EFFECTS OF REMOVING EXTRACELLULAR Ca^{2+} ON EXCITATION AND ADAPTATION IN *LIMULUS* VENTRAL PHOTORECEPTORS

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ABSTRACT In *Limulus* ventral photoreceptors, removing extracellular calcium (Ca_o^{2+}) increases the median latency of light-evoked discrete waves. Removal greatly lengthens the time-to-peak of responses in the dark-adapted cell, but not in the light-adapted cell. Removal does not block light-adaptation or the light-induced rise in intracellular calcium (Ca_i^{2+}). These results are interpreted in terms of the hypothesis that both sensitivity and the kinetics of excitation are dependent on Ca_i^{2+}, and that Ca_i^{2+} is dependent on Ca_o^{2+} in the dark-adapted cell, but in the light is dependent largely on Ca^{2+} released from intracellular compartments.

Like other photoreceptors, those of the *Limulus* ventral eye undergo changes in sensitivity to light (i.e. adapt). Several lines of evidence support the hypothesis (1) that light-induced desensitization is signaled by a rise in the intracellular free Ca^{2+} concentration (Ca_i^{2+}): intracellular injection of Ca^{2+} desensitizes the cells (2, 3); injection of Ca^{2+} buffer reduces light-induced changes in sensitivity (4); a light-induced rise in Ca_i^{2+} has been measured (5). The mechanism(s) responsible for the rise in Ca_i^{2+} have not been identified. One possibility is that a light-induced influx of Ca^{2+} from the extracellular space leads to a rise in Ca_i^{2+}. To test this hypothesis we have examined the effect of removal of extracellular Ca^{2+} on the process of light adaptation. The data show that, to the contrary, light adaptation is quite unaffected by removal of external Ca^{2+} (Ca_o^{2+}). One unexpected result of the experiments is the observation that removal of Ca_o^{2+} has a marked effect on the kinetics of excitation in dark-adapted cells. A brief report of the results below has been presented previously (6).

Cells were impaled with two microelectrodes and responses to light measured under voltage-clamp (7). The responses of dark-adapted cells to dim flashes were altered by extracellular perfusion with O-Ca seawater (SW) containing 30 mM of the Ca^{2+} chelating substance, EGTA (Fig. 1 A). In O-Ca SW the responses were longer in duration and had a longer time-to-peak than those in artificial seawater (ASW). These effects were reversible if the exposure to O-Ca SW was less than 4 min. It was

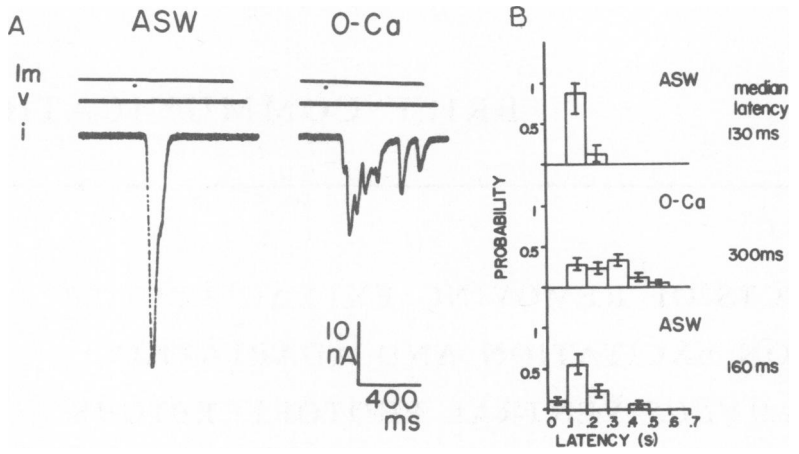

FIGURE 1 (*A*) Responses (light-induced current, *i*) to 10-ms flashes in ASW and O-Ca^{2+} SW. Removing Ca_o^{2+} made the response to the same intensity stimulus longer and less smooth. The responses were measured under voltage-clamp so that the voltage (V) was constant. Im is the light-monitor. (*B*) Probability distribution of the latency of discrete waves before, during, and 3 min after perfusion with O-Ca SW. In O-Ca SW the latency distribution was wider and the median latency longer than in ASW. The discrete waves were evoked by 10-ms flashes given every 3 s. Each flash evoked an average of two discrete waves. ASW is as defined in ref. 12. O-Ca SW was made by substituting 30 mM EGTA (brought to pH 7.8 by addition of NaOH) for 60 mM NaCl and making the solution isosmotic by addition of sucrose. The total time for an experimental run in O-Ca SW was shorter than 4 min. Similar results were obtained in six experiments. These results may be related to an effect of Ca_o^{2+} on the absolute latent period of the response to bright flashes in *Limulus* lateral eyes (14).

possible to repeat such experiments as often as 10 times on the same cell without deterioration of the receptor potentials.

The work of Dodge et al. (8) showed that receptor potentials in *Limulus* photoreceptors are the summation of discrete waves, each of which is evoked by a single photon. Therefore the results in Fig. 1 A could be interpreted to imply that O-Ca SW affects the latency of discrete waves. To examine this question directly, cells were stimulated with dim flashes that evoked, on the average, about two discrete waves. Latency histograms constructed from such experiments indicate that perfusion with O-Ca SW increases both the median latency of the discrete waves and the width of the latency distribution (Fig. 1 B). O-Ca SW had no obvious effect on the shape of the waves nor did it alter the probability of evoking them (the accuracy of this measurement is 20%). Little can be said about how Ca^{2+} might produce this effect on the median latency since the latent period itself is not understood. There are reasons (9) for believing that an internal transmitter couples the isomerization of rhodopsin to the production of discrete waves, but there is no direct evidence for such a transmitter.

Although removing Ca^{2+} had marked effects on the kinetics of excitation, it did not substantially inhibit the process of light adaptation, as shown in Fig. 2. A background

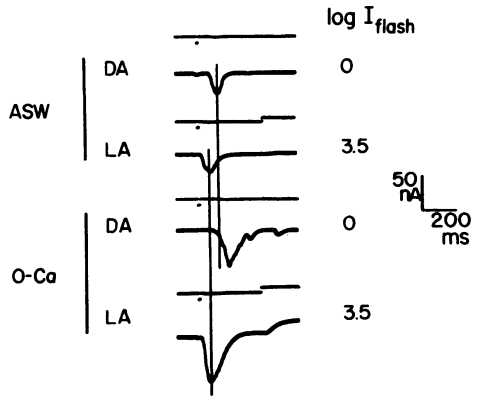

FIGURE 2 Light-adaptation is not blocked in O-Ca SW. In ASW, the test flash intensity had to be 3.5 log units brighter in the light-adapted cell (LA) than in the dark-adapted cell (DA) to produce approximately the same size response. Since light-induced current is linearly related to the intensity of brief flashes (15), small differences in amplitude are of little importance in measuring sensitivity. In O-Ca SW the same background light produced a similar desensitization of the cell. In the dark-adapted cell, removing Ca_o^{2+} increased the time-to-peak (t_p). In the light-adapted state, removing calcium had relatively little effect on t_p. The adapting stimulus had a duration of 1.3 s. Its intensity was 5.6 log units brighter than a stimulus evoking 1 discrete wave per second (approx. 1×10^{-5} W/cm^2). Measurements in O-Ca SW were made about 90 s after the solution change.

light that desensitized the cell by 3.5 log units in ASW produced a similar reduction of sensitivity in O-Ca SW. Experiments with brighter backgrounds showed that the cell could be further desensitized in either solution, but that the loss of sensitivity was somewhat less (1 log unit) in O-Ca SW. These observations bear on the role of intracellular sodium in the adaptation process. It might be argued that the rise in Na_i^+ (10) caused by the light-induced increase in Na^+ conductance (11) stimulates desensitization of the cell. This model is plausible because of the observation (2) that raising Na_i^+ by iontophoresis leads to a desensitization far greater than expected from the change in the Nernst potential for Na^+. However, the desensitization caused by injecting Na^+ does not occur if Ca_o^{2+} is low (2). Therefore, the observation that light-adaptation is largely independent of Ca_o^{2+} (Fig. 2) indicates that, at most, only a small component of light adaptation is stimulated by a rise in Na_i^+.

If light adaptation is related to a rise in Ca_i^{2+} the ability of the ventral photoreceptor to light-adapt in O-Ca SW implies that light must induce the release of Ca^{2+} from an internal compartment. This inference is consistent with the experiments of Brown and Blinks (5) in which changes in Ca_i^{2+} were measured with the Ca-activated photoprotein, aequorin. They detected a light-induced rise in Ca_i^{2+} in ventral photoreceptors externally perfused with seawater containing zero Ca^{2+} and Mg^{2+} and 5 mM EGTA. Fig. 3 (kindly provided by J. E. Brown and J. Blinks) shows that a light-induced rise in Ca_i^{2+} can be detected with aequorin in cells perfused with O-Ca SW

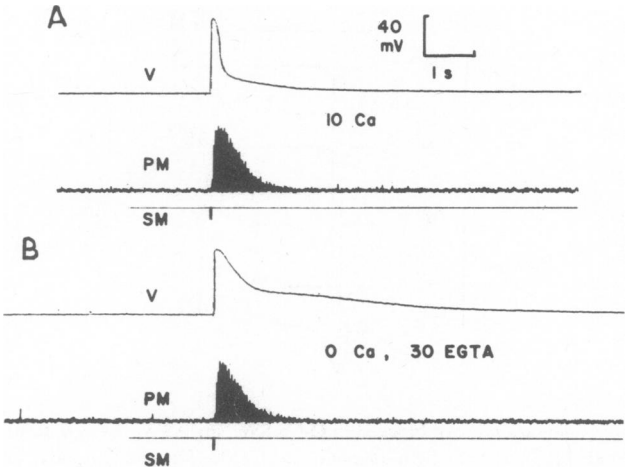

FIGURE 3 (*A*) A light-induced rise in Ca_i^{2+} is detected by aequorin when the cell is in artificial seawater that contains 10 mM Ca^{2+} and (*B*) after 90 s in O-Ca SW containing 30 mM EGTA. PM is proportional to the output of a photomultiplier tube responding to the light emitted from aequorin previously pressure-injected into a photoreceptor. V is the light-induced change in membrane potential. Sm is the stimulus monitor: stimulus was a 40 ms white flash (5×10^{-5} W/cm^2). See ref. 5 for detail of technique, and refs. 4 and 5 for a discussion of the transient nature of the aequorin response. Unlike the O-Ca, O-Mg SW used by Brown and Blinks (5), O-Ca SW does not substantially alter resting potential.

containing even higher concentrations of EGTA (30 mM). Thus, neither the light-induced rise in Ca_i nor the light-induced reduction of sensitivity can be substantially inhibited by removing Ca_o^{2+}.

The vertical lines in Fig. 2 are to aid in comparison of the time-to-peak (t_p) under various conditions. The experiments in Fig. 2 replicate the well-known observation (12) that light-adapting a photoreceptor speeds up the receptor potential (shortens t_p). This occurs both in ASW and in O-Ca SW. What is surprising however, is that whereas removal of Ca_o^{2+} has a large effect on t_p in the dark-adapted cell, it has relatively little effect on t_p in the light-adapted cell. Or, to put it differently, by light-adapting a cell, one can make t_p relatively insensitive to Ca_o^{2+}. A possible explanation of this finding is developed below.

A relationship between Ca_i^{2+} and t_p has been previously demonstrated in *Limulus* by Brown and Lisman (13). They showed that increasing intracellular Ca^{2+} by direct injection causes a *decrease* in t_p, implying that there are intracellular sites that regulate t_p. These intracellular sites may also mediate the *increase* in t_p that occurs when extracellular Ca^{2+} is removed from dark-adapted cells. All that need occur is that lowering Ca_o^{2+} lowers Ca_i^{2+} in the dark-adapted cell, and that lowering Ca_i^{2+} lowers the amount of Ca^{2+} bound to these regulatory sites. The assumption that t_p is controlled primarily by intracellular Ca^{2+} allows a simple explanation for the fact that, in the light, removal of extracellular Ca^{2+} has little effect on t_p (Fig. 2). Namely, the

level of intracellular Ca^{2+} in the light is relatively unaffected by removal of extracellular Ca^{2+} (Fig. 3).

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