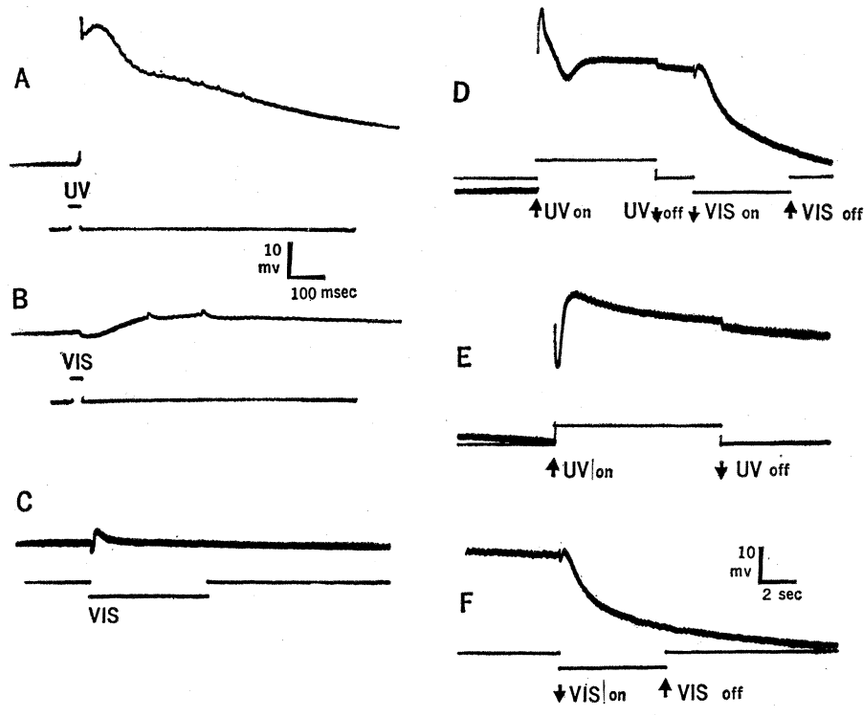

Fig. 1. Receptor potentials recorded intracellularly from an "ultraviolet-type" cell. The upper records are membrane voltage versus time; the lower records are a light monitor. Records (A) and (B) were taken from one cell; (C) through (F), from a different cell. (A) Depolarizing receptor potential to a flash ($\lambda = 375$ nm) of about 50-msec duration. (B) Biphasic receptor potential to a flash ($\lambda > 500$ nm) of about 50-msec duration. Calibration for (A) and (B): 10 mv and 100 msec. (C) Biphasic receptor potential to a long flash to visible light. (D) Response to a long ultraviolet flash followed by a long visible flash. When the visible light is turned on, there is an initial hyperpolarizing receptor potential followed by a rapid recovery toward resting potential. (E) Depolarizing receptor potential to a long ultraviolet stimulus. Note the steady state reached with the light on and the slow decay when the light is removed. (F) Next trace after (E). The repolarization by visible light can be effected at any time along the trajectory of the recovery after the ultraviolet stimulus. Calibration for (C) through (E): 10 mv and 2 sec. *UV*, ultraviolet; *vis*, visible light.

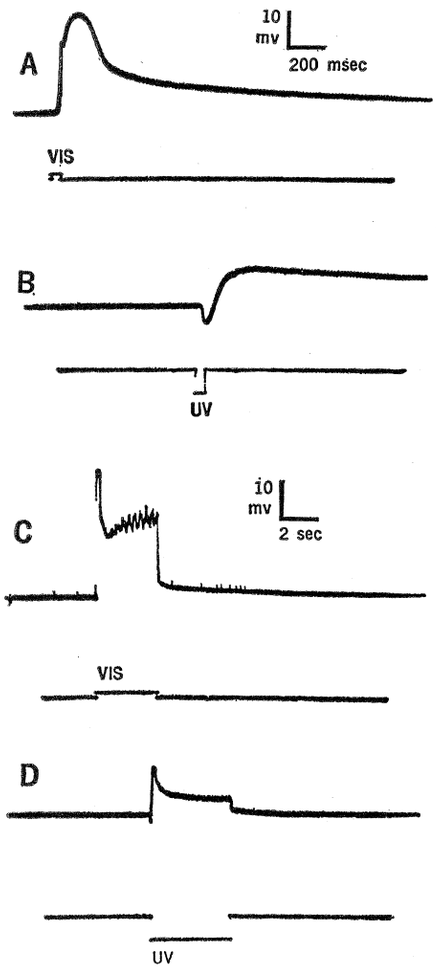

Fig. 2. Receptor potentials recorded from a "visible-type" cell. The upper records are membrane voltage versus time; the lower records are a light monitor. (A) Depolarizing response to a visible light stimulus ($\lambda > 500$ nm). (B) Biphasic response to an ultraviolet stimulus ($\lambda = 375$ nm). Calibration for (A) and (B): 10 mv and 200 msec. (C) Response to a long flash of visible light. Note the rapid return of membrane potential after cessation of the stimulus. (D) Response to a long flash of ultraviolet. The membrane initially hyperpolarizes then reaches a small depolarization in the steady state and decays rapidly at the cessation of the stimulus. Calibration for (C) and (D): 10 mv and 2 sec.

ceptor cells differ only in that the ultraviolet-type depolarizes to an ultraviolet flash, and hyperpolarizes to a visible flash, and the visible-type, vice versa. Both types of receptor cells exhibit phenomena previously reported in other phoreceptors in *Limulus* (*3*); spontaneous "bumps" occur in the dark-adapted ocellar cells; extrinsic current evokes a "spike-like" potential with depolarization or on the release of hyperpolarization; and occasionally, small spikes are superimposed on the depolarizing phases of the receptor potential (as in the lateral eye retinular cells).

The ultraviolet-type cell has one set of distinguishing characteristics. Upon the cessation of a long pulse of intense ultraviolet light to an ultraviolet-type cell, the membrane potential often decays back to resting potential with a time constant of minutes (see Fig. 1E and Fig. 3) whereas the membrane time constant to a pulse of extrinsic current is of the order of 100–200 milliseconds. If a visible light is turned on during this slow decay, the membrane quickly repolarizes back toward resting potential (Fig. 1, D and F; Fig. 3, C and F) and remains at that level even after the visible light is removed. The visible-light stimulus alone produces an initial hyperpolarizing response, which may or may not be followed by a small depolarizing steady-state potential (Fig. 1C). On the other hand, returning the membrane potential back to its resting level with current is not sufficient to insure this recovery of resting potential, that is, the membrane depolarizes again to a level predicted by the trajectory of the long decay when the current is turned off (Fig. 3E).

The initial ultraviolet stimulus need not be removed in order to observe a hyperpolarizing action of visible light. If the ultraviolet stimulus remains on, and the visible stimulus is presented during the steady state of the receptor potential, the membrane hyperpolarizes to a new steady state (Fig. 3B); the amplitude of this hyperpolarization depends on the relative intensities of the ultraviolet and visible stimuli.

The level of membrane potential for a single ultraviolet-type photoreceptor is thus controlled by the intensities of both the ultraviolet and the visible light which impinge on it. Put another way, the depolarization induced by ultraviolet light, that is, the steady-state receptor potential, can be counteracted (at least in part) by light of longer wavelength. We have not as yet observed a visible-type cell in which ultraviolet light either increases the rate of decay from the steady-state receptor potential toward resting potential following cessation of the stimulus, or reverses the depolarization caused by visible light. In general, the receptor potential in a visible-type cell decays to resting potential very rapidly when the stimulus is turned off (Fig. 2C).

The existence of both a depolarizing and an initial hyperpolarizing receptor potential in the same cell might indicate that there are two separate mechanisms in that cell, each controlled by a different photopigment. However, there are two other ways to account for the results if one assumes that any given cell possesses only one photopigment to control the mechanism for generating a depolarizing receptor potential. The first is that the hyperpolarizing receptor potential arises by an electrical interaction between the neighboring photoreceptors in a tightly packed array of cells. That is, during depolarization by ultraviolet light, net positive current would enter an ultraviolet-type receptor cell across its active membrane. If some of this current passed passively through a neighboring visible-type cell, and if the access resistance to the active mem-

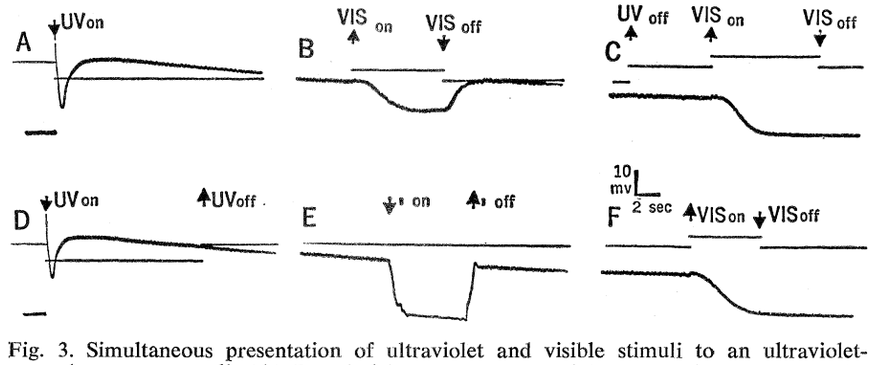

Fig. 3. Simultaneous presentation of ultraviolet and visible stimuli to an ultraviolet-type photoreceptor cell. (A) Depolarizing receptor potential to a steady-state ultraviolet light ($\lambda = 375$ nm). (B) Next sweep. Turning on a visible ($\lambda > 500$ nm) stimulus in the presence of a steady-state ($\lambda = 375$ nm) ultraviolet stimulus causes a partial repolarization of the membrane. (C) Next sweep. After cessation of the ultraviolet stimulus, the visible light elicits a repolarization back to resting potential. (D) Depolarizing receptor potential to a long ultraviolet flash ($\lambda = 375$ nm). (E) Next sweep. Repolarization by extrinsic current (arrow down) and release of current (arrow up). The membrane depolarizes to its original trajectory of recovery. (F) Next sweep. A visible light then repolarizes the membrane to resting potential. Calibration: 10 mv and 2 sec.

brane of the first cell were high, this current could hyperpolarize the visible-type cell. Preliminary experiments designed to test this hypothesis, involving the placement of two separate microelectrodes inside neighboring cells, indicate that such cells are not coupled. This evidence argues against an electrical interaction model.

The second alternative is that the initial hyperpolarizing component of the receptor potential is an inhibitory postsynaptic potential (IPSP) (*5*), that is, the result of an inhibitory chemical synapse of a visible-type cell upon an ultraviolet-type neighbor, or vice versa. Several lines of evidence suggest that this is not the case: we cannot reverse the polarity or significantly change the magnitude of the hyperpolarizing component by hyperpolarizing or depolarizing the cell with extrinsic current; we find no difference in the hyperpolarizing component when K_2SO_4 electrodes are used in place of KCl electrodes; both depolarizing and biphasic responses have comparable latencies; and finally, in the two-electrode experiments referred to earlier, the depolarization of one cell with extrinsic current would be expected to produce an IPSP in a neighboring cell if the presumed synapses existed, but these IPSP's have not been found. Thus, we feel that the evidence to date is most consistent with the hypothesis that two photopigments in each cell are involved in the generation of receptor potentials; alternatively, one pigment in two different states (*6*) would also suffice.

It also seems unlikely that the repolarization of the membrane of one cell can be effected through an electrical interaction by depolarization of a neighboring cell. Here, the electrical interaction hypothesis would require that extrinsic current produce the same effect as light, but this is not the case (Fig. 3E).

The hypothesis that the repolarization is the result of an inhibitory chemical synapse of the visible-type cell onto the ultraviolet-type cell is also unlikely. If the IPSP due to visible light were produced by ion-conductance changes, as has been found for IPSP's elsewhere (*5*), then upon cessation of the visible light stimulus, the potential ought to return again to the trajectory of the slow recovery of potential seen after a bright ultraviolet stimulus; this is not found (Fig. 1, D and F; Fig. 3, C and F). Here again, we feel that the data, showing that visible light both diminishes the depolarization elicited by ultraviolet light and causes the cell to repolarize rapidly to its resting level following cessation of stimulation (despite the small steady-state depolarization which may be produced by visible light), suggest that two photopigments, or two states of the same pigment, are involved in the mechanism producing the receptor potential.

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Genetic Implications of Common Region Sequence Comparisons of Lambda Immunoglobulin Chains Differing at Position 190

Abstract. *The common regions of two lambda chains (amino acid residues 109 to 213) have been partially sequenced. These two human immunoglobulin chains have lysine at position 190, but are otherwise identical in their common-region sequence to four reported lambda chains that have arginine at position 190. The single amino acid interchange at position 190 may be explained either by an ambiguous codon at this position or by a gene duplication so recent that only a single mutational event has occurred.*

Amino acid sequence information from homogeneous immunoglobulins, that is, those produced by plasmacytomas of man and mouse, indicates that immunoglobulin light chains contain a virtually constant COOH-terminal sequence (common region characteristic of chain type lambda or kappa) and an NH_2-terminal sequence specific to each plasma cell clone (*1*). The common region of lambda chains (CL region) from human immunoglobulins extends from residues 109 to 213 [according to the numbering of fully sequenced lambda protein Sh (2)] and has an amino acid interchange (arginine-lysine) at position 190 (Oz marker) (*3*); that is, about 75 percent of 107 lambda (Bence Jones) proteins have arginine at this position [Oz (−)] and 25 percent have lysine [Oz (+)] (*4*). Ein (*4*) has demonstrated that ten randomly chosen normal persons have both the lysine and arginine forms of the common region in their lambda chains and has indicated that this interchange is probably not due to allelic forms of a single gene. One might explain such an interchange by either of two models, (i) the presence of an ambiguous codon at position 190 or (ii) gene duplication followed by mutation. If the gene duplication is not a recent event, one might expect to find additional mutated sites. Thus, if the gene duplication at the common region is not recent, the arginine and lysine common regions should differ from one another at multiple positions just like the beta and delta chains of human hemoglobin which differ at ten positions (*5*).

Only four common regions of lambda chains have been completely sequenced; they are identical, and all have arginine at position 190 (and are termed CL-arg proteins) (*2*, *6*). We set out to see whether or not the common regions from two proteins with lysine at position 190 (that is, CL-lys proteins) were identical to their arginine counterparts (except for the interchange at position 190) in order to distinguish between a very recent gene duplication or translational ambiguity on the one hand and a gene duplication which occurred early in immunoglobulin evolution on the other hand.

Aminoethylated lambda chains (*7*)