Functional Significance of Voltage-dependent Conductances in *Limulus* Ventral Photoreceptors

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ABSTRACT The influence of voltage-dependent conductances on the receptor potential of *Limulus* ventral photoreceptors was investigated. During prolonged, bright illumination, the receptor potential consists of an initial transient phase followed by a smaller plateau phase. Generally, a spike appears on the rising edge of the transient phase, and often a dip occurs between the transient and plateau. Block of the rapidly inactivating outward current, i_A, by 4-aminopyridine eliminates the dip under some conditions. Block of maintained outward current by internal tetraethylammonium increases the height of the plateau phase, but does not eliminate the dip. Block of the voltage-dependent Na^+ and Ca^{2+} current by external Ni^{2+} eliminates the spike. The voltage-dependent Ca^{2+} conductance also influences the sensitivity of the photoreceptor to light as indicated by the following evidence: depolarizing voltage-clamp pulses reduce sensitivity to light. This reduction is blocked by removal of external Ca^{2+} or by block of inward Ca^{2+} current with Ni^{2+}. The reduction of sensitivity depends on the amplitude of the pulse, reaching a maximum at ~+15 mV. The voltage dependence is consistent with the hypothesis that the desensitization results from passive Ca^{2+} entry through a voltage-dependent conductance.

INTRODUCTION

Illumination of photoreceptors produces a receptor potential having a complex waveform. In initial attempts to model this waveform it was assumed that the kinetics of the receptor potential resulted directly from the kinetics of the membrane process directly activated by light (Fuortes and Hodgkin, 1964; Millecchia and Mauro, 1969 *b*). These models followed from the assumption that the photoreceptor membrane was electrically simple, containing only a light-activated conductance and a voltage-independent leakage conductance. Recent evidence, however, shows that the photoreceptor membrane is electrically complex, containing a variety of voltage-dependent conductances. For instance, vertebrate rods contain a Ca^{2+} conductance, a K^+ conductance, and an inward rectifier (for a review see Fain and Lisman, 1981). In invertebrates,

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voltage-dependent conductances have been found in scallop (Cornwall and Gorman, 1979), barnacle (Brown et al., 1970), and *Limulus* photoreceptors (Borsellino et al., 1965). In most cases, the role of these conductances in shaping the receptor potential remains unclear.

Limulus ventral photoreceptors provide a favorable preparation for examining the influence of voltage-dependent conductances on the receptor potential because these photoreceptors can be easily voltage-clamped. It is therefore possible to measure voltage-dependent currents directly and to separate pharmacologically the conductances that give rise to these currents. In a companion paper (Lisman et al., 1982), it was shown that the *Limulus* membrane contains at least three voltage-dependent currents, each of which is activated by depolarization: 1) a Na^+ and Ca^{2+} current that can be blocked by Ni^{2+}; 2) a rapidly inactivating current that strongly resembles the A current of molluscan neurons (Connor and Stevens, 1971) is probably carried by K^+, and is blocked by low concentrations of 4-aminopyridine (4-AP); 3) a K^+ current that resembles the K^+ current of squid axon (Hodgkin and Huxley, 1952) and is blocked by intracellular tetraethylammonium (TEA). The goal of this paper is to study the effects of these conductances on the receptor potential. The effects we have found fall into two categories: first, the direct electrical effects of current through the channels; and second, an indirect effect resulting from a rise of Ca^{2+}_i. Preliminary reports of some of these results have appeared (O'Day et al., 1980 *a* and *b*).

MATERIALS AND METHODS

The method of voltage clamp and the composition of solutions are described in the preceding paper (Lisman et al., 1982).

Throughout this paper, light intensities are described in log units of attenuation, neutral densities (ND). An unattenuated light (0 ND) has an intensity of 4×10^{-4} W/cm^2 at 530 nm, the wavelength used in all experiments.

Measurements of the photoreceptor's sensitivity to light are made using brief test flashes. The magnitude of the peak current evoked by brief flashes has been shown to vary linearly with flash intensity to a good approximation up to ~100 nA (Lisman and Brown, 1975 *a*). Sensitivity could then be defined as the ratio of light-induced current to flash intensity; however, in this paper we use the term "sensitivity" to refer to relative sensitivity. Because the test flashes used to measure sensitivity (see protocol of Fig. 5) produced responses within the linear range, the change in the log of sensitivity could be computed directly from the fractional change in amplitude.

The resting potential of *Limulus* photoreceptors measured with one electrode is typically −60 to −70 mV. After impalement with a second electrode, however, the resting membrane potential often becomes less negative. This reduction in resting potential is most likely due to leakage of current through areas of the membrane damaged by impalement. Therefore, in some experiments, the membrane was repolarized to −70 mV using current injection, which opposed the leakage current. In the experiments of Figs. 1, 3 *a*, and 4 *a*, a small (<2 nA) constant hyperpolarizing current was injected so that resting potential was −70 mV, with the rationale that a uniform resting potential would facilitate comparison of the effects of various manipulations on the receptor potential.

RESULTS

Electrical Influences of Voltage-dependent Conductances on the Receptor Potential

VOLTAGE-DEPENDENT INWARD CURRENTS In *Limulus* photoreceptors, a spike generally can be evoked electrically (Millecchia and Mauro, 1969 *a*) at anode break. The larger quantum bumps evoked by dim lights can also trigger such a spike. With brighter illumination a spike is often only evident as a notch on the rising edge of the receptor potential. Voltage-clamp experiments (Lisman et al., 1982) have shown that depolarization in the dark produces inward current carried by Na^+ and Ca^{2+} and that this current can be blocked by 10 mM Ni^{2+}. Therefore it was of interest to examine how the spikes were affected by Ni^{2+}. As shown in Fig. 1 the spike evoked by brief flashes was blocked by 10 mM Ni_o. The spike generated at anode break was also blocked by Ni^{2+} (Fig 2). Thus, it is reasonable to infer that the spikes are due to voltage-dependent influx of Na^+ and Ca^{2+}.

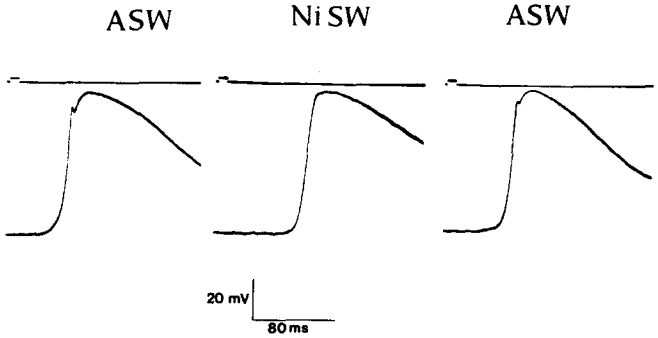

FIGURE 1. Ni^{2+} block of the regenerative event in the receptor potential. The cell was hyperpolarized in the dark to −70 mV by current injection. Moderately bright (2 ND) flashes of 10 ms duration were used. In normal SW a regenerative event was evident as a notch on the rising edge of the receptor potential. With application of 10 mM Ni^{2+} the spike disappeared and did not reappear after longer times even at much brighter flash intensities until return to ASW.

It should be noted in passing that an additional effect of 10 mM Ni^{2+} was to reduce the magnitude of the light-activated conductance and to slow its time-course. However, the disappearance of the light-activated spike in Ni^{2+} was not due to the reduction in light-activated conductance, since the spike could not be restored by raising light intensity.

VOLTAGE-DEPENDENT OUTWARD CURRENTS Intracellular TEA has been shown to block the steady-state outward currents in *Limulus* (Pepose and Lisman, 1978; Lisman et al., 1982). As shown in Fig. 3 *a*, TEA injection increased the height of the plateau phase of the receptor potential, in agreement with the findings of Pepose and Lisman (1978). The waveform of the receptor potential in Fig. 3 *a* shows a dip in the voltage waveform between the transient and plateau. Under the same conditions no dip was present in the light-induced current (Fig. 3 *b*). TEA injection made membrane voltage

during the dip slightly more positive, but the effect was small in comparison with the increase in plateau voltage. The fact that the dip in the receptor potential remained after the block of the delayed rectifier (Fig. 3 *c* and *d*) and that no dip was present in the light-induced current suggests that the dip in this particular cell must arise from the transient outward current, which in Fig. 3 *c* and *d* is shown to be only slightly affected by intracellular TEA at the concentrations used in these experiments.

The voltage-dependent transient outward current (A current) can be blocked by 4-AP (Lisman et al., 1982). Fig. 4 *a* shows that 0.1 mM 4-AP slowed the decline of the transient phase of the receptor potential, eliminated the dip between transient and plateau, and slightly increased the amplitude of the plateau. Voltage-dependent currents were measured in the dark in the same cell. Fig. 4 *c* and *d* shows that the A current was greatly reduced by 4-AP, whereas the maintained outward current was not greatly affected.

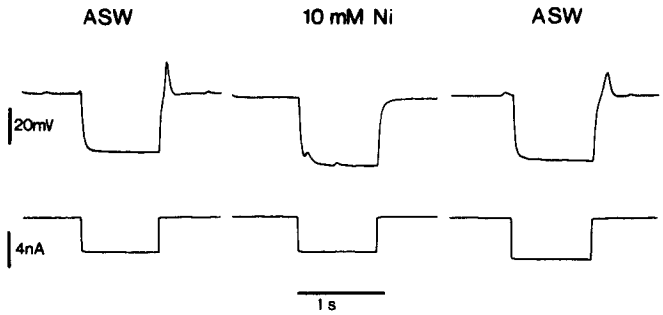

FIGURE 2. Block of anode break excitation by Ni^{2+}. A cell with a resting potential of −53 mV was hyperpolarized in the dark by current injection to −70 mV. Upon repolarization to rest, a regenerative event (anode break excitation) was seen in normal SW, but was blocked in the presence of 10 mM Ni^{2+}. The effect reversed upon return to ASW.

FIGURE 3. Effects of TEA injection. The data in this figure were obtained from one cell. (*a*) Receptor potential. The cell was hyperpolarized in the dark to −70 mV by current injection. A prolonged (20 s) bright (2.0 ND) light was presented to the cell. A higher plateau and a slightly more positive dip resulted after injection of TEA (8×10^{-6} C). Arrow indicates dip. (*b*) Light-induced current. The cell was voltage-clamped in the dark to −70 mV and presented with the same light as in *a*. The light-induced current was only slightly affected by TEA injection. (*c*) Responses to voltage pulses in the dark. The cell was voltage-clamped to −70 mV and presented with voltage pulses (top traces) in 10-mV increments. The resulting currents are shown in the bottom trace. (*d*) Maintained outward current. The current, i_{ss}, measured at the end of the pulse is plotted vs. membrane potential (V). i_{ss} was significantly reduced after TEA injection. (*e*) Transient outward current. The value i_A, the total $[i(t)]$ minus the maintained current (i_{ss}), is plotted vs. membrane potential (V) for $t = 50$ ms. i_A was slightly affected by TEA injection.

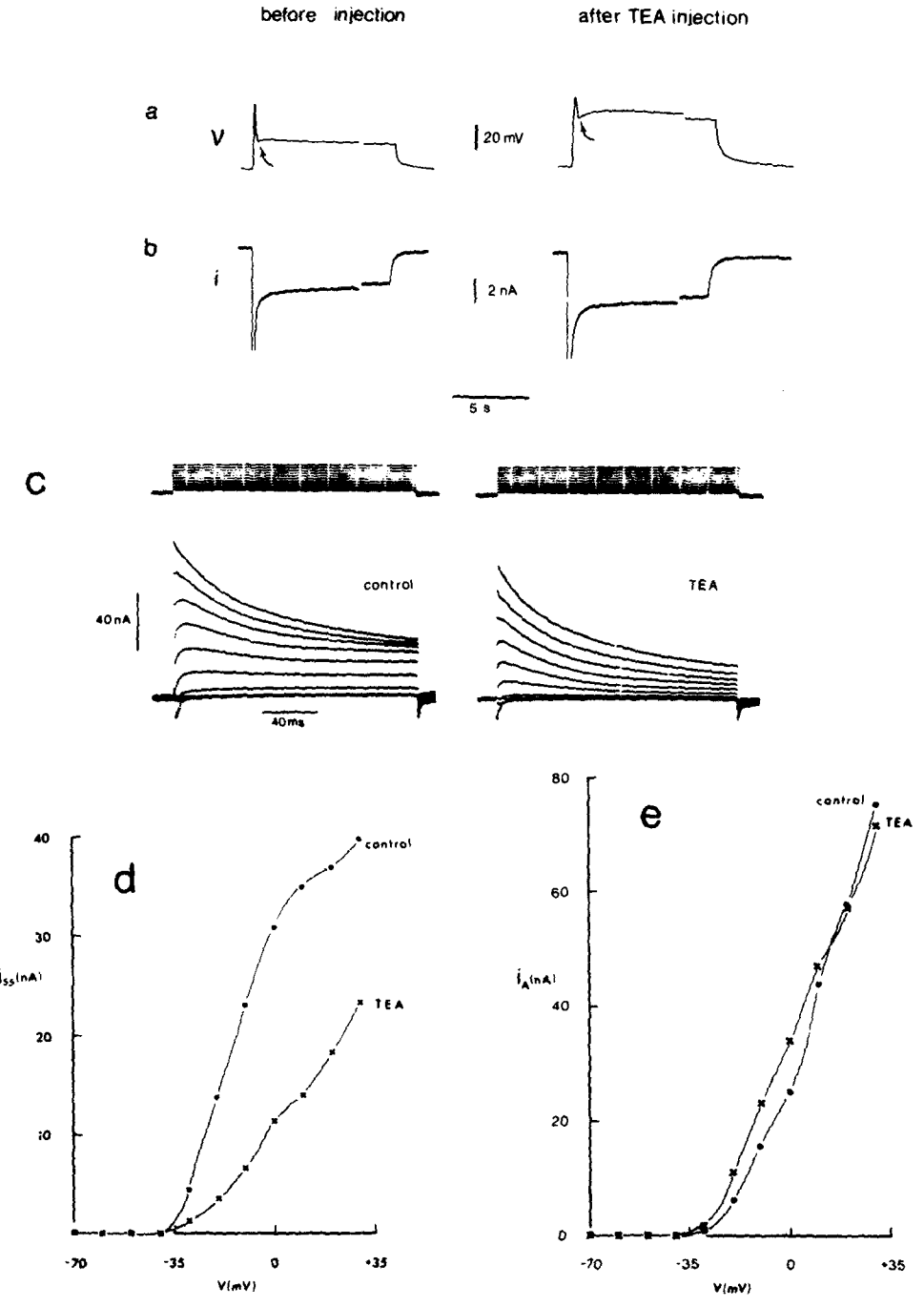
before injection
after TEA injection
a
V
20 mV
b
i
2 nA
5 s
c
40nA
control
TEA
40ms
d
control
TEA
i_{ss}(nA)
40
30
20
10
-70
-35
0
+35
V(mV)
e
control
TEA
i_A(nA)
80
60
40
20
0
-70
-35
0
+35
V(mV)

FIGURE 3.

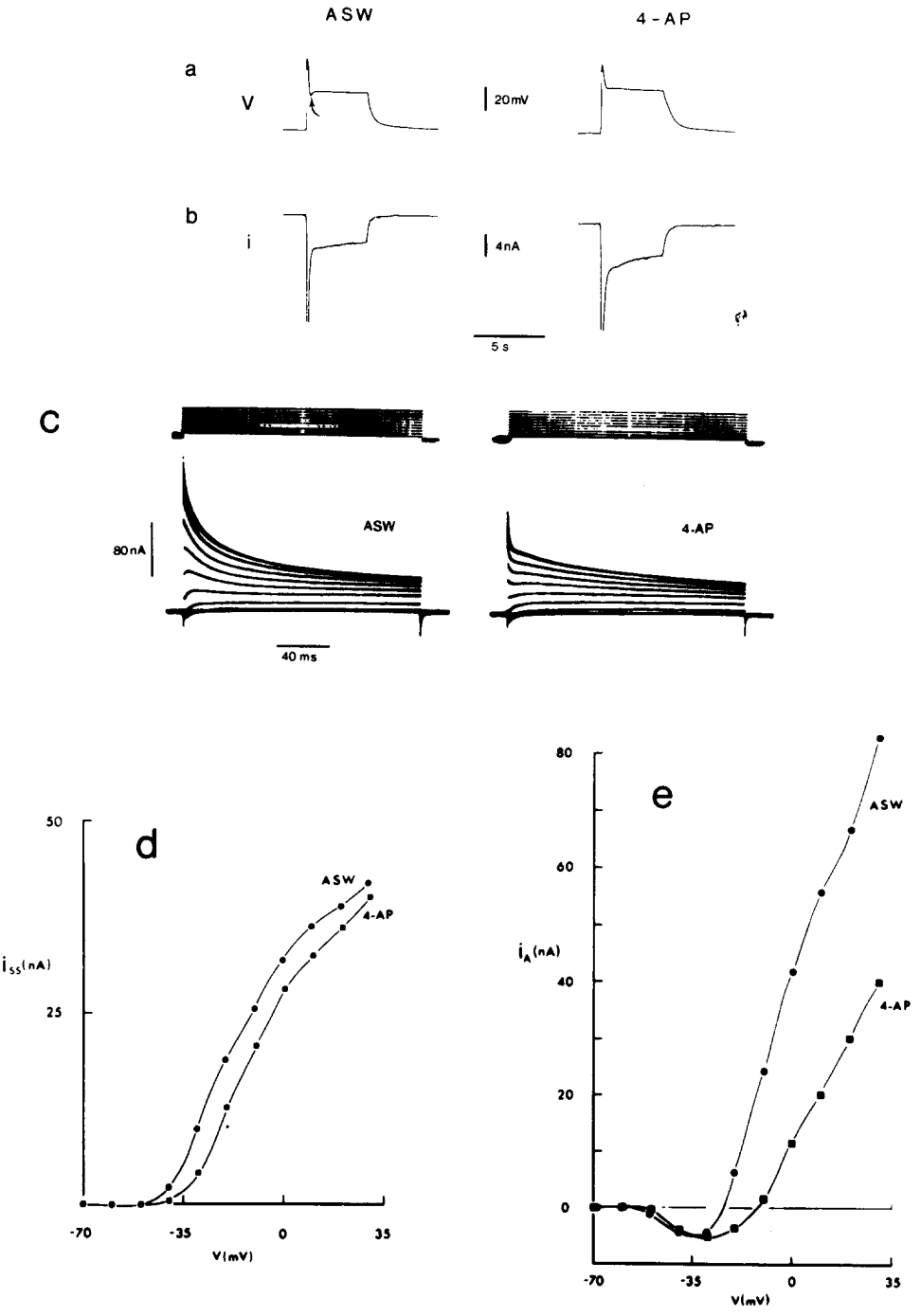
ASW
4-AP
a
V
20mV
b
i
4nA
5 s
c
80nA
ASW
4-AP
40 ms
d
50
25
i_{ss}(nA)
ASW
4-AP
-70
-35
0
35
V(mV)
e
80
60
40
20
0
i_A(nA)
ASW
4-AP
-70
-35
0
35
V(mV)

FIGURE 4.

Although higher concentrations (10 mM) of 4-AP greatly reduce the light-induced current (Pepose and Lisman, 1978), the concentrations used here produced only a small reduction of light-induced currents (not shown) and a slight slowing of kinetics (Fig. 4 *b*). Because no dip was present in the light-induced current (Fig. 4 *b*) and because the dip in the receptor potential was eliminated when the transient outward current was reduced by 4-AP, the dip in this case can be attributed to action of the A current.

In the course of investigating the dip, however, it became clear that other currents besides the A current could sometimes contribute to formation of the dip. The time-course of the light-induced current measured under voltage clamp reflects the kinetics of the light-activated Na^+ conductance, provided the holding voltage, V_H, is more negative than −40 mV (Lisman and Brown, 1971; Fain and Lisman, 1981). We found that many cells exhibited a dip in the light-activated conductance at $V_H = -70$ mV that corresponded in time with the dip in the receptor potential. The presence of the dip in the light-activated conductance depended on the light intensity and on the state of adaptation. Furthermore, the relative size of the dip was voltage dependent. In some cells depolarization eliminated the dip both in the receptor potential and in the light-activated conductance. These data indicate that under some circumstances the kinetics of the light-activated conductance strongly affects the formation of a dip in the receptor potential.

The A conductance is inactivated at voltages more positive than −40 mV (Pepose and Lisman, 1978; Lisman et al., 1982). Therefore, depolarizing the membrane before the light stimulus should eliminate the contribution of transient outward current to the dip. To do this experiment, we selected conditions under which no dip was present in the light-induced current. In two of the cells studied we found that depolarization could reduce the dip, but the dip persisted even at voltages at which the A conductance had been inactivated. In these cells, a conductance other than the light-activated conductance and the A conductance, presumably the delayed rectifier, must have produced the dip. In two other cells we found that the dip could be abolished by depolarization.

FIGURE 4. Effects of 4-AP. The data in this figure were obtained from one cell. (*a*) Receptor potential. The cell was hyperpolarized in the dark to −70 mV resting potential by current injection. A prolonged bright (2.0 ND) light was presented to the cell. 4-AP (0.1 mM) eliminated the dip, slightly increased the plateau, and lengthened the rate of decline from transient to plateau. Arrow indicates dip. (*b*) Light-induced current. The cell was voltage-clamped in the dark to −70 mV and presented with the same light as in *a*. The light-induced current was slightly affected by 4-AP. (*c*) Responses to voltage-clamp pulses in the dark. The cell was voltage-clamped to −70 mV and presented with voltage pulses in 10-mV increments (top traces). The resulting currents are shown in the bottom trace. (*d*) Maintained outward current. The current, i_{ss}, measured at the end of the pulse is plotted vs. membrane potential (V). i_{ss} was somewhat reduced by 4-AP. (*e*) Transient outward current. The value i_A, the total $[i(t)]$ minus the maintained current (i_{ss}), is plotted vs. membrane potential (V) for $t = 50$ ms. i_A was significantly reduced by 4-AP.

On occasion we observed a regenerative event that occurred on the upswing from a large dip. After this event the potential declined to a stable plateau. This regenerative event was probably due to the activation of voltage-dependent inward current (the Na^+ and Ca^{2+} conductance described in the preceding paper) by the depolarizing phase of the dip.

In summary, under some conditions the transient outward current appears to be the principal factor shaping the dip; under other conditions, however, the dip may be influenced by the light-activated conductance, voltage-dependent Na^+ and Ca^{2+} conductances, and the delayed rectifier.

Desensitization Mediated by Voltage-dependent Ca^{2+} Entry

It has been shown previously (Lisman and Brown, 1972) that a rise in Ca_i can reduce the sensitivity of *Limulus* photoreceptors to light. Thus the existence of a voltage-dependent Ca^{2+} conductance raises the possibility that depolarization might cause sufficient Ca^{2+} influx to reduce sensitivity. The following data show that depolarization in the dark can indeed reduce sensitivity and that this desensitization is produced by the voltage-dependent Ca^{2+} conductance. In the final section we show that a component of light-induced desensitization occurs as a consequence of the depolarization caused by light.

Depolarization in the Dark Produces Reduction of Sensitivity

Brief, dim flashes of constant intensity were presented while the cell was voltage-clamped to −70 mV (Fig 5). The light-induced currents (downward deflections) exhibit a characteristic variability in amplitude due to random fluctuations in the number of photons absorbed and the size of the response generated by each absorbed photon. A depolarizing voltage-clamp pulse was applied in an interval between two test flashes. After the pulse, the currents induced by test flashes were reduced in amplitude (Fig. 5 *a*), and they recovered over a period of ~20 s. Similar desensitization produced by depolarization in the dark was reported by Lisman and Brown (1972).

It was of interest to determine whether the reduction in response amplitude after depolarization was accompanied by a speeding of the response kinetics, mimicking light adaptation (Fuortes and Hodgkin, 1964; Fein and Devoe, 1973), or whether depolarization desensitized the cell by a process unrelated to normal light adaptation. Fig. 6 *a* shows that desensitization after a voltage pulse was accompanied by a speeding of kinetics, which suggests that depolarization affects the adaptation of machinery of the cell.

It should be noted that the protocol used in Fig. 6 differs significantly from that used in Fig. 5. The protocol of Fig. 5 precluded accurate measurement of sensitivity because of the large variation in the amplitude of the responses to dim test flashes. In Fig. 6, the relative variation was reduced by increasing test flash intensity; however, such brighter flashes could not be given repetitively because they produced considerable light adaptation, and this reduced the desensitization produced by depolarization (Fig. 9). Therefore, a *single* bright test flash (10 ms) was used to measure sensitivity. This test flash reduced the subsequent sensitivity to light, so that the cell had to be allowed to recover its

sensitivity before the next test flash was presented. Dark adaptation curves (Lisman and Strong, 1979) show that the rate of dark adaptation becomes slower as the cell approaches absolute threshold. By maintaining the cell slightly (~0.3 log units) light-adapted using repetitive dim flashes from a second light, the cell was kept out of the sensitivity range where the recovery rate was inordinately slow. With this procedure, ~5 min were required between test flashes for the cell to recover sensitivity. The protocol for

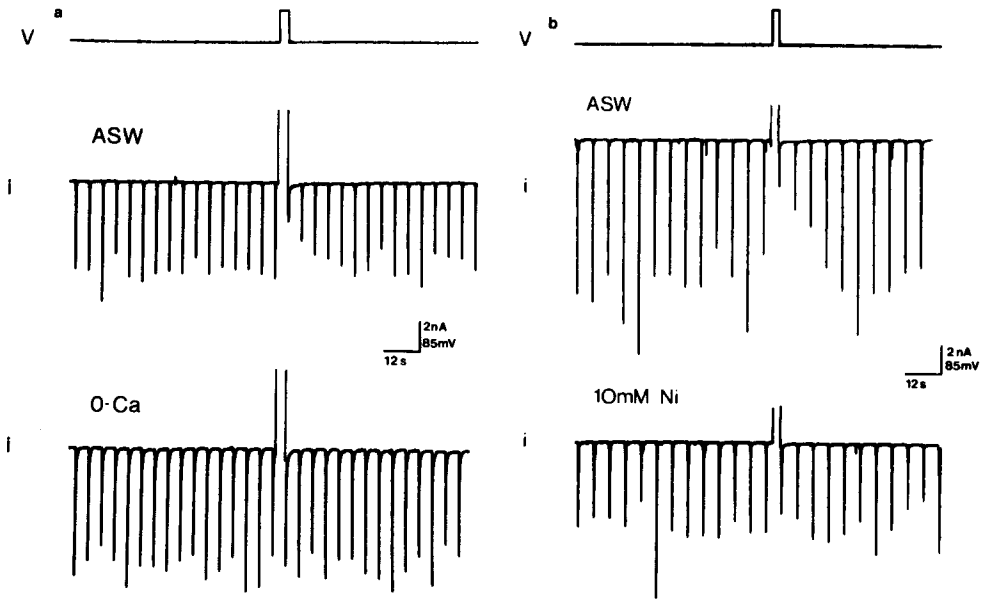

FIGURE 5. Desensitization of photoreceptors induced by depolarizing pulses. (*a*) The cell was voltage clamped to −70 mV holding potential. Brief (10 ms) dim flashes (4 ND) were presented to the cell at 5-s intervals. Light-induced inward currents are shown as downward deflections in the current trace (*i*). After the depolarizing pulse, the light-induced currents were reduced, and then slowly recovered. In 0-Ca SW there was no reduction in light-induced currents after the pulse. (*b*) Under the same protocol in a different cell, 10 mM Ni^{2+} inhibited the desensitization after a voltage pulse.

determining desensitization and the associated changes in kinetics was the following. Brief, dim flashes were presented at regular intervals to light-adapt the cell weakly, and a bright test flash was then presented to monitor sensitivity quantitatively. After the cell had recovered sensitivity (monitored by the average response to eight brief, dim flashes), the cell was presented with the same test flash, but this time it was preceded by a depolarizing voltage-clamp pulse. We have used this protocol in subsequent measurements of sensitivity (Figs. 7 and 8).

Reduction of Sensitivity Produced by Depolarization Depends on Ca_0^{2+}

To determine whether Ca^{2+} influx from the extracellular space during depolarization produces desensitization, we superfused the photoreceptor with 0-Ca seawater (SW). Superfusion with 0-Ca SW resulted in an increase in the

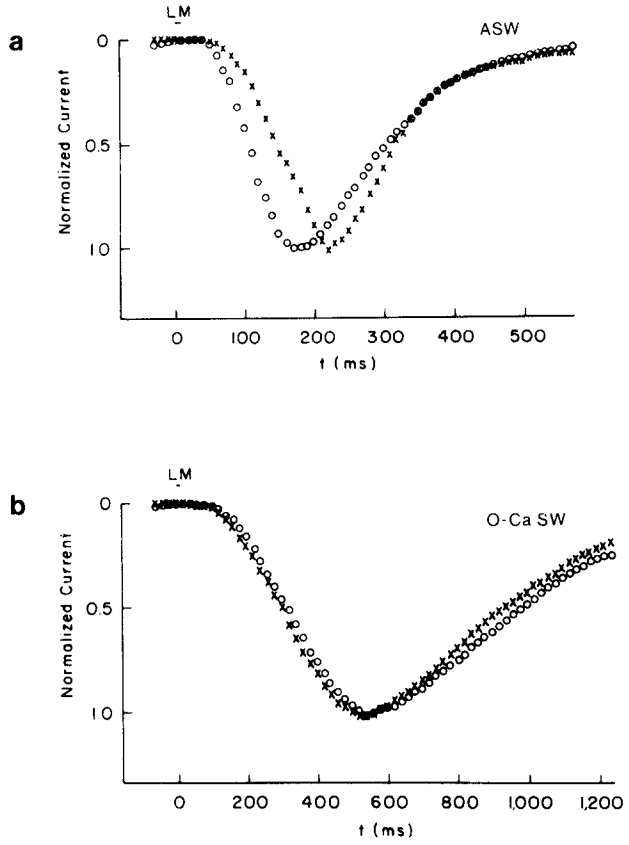

FIGURE 6. Alteration of photoresponse kinetics caused by a depolarizing pulse. (*a*) The cell was voltage-clamped to −70 mV holding potential and a 80-mV depolarizing pulse of 1 s duration was given. 300 ms after the pulse, a 2.6-ND, 10-ms test flash was presented. The kinetics of the normalized light-induced currents were faster after a desensitizing voltage pulse (○) than in the absence of such a pulse (×). (*b*) In 0-Ca SW, the kinetics were not altered by the pulse. Note that the light-responses were slower in 0-Ca SW than in ASW. The absolute magnitudes of the light-induced currents were ASW: 10.2 nA (×), 3.2 nA (○); 0-CaSW: 30.0 nA (×), 29.9 nA (○).

response amplitude and a slowing of the kinetics of the light response. Depolarization in 0-Ca SW produced no reduction in response amplitude (Fig. 5 *a*) or change in the kinetics of the light response (Fig. 6 *a*). Thus the effects of a depolarizing voltage pulse mimicked those of light-adaptation only in the presence of extracellular Ca^{2+}.

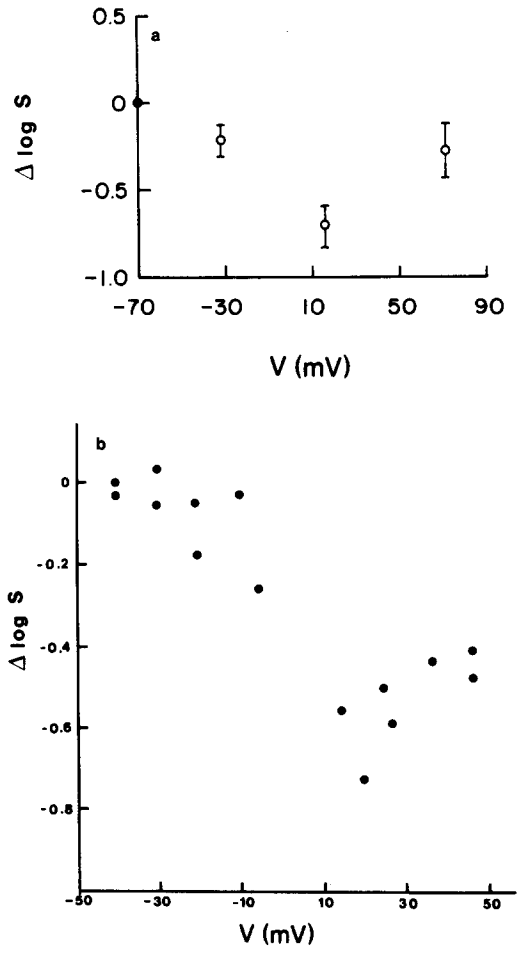

FIGURE 7. Voltage dependence of desensitization induced by a depolarizing pulse. (*a*) The log of sensitivity of the cell measured after a 980-ms pulse (from holding potential of −70 mV) was compared with that measured in the absence of a pulse. The protocol was the same as that used in Fig. 6. Six trials were made at each amplitude (bars indicate SD) using test flashes of 2.3 ND. Desensitization ($\Delta\log S<0$) was greatest with a pulse to +15 mV. (*b*) The log of sensitivity was measured at two different times during identical voltage pulses from a holding potential of −50 mV. Dim flashes were presented as previously, a depolarizing pulse was applied, and, 1.5 s after the onset of the pulse, a test flash was presented to measure sensitivity near the beginning of the depolarization. The cell was repolarized until sensitivity recovered. Then a second voltage step of the same magnitude was applied and a test flash was presented 5 s after the onset of the pulse. The difference between the two measurements ($\Delta\log S$) was plotted vs. pulse amplitude. Desensitization ($\Delta\log S<0$) was greatest with a pulse to ~+15 mV.

Because voltage-dependent inward current is blocked by 10 mM Ni^{2+} (Lisman et al., 1982), Ni^{2+} should also block the depolarization-induced desensitization if the desensitization is due to voltage-dependent Ca^{2+} current. Ni^{2+} did inhibit the desensitization (Fig. 5 *b*). However, Ni^{2+} itself reduced the light-activated conductance, and since depolarization-induced desensitization is smaller when the cell is less sensitive to light (Fig. 9), it could be argued that the decreased effect of depolarization was a secondary consequence of Ni^{2+}-induced desensitization. To settle this question, we light-adapted the photoreceptor in ASW to a sensitivity comparable to that previously measured in Ni SW. The data in Table I show that for approximately the same initial sensitivities, the desensitization resulting from a voltage pulse in Ni SW was

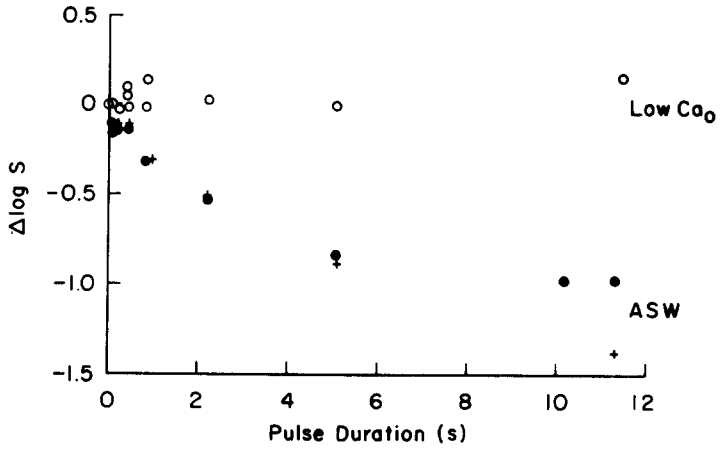

FIGURE 8. Dependence of depolarization-induced desensitization on the duration of the pulse. The log of sensitivity of the cell measured after a 90-mV depolarizing pulse from a holding potential of −70 mV was compared with that measured in the absence of a pulse. The protocol was the same as that used in Fig. 6. Desensitization ($\Delta \log S < 0$) increased with pulse duration up to 10 s in ASW (10^{-2} M Ca) but was absent at all pulse durations in low-Ca SW (10^{-4} M Ca). Low-Ca SW (○), 0-ASW, ASW recovery (+).

small compared with the desensitization resulting from the same pulse in artificial seawater (ASW). This result indicates that the block of desensitization induced by depolarization in Ni SW is not a secondary result of the desensitizing effect of Ni^{2+}. We conclude, rather, that Ni^{2+} acts by blocking voltage-dependent Ca^{2+} entry. Thus, reduction of the Ca^{2+} current, either by Ni^{2+} or by Ca^{2+} removal, inhibits the desensitization induced by a depolarizing pulse.

Desensitization Depends on Pulse Amplitude and Duration

To measure the voltage dependence of the desensitization produced by depolarization, we systematically varied the amplitude of the voltage-clamp pulse and used a bright test flash to measure the resulting reduction in

sensitivity ($\Delta \log S$). Desensitization induced by depolarization was not a monotonic function of voltage (Fig. 7 *a*). The desensitization was greatest for intermediate amplitude pulses (to ~+15 mV) and somewhat less for larger or smaller pulses. Depolarization of the membrane above +75 mV damaged the cells irreversibly.

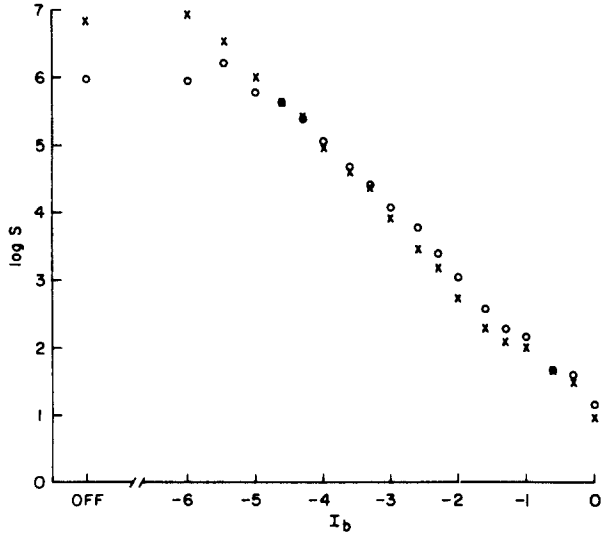

FIGURE 9. Dependence of depolarization-induced desensitization on the intensity of background illumination. Desensitization induced by voltage pulses varied with background illumination (I_b). The cell was voltage-clamped to −70 mV holding potential. A 100-mV, 2-s depolarizing pulse was applied. 250 ms after repolarization, a steady background light was presented, and 800 ms later, a test flash was superimposed on the background light. The log of sensitivity was calculated from the peak amplitude of the incremental response after the pulse (○) and in the absence of a pulse (×). [Two other protocols were used to determine the dependence of desensitization on photoreceptor adaptation: 1) The voltage pulse and the test flash both occurred *during* the background illumination; the test flash was given 250 ms. after the voltage pulse. 2) The background light was terminated 500 ms before the beginning of the voltage pulse, which was followed 250 ms later by the test flash.] Under each protocol, the desensitization induced by depolarization was greatest under dark-adapted conditions (background illumination OFF) and decreased with brighter background illumination.

The voltage dependence shown in Fig. 7 *a* is similar to that of other measures of passive Ca^{2+} entry. For example, direct measurement of intracellular Ca^{2+} accumulation using aequorin in squid giant axon (Baker et al., 1971) shows that Ca_i^{2+} increases with the amplitude of depolarization up to a point and then decreases with larger depolarizations. The interpretation of these results is that Ca^{2+} influx at low depolarizing voltages is small because

the activation of the Ca^{2+} conductance is small; as the depolarizing voltage increases, the conductance becomes more fully activated and Ca^{2+} influx is larger; at still more positive voltages, the Ca^{2+} influx decreases because the driving force for Ca^{2+} entry becomes negligible. The results shown in Fig. 7 *a* are consistent with the idea that desensitization results from passive entry of Ca^{2+} through a voltage-dependent conductance.

In the experiment described in Fig. 7 *a*, sensitivity was measured after the pulse. Because Ca^{2+} can enter the cell after repolarization during the short

TABLE I

EXTRACELLULAR Ni^{2+} BLOCKS DEPOLARIZATION-INDUCED DESENSITIZATION

	Δlog *S*	
Cell	ASW	10 mM Ni SW
1	−0.33	−0.08
	−0.49	−0.08
	−0.60	−0.08
	−0.80	−0.04
	−0.55 ± 0.20*	−0.07 ± 0.02*
2	−0.39	+0.02
	−0.40	−0.06
	—	+0.08
	—	−0.17
	—	−0.03
	−0.40 ± 0.01*	−0.03 ± 0.09*
3	−0.43	−0.19
	−0.50	−0.06
	—	+0.05
	—	−0.17
	—	+0.07
	−0.47 ± 0.05*	−0.06 − 0.12*

The pre-pulse sensitivity of each photoreceptor was reduced by light adaptation in ASW to match its pre-pulse sensitivity in Ni SW. 3.0-s depolarizing pulses from −70 mV holding potential to +30 mV were then presented. Sensitivity after the pulses was measured using a bright test flash. As shown below the desensitization (Δlog *S*) caused by the pulse was greater in ASW than in 10 mM Ni^{2+} SW.

* Average ± standard deviation.

time that the Ca^{2+} conductance remains activated, the desensitization may reflect the combined influx of Ca^{2+} during and after the pulse. It was therefore desirable to use a different protocol in which sensitivity was compared at early and late times *during* voltage-clamp pulses. Using this protocol, a more detailed description of the voltage dependence of the desensitization due to depolarization was generated (Fig. 7 *b*). Between −30 and +15 mV, desensitization increased monotonically with voltage. The desensitization then decreased as the depolarization was made still larger. Thus, with either protocol a maxi-

mum appears in the desensitization vs. voltage curve, consistent with the hypothesis that the desensitization is mediated by passive Ca^{2+} entry.

To characterize further the desensitization produced by voltage, we varied the duration of a voltage-clamp pulse. Fig. 8 shows that the desensitization produced by a 90-mV pulse increased with duration for times up to 10 s. This result suggests that the Ca^{2+} conductance does not completely inactivate, at least within 10 s after depolarization. A Ca^{2+} current of a similarly long duration has been described by Baker et al. (1973) in squid giant axon.

Desensitization Induced by Depolarization Depends on the Adaptational State of the Cell

When the level of Ca_i in a photoreceptor is raised by illumination (Brown and Blinks, 1974; Brown et al., 1977), the relative increase in Ca_i due to voltage-dependent Ca^{2+} entry should be reduced. Because sensitivity depends on Ca_i, the desensitization produced by voltage-dependent Ca^{2+} entry should be smaller in the light-adapted state than the dark-adapted state. Fig. 9 demonstrates that this prediction is correct. In this experiment the adaptational level of the cell was varied by presenting a series of background lights. The additional reduction of sensitivity produced by a voltage-clamp pulse that immediately preceded the background light was measured. As the intensity of the background light was increased, the desensitizing effect of the voltage-clamp pulse disappeared. Depolarization even sensitized the cell slightly at high background intensities. Results using three different protocols in which the sequence of voltage pulse, adapting light, and test flash were varied (see caption to Fig. 9) all showed that depolarization produced greater desensitization when cells were dark-adapted than when they were light-adapted.

Depolarization during a Normal Light Response Can Cause Desensitization

The previous experiments demonstrate that depolarization produced under voltage clamp can desensitize the photoreceptors. Light, of course, depolarizes the cell, but it remains to be shown whether this depolarization is itself important in the process of light adaptation. Certainly, extensive light adaptation can occur without any change of voltage (Lisman and Brown, 1975 *a*). If voltage-dependent Ca^{2+} entry during the receptor potential can contribute to further light adaptation, we would expect greater desensitization when the illuminated cell is allowed to depolarize than when it is clamped to a constant voltage.

The experiment shown in Fig. 10 demonstrates that the desensitization produced by light was indeed greater when the cell was allowed to depolarize. The cell was voltage-clamped to resting potential, and the sensitivity of the cell was monitored qualitatively using brief, dim flashes (not shown). The clamp was then turned off, and a brief adapting light was presented to the cell. After the resulting receptor potential was completed, the clamp was turned on. Then, 400 ms after the end of the adapting light, a bright test flash was presented to measure sensitivity quantitatively. After a recovery period, an identical adapting light was presented to the cell, but this time the voltage during the adapting light was held constant by the clamp. A bright test flash

was again presented 400 ms after the end of the adapting light to measure sensitivity. The difference between the two sensitivity measurements indicated the additional desensitization that occurred because the cell was allowed to depolarize during an adapting flash. In Fig. 10, $\Delta \log S$ due to depolarization was ~0.16. Similar experiments are summarized in Table II.

DISCUSSION

Effect of Voltage-dependent Conductances on Waveform of the Receptor Potential

The receptor potential in *Limulus* has a complex waveform that is due in part to the time dependence of the light-induced current (Millecchia and Mauro,

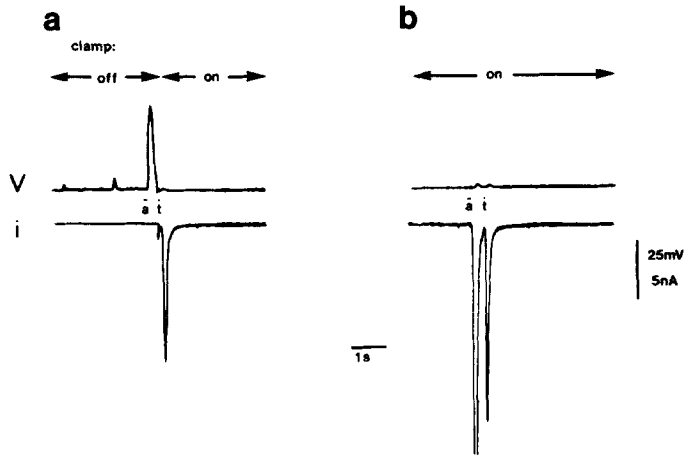

FIGURE 10. Desensitization produced by light-induced depolarization. An adapting flash (3.0 ND, 50 ms) was followed 400 ms later by a 10-ms brief bright (1.3 ND, 10 ms) test flash to measure sensitivity. The response to the test flash was always measured under voltage-clamp. In *a* the clamp was briefly turned off during the response to the adapting light. In *b* the cell was clamped to rest during the adapting light. (V_H = −46 mV). The sensitivity was significantly smaller (0.16 log units) when the cell was allowed to depolarize during the adapting flash (*a*) than when the cell was voltage-clamped to rest (*b*) during the adapting flash. The labels "a" and "t" are the light monitors for the adapting and test flashes, respectively.

1969 *b*). We have shown here that this waveform is also influenced by voltage-dependent conductances. Specifically, we have found that the spike on the rising edge of the receptor potential, the dip that occurs between the transient and plateau phases of the receptor potential, and the relative sizes of the transient and plateau phases are determined in part by the voltage-dependent properties of the membrane. The role that each of the identified voltage-dependent conductances plays in shaping the receptor potential is discussed separately below.

Inward Na^+ and Ca^{2+} Current

Neither the spike in *Limulus* ventral photoreceptors nor that in the retinula cells of the lateral eye is blocked by high concentrations of tetrodotoxin (Dowling, 1968; Bayer and Barlow, 1978) and is thus not generated by Na^+ channels of the kind found in axons. The spikes are blocked by Ni^{2+} (Fig. 2) at a concentration that was shown previously (Lisman et al., 1982) to block the voltage-dependent inward currents measured during depolarizing voltage-clamp pulses. Because this current is carried by Na^+ and Ca^{2+}, it is reasonable to conclude that the spikes are generated by the influx of both these ions. The results of Wulff and Fahy (1979), showing that similar spikes in lateral eye retinula cells have amplitudes that vary with the extracellular Ca^{2+} concentration, are consistent with the conclusion that Ca^{2+} influx contributes to the formation of the spikes.

TABLE II

DENSENSITIZATION PRODUCED BY LIGHT-INDUCED DEPOLARIZATION

Intensity of adapting background light	Additional desensitization due to depolarization	Receptor potential (ΔV)	Resting potential
I_a (*ND*)*	$\Delta \log S_u - \log S_c$‡	*mV*	*mV*
4.0	0	11	−44
3.3	0	32	−50
3.0	0.40	33	−44
3.0	0.15	34	−42
3.0	0.12	31	−41
2.6	0.16	29	−40
2.3	0.25	28	−37
2.0	0.12	30	−34

* Not all data from the same cell.

‡ Protocol was as described in caption of Fig. 10.

S_u is sensitivity measured after unclamped response to adapting light.

S_c is sensitivity measured after clamped response to adapting light.

A puzzling feature of the spikes in the ventral and lateral eyes is that their peaks ($\backsim -10$ mV) are much lower than the Na^+ and Ca^{2+} equilibrium potentials. Because the inward currents generating the spike are not located in the distant regions of axon (Lisman et al., 1982), it is unlikely that the spike amplitude is attenuated by electronic spread. A possible explanation is that the inward Na^+ and Ca^+ currents are counterbalanced by voltage-dependent outward currents. Bayer and Barlow (1978) have argued that the reversal potential for the channels carrying early inward currents is low because the channels also allow efflux of K^+. A low reversal potential would account for the small size of the spikes; however, such nonspecific channels of this kind have no precedence in the literaure. Further work will be required to clarify this problem.

Outward Currents (A Current and Delayed Rectifier)

From the properties of the A current (Lisman et al., 1982) one might expect *a priori* that this current would be activated by the transient phase of the receptor potential and that the resulting outward current would rapidly repolarize the cell. Then, as the outward current inactivated, the cell would depolarize slightly, thereby forming a dip in the receptor potential. In Fig. 4 we show an example of a cell that had a dip in the receptor potential, but no dip in the underlying light-induced currents. Blocking the A current with 4-AP reduced the rate of repolarization and eliminated the dip, in accordance with the model of the dip presented above. In instances such as this, the model of Fuortes and Hodgkin (1964) (see their Fig. 13) is clearly incorrect in that the dip cannot be attributed to oscillations of the light-activated conductance itself but rather appears to be due to the voltage-dependent A current.

In general we have found that the formation of the dip can be much more complex than in the example of Fig. 4. In some cases a dip in the light-activated conductance strongly influences the dip in the receptor potential. Delayed rectifier and voltage-dependent inward currents may also influence the dip (see pg. 217). In barnacle photoreceptors it has been shown that the dip is due to a large increase in K^+ permeability, and it has been suggested that the K^+ conductance increase is activated by a rise in Ca_i (Hanani and Shaw, 1977). This is unlikely to be the case in *Limulus* because injection of Ca^{2+} does not activate a K^+ conductance (Pepose and Lisman, 1978).

The amplitude of the plateau phase of the receptor potential is not greatly affected by blocking the A current (Fig. 4 *a*). However, blocking the delayed rectifier with TEA produces a large increase in the amplitude of plateau phase (Fig. 3 *a*). Similar results were previously reported by Pepose and Lisman (1978), and the reader is referred to their paper for a more complete account of the role of the delayed rectifier and the kinetics of the light-induced conductance in determining the amplitude of the transient and plateau phase of the receptor potential.

Desensitization Produced by Voltage-dependent Ca^{2+} Entry

There is now substantial evidence that light increases Ca_i in *Limulus* (Brown and Blinks, 1974) and that this rise mediates the normal process by which light reduces sensitivity (Lisman and Brown 1972, 1975 *b*). The light-induced rise in Ca_i appears to be due to three independent mechanisms. First, a light-induced release of Ca^{2+} from intracellular stores was demonstrated by Brown and Blinks (1974), who recorded a rise in Ca_i even after long periods of superfusion with 0-Ca SW (see also Lisman, 1976). Second, evidence for voltage-independent influx of Ca^{2+} from the extracellular space during illumination was presented by O'Day and Lisman (1979): under constant voltage, light adaptation was slightly reduced by removal of extracellular Ca^{2+}. This voltage-independent Ca^{2+} entry might be due to an effect of the light-induced rise in Na_i (Brown and Lisman, 1972) on Na^+-Ca^{2+} exchange (see Fain and Lisman, 1981). Finally, in this paper we present evidence for voltage-dependent entry of Ca^{2+} from the extracellular space.

The contribution of the depolarization-induced rise in Ca_i to adaptation may not be significant in many cases. For instance, Fig. 9 shows that even very large voltage-clamp pulses fail to desensitize the cell under light-adapted conditions. Thus, desensitization produced by voltage-dependent Ca^{2+} entry is likely to be important only under relatively dark-adapted conditions. A likely explanation is that increases in Ca_i due to the other mechanisms are much larger than those resulting from voltage-dependent entry. Brown et al., (1977), using Arsenazo III, have found that the light-induced rise in Ca_i in *Limulus* can be very large (100–650 μM). Although a comparable measure of voltage-dependent increases in Ca_i is not available for *Limulus*, we can estimate from Arsenazo III records of Gorman and Thomas (1978) and Ahmed and Connor (1979) that Ca_i might rise only as high as 2 μM during a 2-s voltage-clamp pulse to 0 mV in molluscan neurons. Thus, our finding that voltage-dependent desensitization is negligible under light-adapted conditions seems quite reasonable.

Role of Voltage-dependent Channels in Transduction

Limulus photoreceptors have the remarkable ability to generate large signals (up to 50 mV) in response to single photons, and yet they can respond over an intensity range of 10^9 without becoming saturated (Barlow and Kaplan, 1971). In this context, each of the voltage-dependent conductances seems to serve the common goal of maximizing the response to dim lights while minimizing the response to bright lights.

Dim lights evoke "quantum bumps" (Fuortes and Yeandle, 1964), the larger of which initiate a spike. As a result, the amplitude of the single photon response is increased severalfold. In the lateral eye this increase is necessary for initiation of a spike in the optic nerve (Dowling, 1968). If K^+ channels were open at or near resting potential (−60 to −70 mV) they would reduce the size of the light-induced depolarization. However, voltage-dependent K^+ channels are not activated until the voltage becomes more positive than −40 mV. Furthermore, the time dependence of K^+ channels is such that when the voltage becomes more positive than −40 mV, the channels are activated more slowly than the voltage-dependent inward currents that give rise to the spike. Thus, under conditions of very dim illumination the voltage-dependent inward currents serve an amplifying role, whereas the voltage-dependent K^+ currents do not reduce the response until after a spike is initiated.

With brighter illumination the situation is different. The maintained response is more positive than −40 mV. However, for reasons that remain unclear, repetitive spiking does not occur, which indicates that the response is not significantly amplified by voltage-dependent inward currents. On the contrary, inward Ca^{2+} currents may decrease the response by producing a rise in Ca_i. Similarly, voltage-dependent K^+ channels reduce the size of the response (Fig. 3) and prevent saturation during very bright lights (Pepose and Lisman, 1978). Thus, under conditions of moderate and bright illumination, inward currents do not amplify the responses and may serve to decrease the response, as do the voltage-dependent K^+ conductances.

MODELING THE RECEPTOR POTENTIAL

A quantitative description of the functional role of voltage-dependent conductances in shaping the receptor potential is desirable. One approach would be to separately model the light-activated conductance and each of the voltage-dependent conductances and then to predict the receptor potential from the voltage-dependent interactions between the conductances. However, we now know that the interactions are more complex than this. We have shown here that the light-activated conductance and the voltage-dependent Ca^{2+} conductance are coupled by a feedback loop that involves Ca_i. Furthermore, Leonard and Lisman (1981) have recently shown that the delayed rectifier is affected not only by voltage, but is also modulated by light through some unknown pathway. Thus, although the rough outlines of the factors shaping the receptor potential are becoming evident, a quantitative theory for the receptor potential that includes all the known interactions is still a distant goal.

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