Physiological Roles of Na^+/Ca^{2+} Exchange in *Limulus* Ventral Photoreceptors

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ABSTRACT In previous work we have presented evidence for electrogenic Na^+/Ca^{2+} exchange in *Limulus* ventral photoreceptors (1989. *J. Gen. Physiol.* 93:473–492). This article assesses the contributions to photoreceptor physiology from Na^+/Ca^{2+} exchange. Four separate physiological processes were considered: maintenance of resting sensitivity, light-induced excitation, light adaptation, and dark adaptation. (*a*) Resting sensitivity: reduction of $[Na^+]_o$ caused a $[Ca^{2+}]_o$-dependent reduction in light sensitivity and a speeding of the time courses of the responses to individual test flashes; this effect was dependent on the final value to which $[Na^+]_o$ was reduced. The desensitization caused by Na^+ reduction was dependent on the initial sensitivity of the photoreceptor; in fully dark-adapted conditions no desensitization was observed; in light-adapted conditions, extensive desensitization was observed. (*b*) Excitation: Na^+ reduction in fully dark-adapted conditions caused a Ca_o^{2+}-dependent depolarizing phase in the receptor potential that persisted beyond the stimulus duration and was evoked by a bright adapting flash. (*c*) Light adaptation: the degree of desensitization induced by a bright adapting flash was Na_o^+ dependent, being larger with lower $[Na^+]_o$. Na^+ reduction enhanced light adaptation only at intensities brighter than 4×10^{-6} W/cm^2. In addition to being Na_o^+ dependent, light adaptation was Ca_o^{2+} dependent, being greater at higher $[Ca^{2+}]_o$. (*d*) Dark adaptation: the recovery of light sensitivity after adapting illumination was Na_o^+ dependent. Dark adaptation after bright illumination in voltage-clamped and in unclamped conditions was faster in normal-Na^+ saline than in reduced Na^+ saline. The final sensitivity to which photoreceptors recovered was lower in reduced-Na^+ saline when bright adapting illumination was used. The results suggest the involvement of Na^+/Ca^{2+} exchange in each of these physiological processes. Na^+/Ca^{2+} exchange may contribute to these processes by counteracting normal elevations in $[Ca^{2+}]_i$.

INTRODUCTION

Na^+/Ca^{2+} exchange serves important physiological roles by regulating $[Ca^{2+}]_i$, the intracellular Ca^{2+} concentration (Reuter and Seitz, 1968; Baker et al., 1969; Blaustein

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and Hodgkin, 1969; DiPolo and Beaugé, 1983). The exchange can occur in the forward direction to extrude Ca^{2+} or in the reverse direction, yielding Ca^{2+} influx. The maintenance of a low intracellular Ca^{2+} concentration and of the large transmembrane Ca^{2+} electrochemical energy gradient is essential for normal neuronal function. Na^+/Ca^{2+} exchange is important in vision, operating in photoreceptors of many different organisms, vertebrate and invertebrate (Yau and Nakatani, 1984; McNaughton et al., 1986; Minke and Tsacopoulos, 1986; O'Day and Gray-Keller, 1989), and it can change $[Ca^{2+}]_i$ sufficiently fast to influence photoreceptor function.

In *Limulus* ventral photoreceptors, Ca^{2+} appears to play an essential role in neural excitation by light (Bolsover and Brown, 1985; Payne et al., 1986*a*, *b*) and in light adaptation, the desensitization induced by bright illumination (Brown and Lisman, 1975). In light adaptation, Ca^{2+} is released from intracellular stores in response to illumination (Brown and Blinks, 1974; Payne and Fein, 1987), and the resulting rise in $[Ca^{2+}]_i$ leads to a reduction in the sensitivity of the cell to subsequent illumination, measured as a reduction in electrical response amplitude (Lisman and Brown, 1972). Since the Ca_i^{2+} appears to be essential for physiological function, the regulation of intracellular levels and distribution of Ca^{2+} must also be important for the physiology.

In this article, we examine the contribution of Na^+/Ca^{2+} exchange to resting sensitivity, excitation, light adaptation, and dark adaptation, the recovery of sensitivity after light adaptation. We suggest that Na^+/Ca^{2+} exchange helps to maintain resting sensitivity of the cell by keeping $[Ca^{2+}]_i$ low, and we suggest that it may act to influence the waveshape of the receptor potential. We also suggest that Ca^{2+} extrusion by Na^+/Ca^{2+} exchange influences both light adaptation and dark adaptation.

Some of the results have been reported previously in abstract form (O'Day and Lisman, 1979; Keller and O'Day, 1986; O'Day and Keller, 1986, 1987*a*, *b*; Gray-Keller et al., 1989).

METHODS

Preparation

Limulus polyphemus were obtained from Marine Biological Labs, Woods Hole, MA. Ventral nerves were removed, desheathed, and treated with pronase (2% for 1 min). Physiological measurements, microelectrode preparation, two-electrode voltage clamp, and current clamp are described in Lisman et al. (1982). Because of the large size of the photoreceptors, whole-cell single-electrode voltage clamp was not possible. During dissection and desheathing, the tissue was bathed in normal saline (or artificial seawater, ASW): 425 mM NaCl, 10 mM KCl, 22 mM $MgCl_2$, 26 mM $MgSO_4$, 10 mM $CaCl_2$, 15 mM Tris Cl, pH 7.8. Each experiment required salines with Na^+, Ca^{2+}, and Mg^{2+} concentrations that differed from those in ASW. "Normal" saline designates NaCl of 425 mM and $CaCl_2$ of 10 mM as in ASW; "0 Na^+" indicates that the NaCl was replaced mole-for-mole with LiCl or with Tris Cl (TRIZMA, Sigma Chemical Co., St. Louis, MO). "0 Ca^{2+}" indicates that no $CaCl_2$ or EGTA was added to the solution and that 10 mM additional $MgCl_2$ was added. We measured the $[Ca^{2+}]$ of 0-Na, 0-Ca saline with a Ca^{2+}-selective electrode to be 4 μM. Salines with $[Ca^{2+}]$ of 10^{-7} and 10^{-8} M were made using EGTA and adjusting $MgCl_2$ to compensate for adjustments in $CaCl_2$. The time to complete solution changes was 5–15 s.

Sensitivity Measurements

Sensitivity of the photoreceptors to light was measured in two ways:

Voltage clamp technique. Measurements of receptor current evoked by brief dim test flashes were made and related to photoreceptor sensitivity as in O'Day et al. (1982). Light intensities are described in $\log_{10}$ units of attenuation, neutral densities (ND). An unattenuated light (0 ND) had an intensity of 4×10^{-4} W/cm^2 at 530 nm, the wavelength used in all experiments. The peak current evoked by brief flashes under constant voltage clamp has been shown to vary nearly linearly with flash intensity up to about 100 nA (Lisman and Brown, 1975). The peak light-induced current under constant voltage clamp is proportional to the peak light-induced conductance change. We then define sensitivity as the ratio of the conductance change induced by test flashes to the intensity of those test flashes. In the text, we have used the term Log S to mean the $\log_{10}$ of the ratio of the measured sensitivity to the sensitivity of a photoreceptor that responds to a 0.0-ND, 10-ms, 530-nm test flash with a 1-nA peak current.

Threshold test flash intensity technique. In some experiments we have compared relative sensitivities by giving the difference in the log of the intensity of test flashes needed to elicit a criterion response, similar to Fein and Charlton (1977).

Li^+ Substitution

Li^+ serves as an ideal substitute for Na^+ for a study of Na^+/Ca^{2+} exchange in two ways. First, Li^+ substitution does not alter E_{hv}, the reversal potential of the light response (Lisman and Brown, 1972; Brown and Mote, 1974; O'Day and Gray-Keller, 1989). This means that we need not use voltage-clamp currents to correct for the change in E_{hv} when we want to determine light sensitivity. Secondly, our results suggest that Li^+ does not participate to any great extent in Na^+/Ca^{2+} exchange (O'Day and Gray-Keller, 1989). However, in some experiments, procedures in which large influxes of Li^+ and reduction of $[Na^+]_i$ would be expected caused irreversible inhibitory effects (O'Day and Keller, 1987*b*). For this reason, we have attempted to minimize the effects of these procedures, and we have considered only effects of Na^+ removal with Li^+ substitution that were readily reversible.

RESULTS

The presence of Na^+/Ca^{2+} exchange across the photoreceptor plasma membrane should have specific consequences for normal physiological function because of the important role of Ca^{2+} in controlling sensitivity of photoreceptors to light (Lisman and Brown, 1975). To investigate the extent of these consequences, we asked whether four quantifiable physiological characteristics were affected by procedures designed to alter Na^+/Ca^{2+} exchange: (*a*) dark (resting) sensitivity, (*b*) receptor potential (the electrical response to light), (*c*) light adaptation (light-induced desensitization), and (*d*) dark adaptation (recovery of sensitivity after light adaptation).

Resting Dark Sensitivity

If Na^+/Ca^{2+} exchange is important in maintaining high resting sensitivity by keeping $[Ca^{2+}]_i$ low, we would expect that sensitivity would fall during reversal of Na^+/Ca^{2+} exchange with $[Na^+]_o$ reduction (O'Day and Gray-Keller, 1989). Fig. 1 illustrates the results of an experiment designed to test this prediction. We held a photoreceptor under constant voltage clamp (−75 mV) while monitoring sensitivity using test flashes (−4.0 ND, 10 ms) presented at 5-s intervals. We then lowered $[Na^+]_o$ to 0 mM. Fig. 1 *A* illustrates that, in a cell with moderately high initial resting light-sensitivity, Na^+

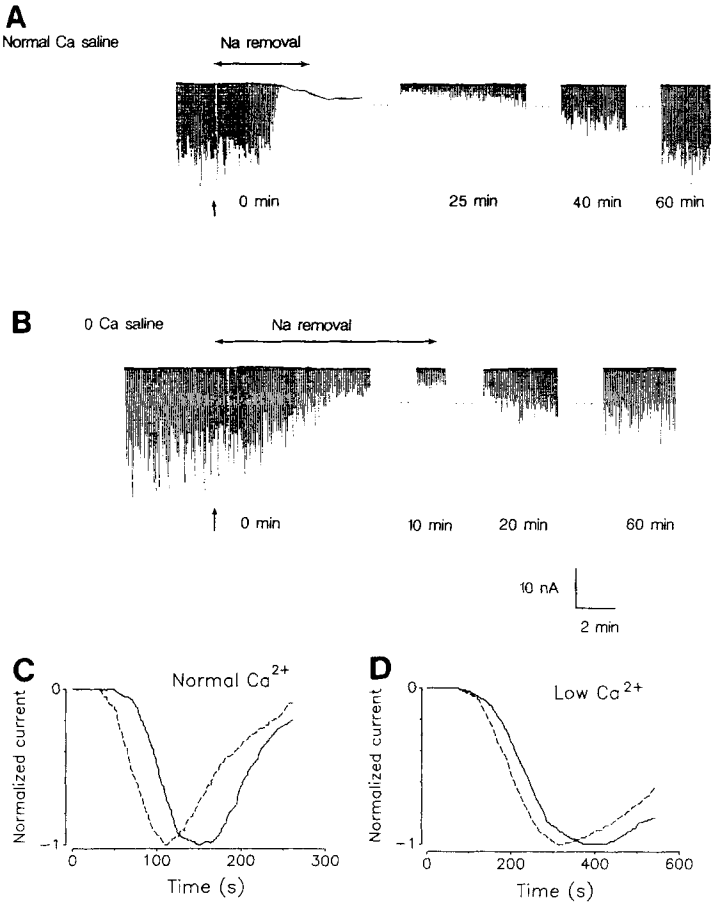

FIGURE 1. Effects of Na^+ removal on resting (dark) sensitivity under constant voltage clamp. Na^+-removal caused a reversible Ca_o^{2+}-dependent reduction in light sensitivity. The cell was stimulated with a test flash (−4.0 ND, 10 ms) every 4 s to monitor sensitivity by the voltage clamp technique (see Methods). The cell was held under constant voltage clamp to −70 mV to ensure that voltage-dependent channels would not open (Lisman et al., 1982). The cell was not fully dark-adapted ($\log_{10}S = \sim 4.3$ [see Methods]); results from fully dark-adapted cells are presented below (Fig. 3). (*A*) Desensitization was more than five $\log_{10}$ units in normal Ca^{2+} saline. (*B*) Desensitization was 0.5 $\log_{10}$ units in low-Ca^{2+} (10^{-4} M) saline. The substituting ion for Na^+ was Li^+. Calibration bars apply to *A* and *B*. In both *A* and *B*, test flash responses were in the linear (vs. intensity) range (see Methods). (*C, D*) Na^+ removal caused a $[Ca^{2+}]_o$-dependent speeding of individual response kinetics. Normalized individual test flash responses from the data of *A* and *B* were digitized and compared. Responses before Na^+ removal are shown as a solid line, those after Na^+ removal as a dashed line. Responses after Na^+ removal are not in the steady state but are taken during the decrease in sensitivity. Peak amplitude in normal-Ca^{2+}, normal-Na^+ saline was 19.2 nA, that in normal Ca^{2+}, 0-Na^+ saline was 3.4 nA; peak amplitude in low-Ca^{2+}, normal-Na^+ saline was 24.0 nA, that in low-Ca^{2+}, 0-Na^+ saline was 7.2 nA. Responses in normal Ca^{2+} were measured 3.0 min after Na^+ removal; responses in low Ca^{2+} were measured 6.5 min after Na^+ removal.

reduction caused a large reversible decrease in sensitivity, as indicated by a decline in peak light-induced currents. A much smaller loss of sensitivity owing to Na^+ removal was observed in low-Ca^{2+} saline (Fig. 1 *B*). These observations are similar to those reported by Millecchia and Mauro (1969*a*, *b*), Brown and Mote (1974), and Stieve et al. (1984) and suggest that the desensitization induced by Na^+ removal resulted from a rise in $[Ca^{2+}]_i$. Supporting this idea is the observation (Fig. 1, *C* and *D*) that Na^+ removal shortened the latency and the time-to-peak of the individual test flash responses, an effect similar to that induced by raising $[Ca^{2+}]_i$ (Lisman and Brown, 1972). In similar experiments we found that Na^+ removal caused a desensitization of

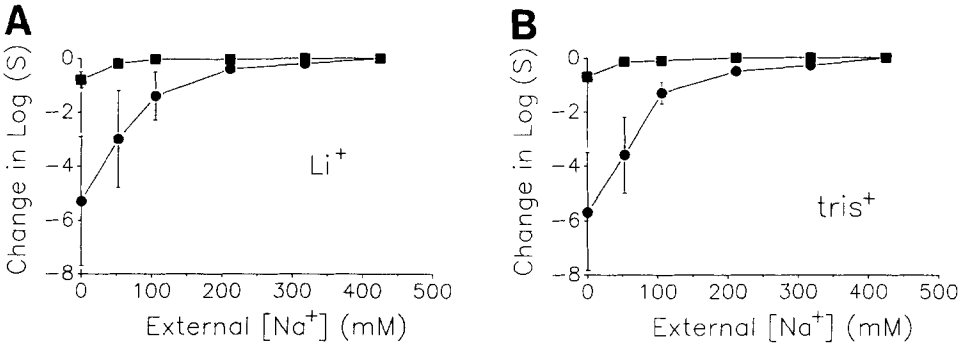

FIGURE 2. In moderately sensitive cells, resting dark sensitivity was dependent on $[Na^+]_o$. The desensitization owing to Na^+ reduction is plotted vs. the $[Na^+]_o$ at which final sensitivity was measured. As in Fig. 1, the initial cell sensitivity was moderately high before each manipulation of Na_o^+, but the cells were not fully dark-adapted. (Log_{10} S was generally between 4 and 5 [see Methods]). In each case, Na^+ was lowered from 425 mM to the final value plotted. Error bars show standard deviation. (*A*) Substituting ion for Na^+ was Li^+. Data from six cells are plotted. (*B*) Substituting ion for Na^+ was $Tris^+$. Data are from three cells. (●) Normal-Ca^{2+} saline; (■) 0-Ca^{2+} saline. In experiments with $Tris^+$ as the substituting ion, the reversal potential of the light response, E_{hv}, varied with the amount of substitution (Brown and Mote, 1974). In the calculation of sensitivity from the light-induced currents, correction was made for the change in E_{hv} (i.e., sensitivity = $I^{-1}*i_{hv}/(V - E_{hv})$, where i_{hv} is the light-induced current, V is the membrane potential under constant voltage clamp to −70 mV, and I is the test flash intensity). No correction was made for the change in light-induced conductance owing to the reduction in concentration of permeant ions. Test flash intensities were −4.0 ND in control (normal $[Na^+]_o$) saline. The test flash intensity in test conditions varied to achieve a criterion test flash response amplitude (5–15 nA).

6.1 ± 2.2 log units (SD, n = 6) in normal-Ca^{2+} saline and a desensitization of 0.45 ± 0.21 log units (SD, n = 6) in low-Ca^{2+} saline.

To examine further the Na_o^+ dependence of desensitization, we repeated the general procedure used for Fig. 1 using different concentrations of Na^+ in reduced-Na^+ saline. A plot of the desensitization vs. the test value of $[Na^+]_o$ is shown in Fig. 2. It illustrates that desensitization was greatest after large Na^+ reductions and that the degree of desensitization was dependent on the degree of Na^+ reduction. The fact that this was observed using $Tris^+$ or Li^+ as the substituting ion suggests that the desensitization was due to Na^+ removal rather than $Tris^+$ or Li^+ specifically. The rates

of recovery of sensitivity after Na_o^+ restoration depended on the degree of desensitization (not shown); sensitivity recovered faster from smaller desensitizations. The desensitizations induced by Na^+ reduction in low-Ca^{2+} saline were much smaller than in normal saline. This may be a result of the fact that much less Ca^{2+} could enter the cell because of the greatly reduced transmembrane electrochemical Ca^{2+} gradient. These results suggest that Na^+ reduction resulted in desensitization owing to Ca^{2+} entry.

Our previous results (O'Day and Gray-Keller, 1989) suggest that Na^+/Ca^{2+} exchange operates less effectively at lower intracellular [Ca^{2+}]. Thus, we would expect that a desensitization induced by Na_o^+ reduction should depend on the initial level of intracellular [Ca^{2+}] if the desensitization is related to Na^+/Ca^{2+} exchange; and, since sensitivity is related to $[Ca^{2+}]_i$ in *Limulus* ventral photoreceptors (Lisman and Brown, 1972), we would expect also that the degree of desensitization should be dependent on the initial sensitivity of the cell to light. The results of others suggested that this is actually the case: Na^+ removal had only a small effect on resting sensitivity in dark-adapted (high sensitivity) conditions (Fein and Charlton, 1978), but in conditions of lower initial sensitivity, Na^+ removal caused a substantial desensitization (Brown and Mote, 1974; Stieve et al., 1984). We have examined this phenomenon in individual cells (Fig. 3) with the aim of quantifying the effects of Na^+ removal. We used flash intensities ranging from very dim to moderately bright and presented several challenges of low-Na^+ saline, each during a different continual presentation of repeated light flashes (−6.0, −4.3, −3.0, −1.0 ND in Fig. 3 *A*). Because it is easier to maintain a high dark-adapted sensitivity with one impaling microelectrode than with two (O'Day and Lisman, 1985), we chose to monitor sensitivity by means of the threshold response protocol (see Methods) rather than the voltage clamp method used for Fig. 1. We found that when dim flashes were used, Na_o^+ removal had only a small effect on resting sensitivity, in contrast to the effects when moderate (−3.0 ND) flashes were used, or when bright (−1.0 ND) flashes were used, where desensitization was generally too large to quantify reliably. As before, Na^+ removal had a much smaller effect in low-Ca^{2+} saline (Fig. 3 *B*). A plot of the desensitization vs. flash intensity measured from four cells in normal-Ca^{2+} saline is presented in Fig. 3 *C*. The overall results of Fig. 3 suggest that Na_o^+ removal reduced reversibly the resting sensitivity of the cell when $[Ca^{2+}]_i$ was elevated by light adaptation but did not affect the resting sensitivity in the highly dark-adapted photoreceptor.

In some cells, where very bright illumination was used, the desensitization owing to prolonged exposure to 0-Na^+ saline (Li^+ substitution) was not reversible either in normal saline or in 0-Ca^{2+} saline. It is possible that the normal process of excitation may be inhibited by our procedures designed to block Na^+/Ca^{2+} exchange. Therefore, in the present experiments, we have tried to minimize the duration of low-Na_o^+, high-Li_o^+ exposure during illumination.

The massive desensitization observed after Na^+ removal in normal saline during illumination with bright (−1.0 ND) test flashes was accompanied by a sudden and sustained depolarization of resting membrane potential (Fig. 3 *A*). The depolarization was invariably initiated during the response to an individual test flash, as if the cell failed to recover its normal resting level after a depolarizing response.

To examine further the effect of Na^+ removal on resting sensitivity in a highly

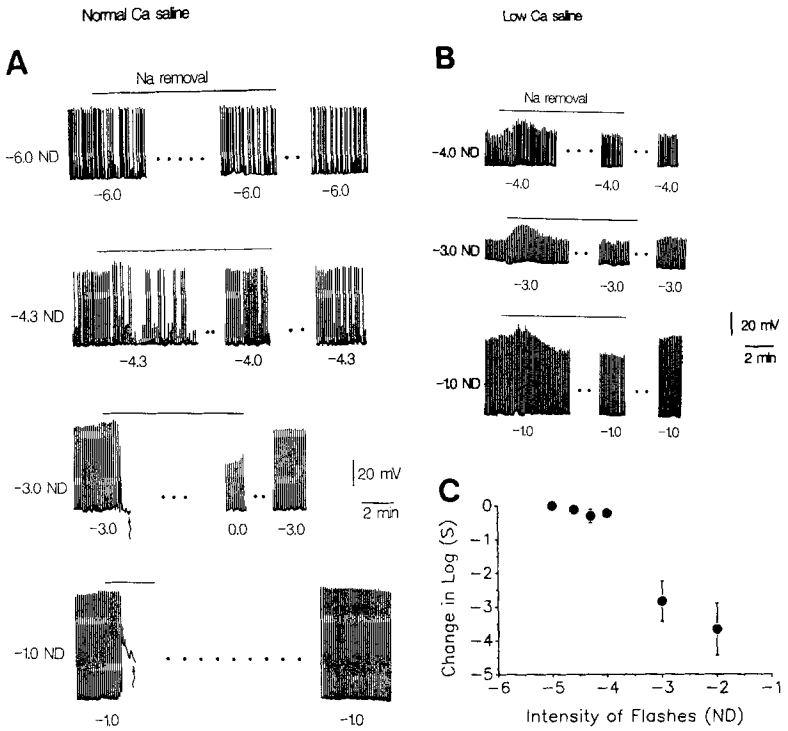

FIGURE 3. The desensitization induced by Na^+ removal was dependent on the initial sensitivity of the photoreceptor to light. The effects were $[Ca^{2+}]_o$ dependent and reversible. Substituting ion for Na^+ was Li^+ (final $[Na^+]_o$ = 0 mM). Photoreceptor sensitivity was maintained at steady state levels (before Na^+ removal) using periodic flashes. (*A*) Normal-Ca^{2+} saline. A variety of flash intensities was used to create different initial conditions of resting photoreceptor sensitivity. Four are shown (−6.0, −4.3, −3.0, and −1.0 ND) with different qualitative effects. When −6.0-ND flashes were used, Na^+ removal had a negligible effect on photoreceptor responses; with −4.3-ND flashes, Na^+ removal had small effects. With −3.0-ND flashes Na^+ removal caused a large reduction in response size (*arrow*), and even very bright flashes (*middle trace*, 0.0 ND) did not evoke comparable responses after Na^+ removal. With −1.0-ND flashes, Na^+ removal reduced responses completely (*arrow*). The gaps in the traces correspond to the following time intervals: At −6.0 ND: first gap, 10 min; second gap, 5 min. At −4.3 ND: first gap, 10 min; second gap, 5 min. At −3.0 ND: first gap, 5 min; second gap, 5 min. At −1.0-ND gap: 15 min. (*B*) Low-Ca^{2+} saline, same cell as in *A*. In contrast to the results in normal-Ca^{2+} saline, the light responses were not greatly affected in any trials. Flash intensities were −4.0, −3.0, and −1.0 ND. The gaps in the traces correspond to the following time intervals—at all intensities: first gap, 5 min; second gap, 10 min. (*C*) Normal-Ca^{2+} saline. Na^+ reduction caused small desensitization when photoreceptors were sensitive, but large desensitization resulted when they were initially desensitized by brighter flashes. The desensitization induced by Na^+ removal is plotted vs. the flash intensity for experiments like that in *A*. Averages and standard deviations are shown for four cells. The threshold response protocol was used to quantify light sensitivity (see Methods). Criterion response size was 5 mV average for 10 test flashes. Note: In general, when the cell membrane potential is quite negative, the responses in the dark-adapted cell will contain a regenerative spikelike component owing to voltage-gated inward currents (O'Day et al., 1982). Thus, in the cell depicted in this figure, the responses to dim test flashes (−6.0 ND) have roughly the same peak amplitudes as responses to brighter flashes (−3.0 ND). Dark-adapted cells, in addition, exhibit more spontaneous activity (−6.0 ND). Dimmer test flashes result in fewer superthreshold responses. Hence the higher rate of response "failures" at −4.3 and −6.0 ND. Desensitization at dimmer light intensities will appear as a reduction in the frequency of superthreshold responses. Desensitization at brighter lights will appear as a reduction in response sizes.

dark-adapted photoreceptor, we examined the changes in quantum bump amplitude and frequency induced by Na^+ removal. Quantum bumps (also called discrete waves) are smooth discrete transient depolarizations (or inward currents under voltage clamp) elicited by absorption of a single photon (Fuortes and Yeandle, 1964). During illumination, the amplitude of quantum bumps is reduced by light adaptation (Dodge et al., 1968; Wong, 1978), but the quantum bump frequency remains the same for a given light intensity (Adolph, 1964). Chemicals that affect phototransduction can do so by affecting quantum bump frequency, in effect reducing quantum efficiency (Bolsover and Brown, 1982; Corson and Fein, 1983). Fig. 4 illustrates that, for a fully dark-adapted photoreceptor, Na^+ removal had no effect on quantum bump frequency, but it significantly reduced quantum bump amplitude, similar to the effect of normal light adaptation. Our results illustrate that Li^+ substitution itself does not diminish the voltage-dependent inward currents (Lisman et al., 1982) responsible for the regenerative quality of large quantum bumps (O'Day, P. M., and C. L. Phillips, manuscript submitted for publication). This result rules out the idea that the reduction in quantum bump amplitude by Na^+ removal was caused by reduction of voltage-activated activity. Thus, the result of Fig. 4 is consistent with the idea that, in a fully dark-adapted cell, Na^+ removal did not affect quantum efficiency, and that it had a small effect on sensitivity. The reduction in quantum bump amplitude was fully reversible (not shown).

Excitation

Since Na^+/Ca^{2+} exchange appears to operate on a fairly fast time scale in *Limulus* ventral photoreceptors (a 10-fold reduction of $[Ca^{2+}]_i$ in 9 s; O'Day and Gray-Keller, 1989) and since intracellular Ca^{2+} influences excitation, it is possible that Na^+/Ca^{2+} exchange contributes to shaping the receptor potential. Some evidence exists for this possibility. Na^+ reduction alters the receptor potential waveshape (Millecchia and Mauro, 1969*a*). Fein and Charlton (1978) observed that a depolarizing afterpotential persisted for long times after the termination of the excitation light in reduced Na_o^+. Changes in $[Ca^{2+}]_o$ also dramatically change the waveshape of the light responses (Lisman, 1976; Stieve and Bruns, 1978; Wulff and Fahy, 1979; Stieve et al., 1985). These effects may be due in part to the action of Na^+/Ca^{2+} exchange.

To investigate whether changes in waveshape would be evident in cells that had the same preillumination sensitivity, we examined the effect of Na^+ removal and of Ca^{2+} removal on receptor potentials elicited by bright lights under highly dark-adapted conditions. We compared the receptor potentials evoked by brief, bright (20 ms, −2.0 ND) flashes presented to the fully dark-adapted photoreceptor in normal saline to those in 0-Na^+ saline. At early times, the receptor potentials were very similar in normal-Na^+ and low-Na^+ saline. But the photoreceptor remained depolarized for much longer times in low-Na^+ saline than in normal-Na^+ saline (similar to Fein and Charlton, 1978). In low-Ca^{2+} saline the persistent depolarization was much less pronounced. No persistent depolarization was observed in identical low-Ca^{2+} experiments using Tris or choline substitution instead of Li^+. The comparisons of waveshapes are shown in Fig. 5 where receptor potentials have been superposed. These results are consistent with the idea that Na^+/Ca^{2+} exchange influences the

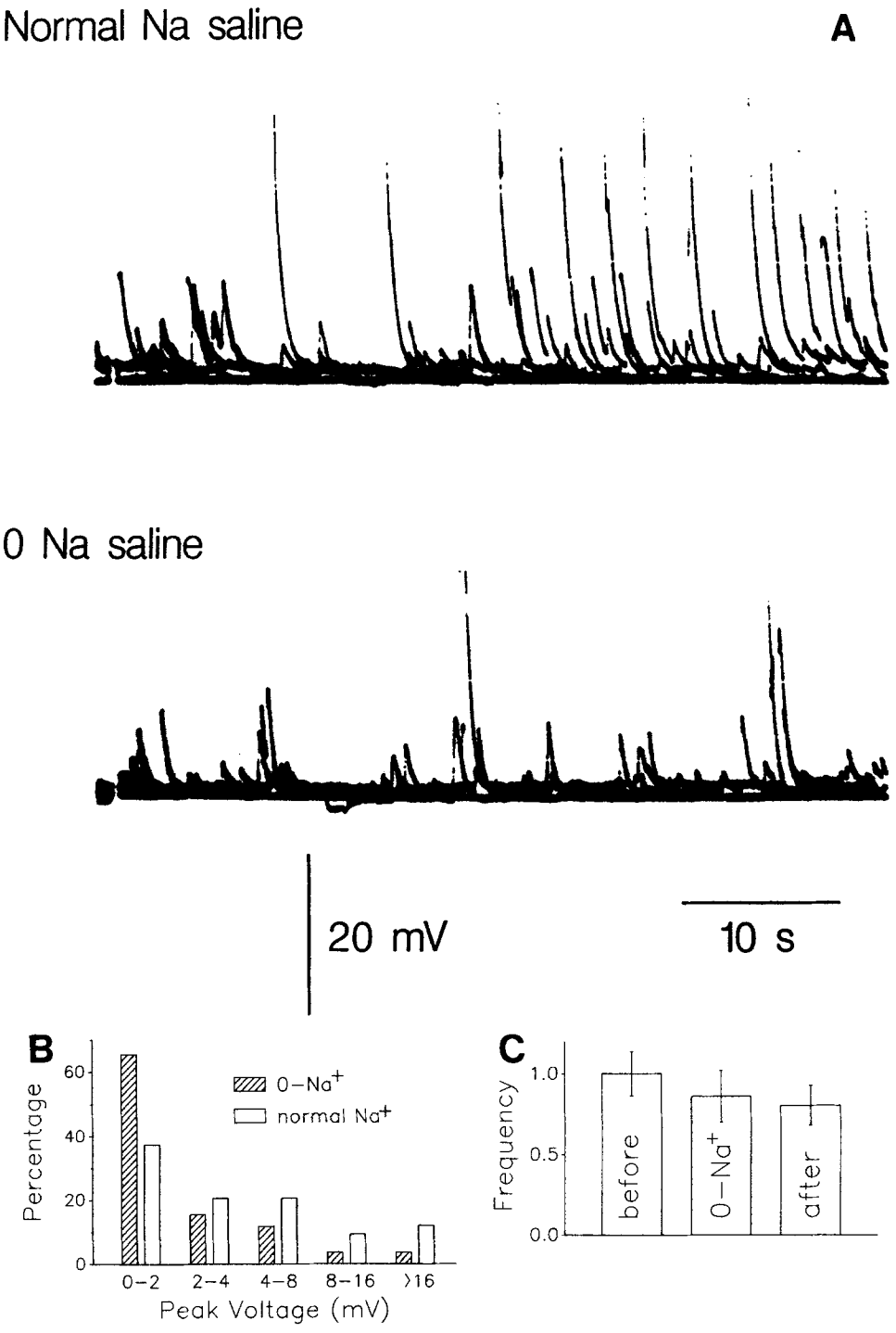

FIGURE 4. Na^+ removal in fully dark-adapted photoreceptor had no effect on quantum bump frequency but caused a large reduction in quantum bump amplitudes. (*A*) Oscilloscope traces of quantum bumps in two conditions, normal-Na^+ and 0-Na^+ salines (Li^+ substitution). Each trace is a superposition of four sweeps. (*B*) Histograms of quantum bump amplitudes recorded from the cell described in *A*. The ordinate is the percentage of total quantum bumps falling in the range of amplitudes plotted on the abscissa. In the test (0-Na^+ saline) trial, the amplitude distribution is skewed toward lower amplitude quantum bumps compared with the control (normal saline) trial. (*C*) Average and standard deviation of quantum bump frequencies are displayed for three cells.

shape of the receptor potential, although we do not know the mechanism of the change in receptor potential shape.

Light Adaptation

If Na^+/Ca^{2+} exchange affects light adaptation, we would expect the degree of light adaptation to be dependent on both $[Na^+]_o$ and $[Ca^{2+}]_o$. With this in mind, we examined the possibility that Na^+/Ca^{2+} exchange can influence light adaptation, asking two general questions: (*a*) Does Na_o^+ reduction affect the degree of light-induced desensitization? and (*b*) Is the degree of light-induced desensitization dependent on $[Ca^{2+}]_o$?

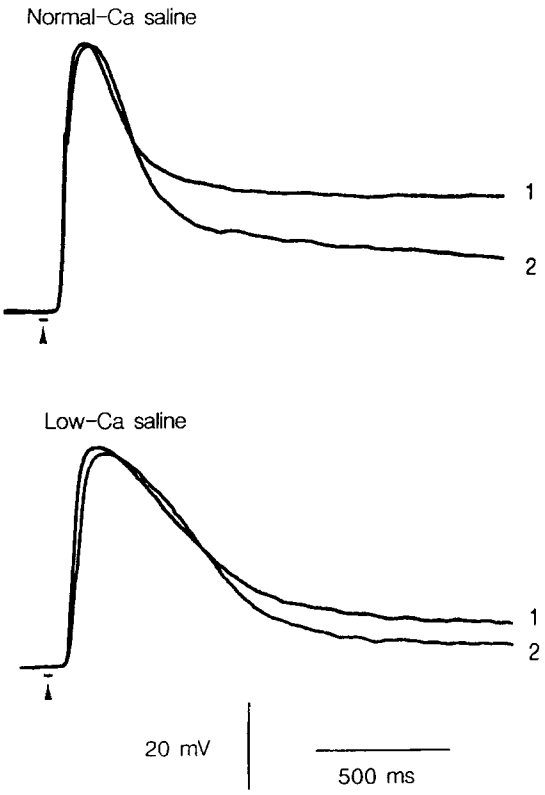

FIGURE 5. Na^+ reduction altered individual receptor potentials evoked by a bright flash. (*A*) Two receptor potentials are shown superimposed. Trace *1* was recorded in normal-Na^+, normal-Ca^{2+} saline, and trace *2* was recorded in normal-Ca^{2+}, low-Na^+ (106.3 mM) saline; in both traces $[Ca^{2+}]_o$ was 10 mM (normal-Ca^{2+}). In the low-Na^+ trial there was a prolonged depolarization at long times (> 1 s) after the light flash (−2.0 ND, 20 ms). (*B*) Two receptor potentials are shown superimposed. Trace *1* was evoked in 0-Ca^{2+}, normal-Na^+ saline, and trace *2* was recorded in 0-Ca^{2+}, low-Na^+ (106.3 mM) saline. In the low-Na^+ trial, an after-depolarization was observed that was smaller than that observed in *A*.

Na_o^+ dependence of light adaptation. We compared the degree of light adaptation measured in reduced-Na^+ saline with that measured in normal-Na^+ saline, using the procedure outlined in Fig. 6*A*. We dark-adapted photoreceptors that had been voltage-clamped to −75 mV and bathed in low-Ca^{2+} saline to minimize Ca^{2+} entry through reverse exchange after Na^+ removal (O'Day and Gray-Keller, 1989). We then monitored light sensitivity continuously using dim flashes, and we took advantage of the observation (see Fig. 3) that the sensitivity of a fully dark-adapted cell was not much affected by Na^+ reduction. After full dark adaptation in low-Na^+ saline, a brief

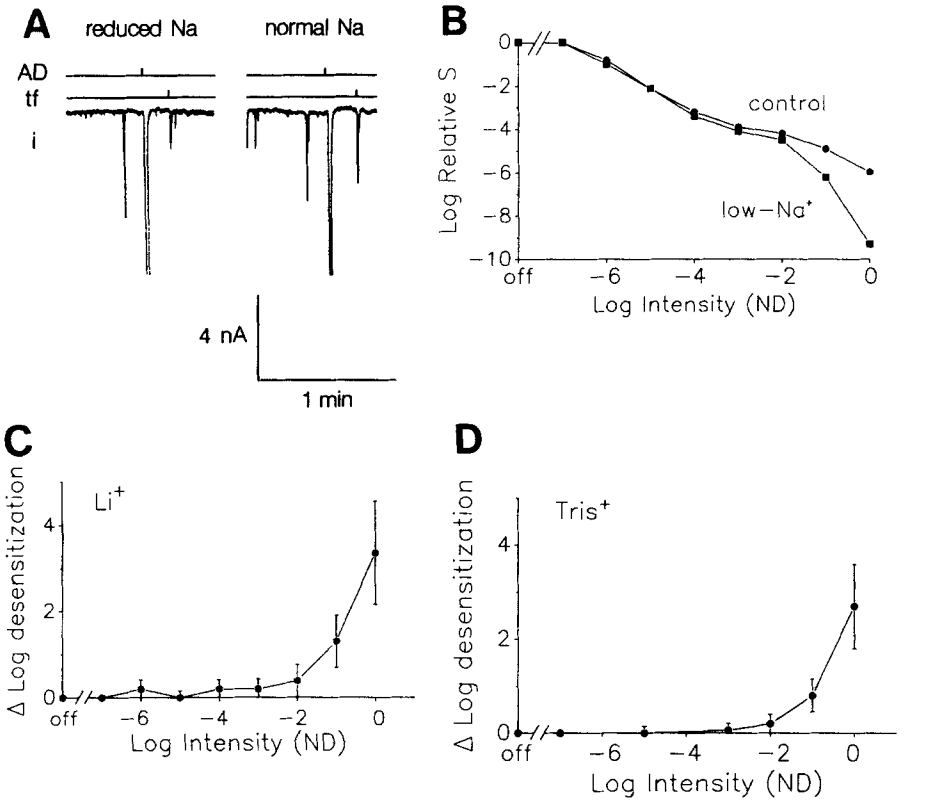

FIGURE 6. Na^+ reduction enhanced light adaptation at adapting intensities. (*A*) Protocol for quantifying the effects of Na^+ reduction on light adaptation. The fully dark-adapted (8.9-nA peak average over 10 responses) photoreceptor was bathed in low-Ca^{2+} (10^{-4} M) saline under constant voltage-clamp to −75 mV. An adapting light (*AD*, 20 ms) was presented as shown and it was followed by a test flash (*tf*, 10 ms) of sufficient intensity to evoke a measurable response. In this example the dark-adapted sensitivity in reduced-Na^+ (26.6 mM) saline was $\log_{10} S$ = 6.95. After *AD* (−2.0 ND) the *tf* (−2.6 ND) evoked a peak inward current of 1.79 nA ($\log_{10} S$ = 2.85). In normal-Na^+ saline, the dark-adapted sensitivity was $\log_{10} S$ = 6.94. After *AD* (−2.0 ND) the *tf* (−2.6 ND) evoked a peak inward current of 3.43 nA ($\log_{10} S$ = 3.14). Thus, the desensitization in reduced-Na^+ saline was 4.1 log units and that in normal-Na^+ saline was 3.8 log units. The interval between the adapting flash and the test flash was chosen experimentally to obtain a measurable postadaptation response within a very short time after the adapting flash. (*B*) Light adaptation was more extensive in low-Na^+ (26.6 mM) saline than in normal-Na^+ saline only at bright *AD* intensities. Using protocol of *A*, we measured light-induced desensitization over a wide range of *AD* intensities in low-Na^+ and normal-Na^+ saline. The ordinate is a plot of the $\log_{10}$ of the relative sensitivity (= Log $S_{(\text{trial})}$ − Log $S_{(\text{dark-adapted})}$). (■) low-$Na^+$ saline; (●) normal-Na^+ saline. (*C*) Intensity dependence of Na^+-dependent changes in light adaptation. The ordinate is the difference between the $\log_{10}$ of the relative sensitivity measured in low-Na^+ saline and that measured in normal saline. In low-Na^+ saline, the substituting ion was Li^+. Data are from three cells. (*D*) Same as *C* except substituting ion was $Tris^+$ (26.6 mM Na^+, 398.4 mM $Tris^+$). Data are from three cells. Reversal voltage of the light-induced currents in $Tris^+$ was −32 mV. As in Fig. 2, the sensitivity was calculated to correct for changes in reversal potential.

(20 ms) bright light was presented to light-adapt the cell, and the sensitivity after the adapting light was subsequently measured with a brief test flash. In the experiment depicted in Fig. 6, the −2.0-ND adapting light caused a desensitization of 4.1 log units in low-Na^+ saline. After light adaptation, the cell was allowed to dark-adapt and returned to normal-Na^+ saline. We repeated this procedure to measure the light-induced desensitization in normal-Na^+ saline and observed a desensitization of 3.8 log units. We used this general approach to compare the intensity dependence of light adaptation in normal-Na^+ saline with that in low-Na^+ saline. For brighter adapting lights, we increased the intensity of the test flashes after light adaptation to measure the larger desensitization. A summary of our results is shown in Fig. 6 *B*; this is a quantification of the light-induced desensitization vs. the intensity of the adapting light in two cases: (*a*) in normal-Na^+ saline and (*b*) in reduced-Na^+ (26.6 mM) saline. This figure illustrates that Na^+ removal affected light-induced desensitization only at fairly bright to very bright adapting intensities; in the range of intensities −2.0 ND and below, the effect of Na^+ reduction was small or negligible. This is shown more

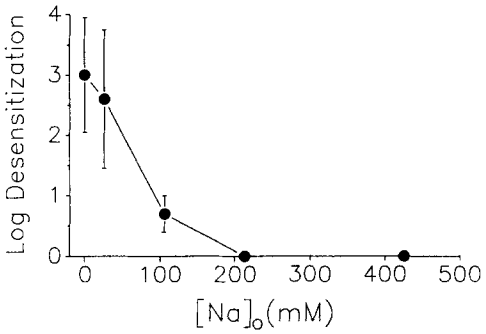

FIGURE 7. Effect of Na^+ reduction on light adaptation depended on final $[Na^+]_o$. Cells were voltage-clamped to −75 mV and the procedures used for Fig. 6 were used to quantify the change in light adaptation owing to Na^+ reduction (the substituting ion was Li^+). The adapting light (*AD*) throughout the experiment was −1.0 ND. The ordinate is the difference between the log of light-induced desensitization measured in the test Na^+ saline and that measured in normal-Na^+ saline. Results are from six cells (average ± SD).

clearly in Fig. 6 *C* in which is plotted the difference between the desensitization in normal-Na^+ saline and that in reduced-Na^+ saline.

Since the effect in Fig. 6 was intensity dependent, we examined whether it might be due to a desensitization induced by Li^+ entry, which should also be intensity dependent, since Li^+ can permeate the light-activated conductance (Brown and Mote, 1974). Brown and Mote (1974) have presented evidence that $Tris^+$ is only slightly permeant through the light-activated conductance; so we repeated the experiment using $Tris^+$ rather than Li^+ as the substituting ion during Na^+ reduction. Fig. 6 *D* illustrates that $Tris^+$ substitution during Na^+ reduction had an effect similar to the effect of Li^+ substitution. This observation suggests that the desensitizing effects of Na^+ removal in Fig. 6 *B* were not due simply to Li^+ entry.

To examine the $[Na^+]_o$ dependence of this phenomenon, we repeated the general protocol used to generate Fig. 6 using a light intensity of −1.0 ND at several different $[Na^+]_o$ levels. Fig. 7 illustrates that the degree of light adaptation was $[Na^+]_o$

dependent; light desensitized the photoreceptors to a greater extent the lower $[Na^+]_o$ was reduced <200 mM.

Ca_o^{2+} dependence of light adaptation. To examine the Ca_o^{2+} dependence of light adaptation, we compared the degree of light adaptation measured in normal-Ca^{2+} saline with that measured in reduced-Ca^{2+} saline. We dark-adapted the cells fully, to take advantage of the result that dark-adapted cells have the same sensitivity to light in normal-Ca^{2+} saline as in low-Ca^{2+} saline (O'Day et al., 1982). We then presented a long-duration adapting background light and continuously monitored light sensitivity using superimposed brief dim flashes under voltage clamp (Fig. 8 *A*). We observed that presentation of the adapting light desensitized the cell to a lesser extent in reduced $[Ca^{2+}]_o$; the lower $[Ca^{2+}]_o$ was, the smaller the light-induced desensitization (Fig. 8 *B*). Results at two adapting light intensities are shown. This effect was dependent on the intensity of the adapting light (Fig. 8 *C*); the brighter the adapting light was, the greater was the difference between the normal and the low-Ca^{2+} curves for intensities -2.0 ND and brighter. Results are shown for two values of $[Ca^{2+}]_o$.

The overall results, the $[Na^+]_o$ dependence of light adaptation and the $[Ca^{2+}]_o$ dependence of light adaptation, suggest that Na^+/Ca^{2+} exchange mitigated the desensitizing effects of light.

Dark Adaptation

Fein and Charlton (1978) showed clearly that *Limulus* ventral photoreceptors bathed in low-Na^+ saline underwent slower recovery from light adaptation, in both voltage-clamped and unclamped conditions, than those in normal-Na^+ saline. It was not clear in their experiments, however, whether the degree of light adaptation was the same in 0-Na^+ and control salines, nor was it clear whether the cell possessed comparable sensitivities before light adaptation in control and low-Na^+ salines. For the purposes of examining the contribution of Na^+/Ca^{2+} exchange to dark adaptation, we deemed it important to maintain identical conditions before and during light adaptation so that the only experimental variable was the $[Na^+]_o$ during dark adaptation.

We did this in two ways: (*a*) we light-adapted cells in normal saline and followed recovery, comparing recovery times for trials in which Na^+ was removed after light adaptation vs. trials in which Na^+ was not removed; and (*b*) we light-adapted cells in 0-Na^+, low-Ca^{2+} saline and followed recovery, comparing recovery times for trials in which Na^+ was restored after light adaptation vs. trials in which Na^+ was not restored.

(*a*) Fig. 9 illustrates our first approach. In this experiment the cell was bathed for 30 min in normal saline and then presented with a bright adapting light for ~ 2 min. At the offset of the adapting illumination, the saline was changed: in trial *a* the $[Ca^{2+}]_o$ was lowered to 100 μM and $[Na^+]_o$ was left at its normal level (425 mM); in trial *b* the $[Ca^{2+}]_o$ was lowered to 100 μM and $[Na^+]_o$ was lowered to 0 mM. After allowing many minutes for recovery, the photoreceptor was returned to normal saline in both cases. The results of Fig. 9 illustrate that the recovery of responsiveness was faster and more extensive in the presence of Na_o^+ than in its absence.

(*b*) Our second approach is illustrated in Fig 10. In this experiment, the cells were light-adapted while they were bathed in 0-Na^+, 0-Ca^{2+} saline in both control and test trials. In the control trials, dark adaptation proceeded after Na^+ restoration; in the test trials, dark adaptation proceeded in 0-Na^+, 0-Ca^{2+} saline. We began this

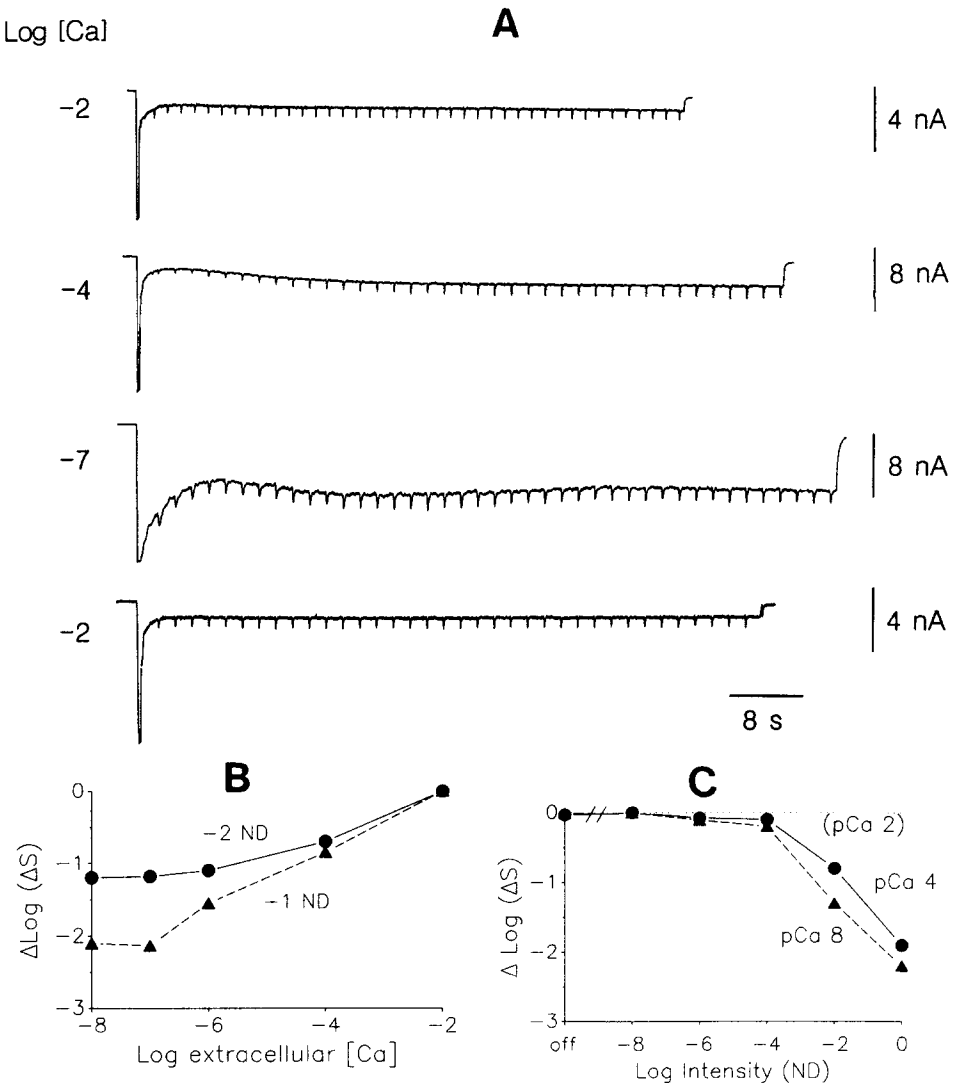

FIGURE 8. Ca_o^{2+} reduction diminished the adapting effect of light. (*A*) Light adaptation in salines with different $[Ca^{2+}]$. The photoreceptor was voltage-clamped to −70 mV and dark-adapted fully ($\log_{10} S = 7.1$) in normal saline. Then low-Ca^{2+} saline was introduced; after 5 min, an adapting background light of −2.0 ND was presented, during which time test flashes (−2.15 ND, 10 ms) were presented at regular intervals. The traces shown are the resulting light-induced currents. (In the bottom trace, the test flash intensity was −2.0 ND). The $\log_{10}$ molar $[Ca^{2+}]_o$ is shown to the left of each trace. (*B*) Ca_o^{2+} dependence of light adaptation. Photoreceptor sensitivity was measured at 20 s after the start of the adapting illumination. The light-induced desensitization was calculated, normalized to that at pCa 2, and plotted vs. extracellular $[Ca^{2+}]$. Data were from a different cell than that depicted in *A*. The ordinate is the $\log_{10}$ of the light-induced desensitization at each $[Ca^{2+}]$ relative to that measured at $\log_{10}$ molar $[Ca^{2+}]_o = 2.0$. Results at two intensities of adapting light are displayed: (●) −2.0 ND; (▲) −1.0 ND. The effect of low Ca_o^{2+} on light-adaptation was greater at brighter adapting light intensity. (*C*) Intensity dependence of the effect of Ca^{2+} reduction on light adaptation. For a different cell than that depicted in *A*, the difference between light-induced desensitization in normal-Ca^{2+} saline and that in low-Ca^{2+} saline is plotted vs. intensity of the background light. (●) Low $Ca^{2+} = 10^{-4}$ M; (▲) low $Ca^{2+} = 10^{-8}$ M.

experiment by dark-adapting the photoreceptors for ~15 min in normal saline presenting dim test flashes (−4.0 ND) every few seconds (Fig. 10*A*); we then reduced Ca^{2+} and then Na^+ in the saline. After bathing the cells for 5 min in low-Ca^{2+}, low-Na^+ saline, we presented an adapting flash (−0.9 ND, 20 ms), immediately restored normal-Na^+ to the saline, and observed recovery for ~5 min using a test flash intensity of −3.6 ND (Fig. 10*A*, *left trace*). (The brighter (−3.6 ND) test flash intensity was used after the light-adapting flash to elicit larger, more easily measurable light-induced currents so that we could monitor sensitivity more reliably.) After the cell had recovered for 30 min in normal saline in the dark, we repeated this

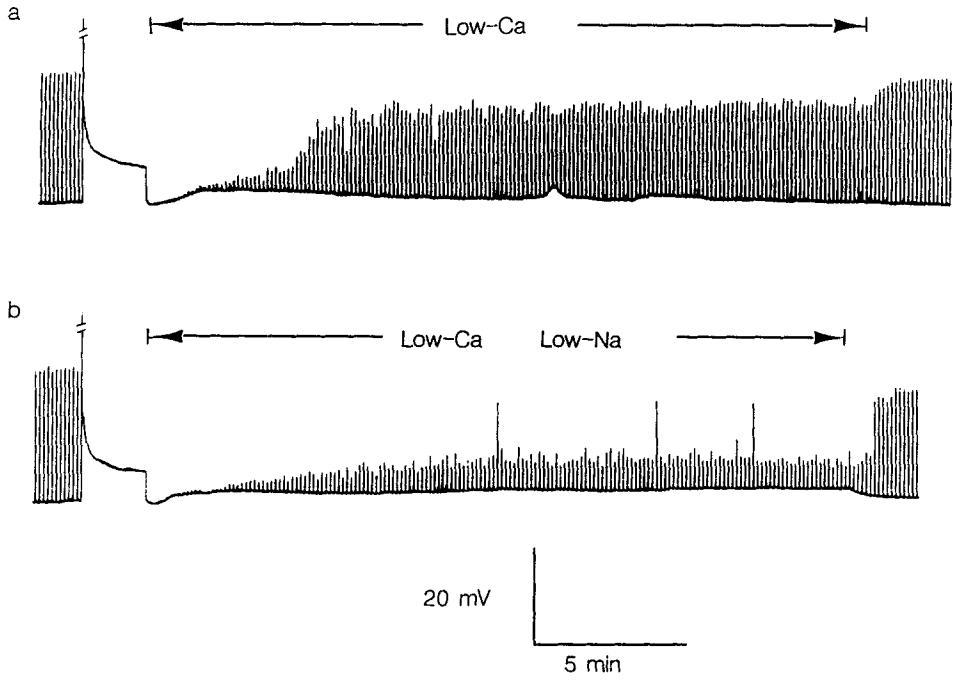

FIGURE 9. Recovery from light adaptation was inhibited by Na^+ reduction. The photoreceptor was presented with −3.0 ND test flashes at regular intervals (8.3 s). A bright adapting light (−0.3 ND) was presented for 2.1 min. At the end of illumination [Ca^{2+}] in the saline was reduced to 10^{-4} M in traces *a* and *b*; additionally, in trace *b* [Na^+] in the saline was reduced to 0 mM. Restoration of Ca_o^{2+} and Na_o^+ led to full recovery. Substituting ion for Na^+ was Li^+.

procedure with the following modification: normal Na^+ was not immediately restored to the saline after the light-adapting flash (Fig. 10*A*, *right trace*). This protocol allowed us to desensitize the photoreceptor under the same conditions in control and test trials (i.e., in both cases, the light-adapting flash was presented while the cell was bathed in 0-Na^+, 0-Ca^{2+} saline). Fig. 10*A* shows that dark adaptation after Na^+ removal exhibited two prominent features: the rate of recovery slowed and the final steady-state level of recovery was reduced. To assess the effect of Na_o^+ removal on the rate of dark adaptation, however, it was desirable to compare situations in which the steady-state recovery levels are comparable. To achieve this situation, we repeated

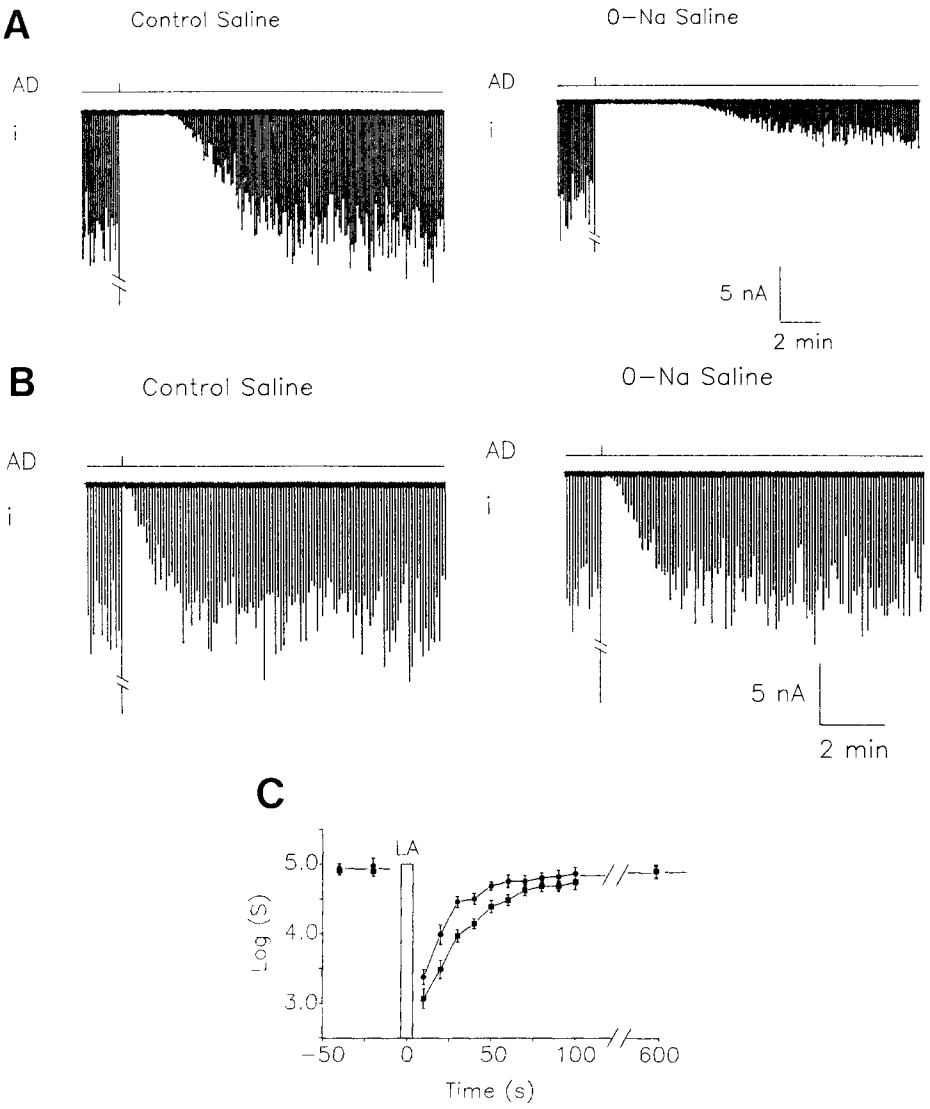

FIGURE 10. Dark adaptation was enhanced by Na^+ restoration. (*A*) The dark-adapted photoreceptor was voltage clamped to −70 mV and stimulated with −4.0-ND, 10-ms, 530-nm test flashes at 5-s intervals. The resulting light-induced current trace is shown. A bright (−0.9 ND, 50 ms) adapting flash was presented (the resulting large inward current went off scale). After the adapting flash, the test flash intensity was raised to −3.6 ND. In the left trace, the Na^+ was restored to the saline immediately after the adapting flash, and the photoreceptor recovered sensitivity in normal-Na^+ saline. In the right trace, the photoreceptor recovered sensitivity in low-Na^+ (26 mM) saline. Na^+ was not restored to saline until several minutes after the adapting flash (not shown). (*B*) The experiment of Fig. *A* was repeated using a dimmer adapting light flash (−1.9 ND, 50 ms). (*C*) The data of *A* were quantified for the first few minutes of dark-adaptation. The log of the sensitivity is plotted vs. time relative to the adapting flash for the two cases: (●) normal-Na^+ saline (*left trace, A*); (■): low-Na^+ saline (*right trace, B*). The data were acquired digitally; peak currents were recorded in 500-ms bins; baseline current changes (drift) were subtracted. Data were averaged every 10 s for time < 15 s, 20 s for 15 s < time < 100 s, and every 200 s for time > 100 s. Error bars correspond to standard deviation.

the experiment of Fig. 10 *A* using a dimmer light-adapting flash (−1.9 ND). We found that the recovery levels were nearly the same in 0-Ca^{2+} before and after Na^+ removal (Fig. 10 *B*). In contrast, recovery was significantly faster in control (*left trace*) than in 0-Na^+ saline (*right trace*), although the difference was not as profound as with brighter adapting illumination. These results illustrate that recovery from light adaptation was slower and less complete in the absence of Na_o^+ than in its presence. In the test trial the photoreceptor recovered dark-adapted sensitivity when Na^+ was restored to the saline (not shown). In Fig. 10 *C*, we have plotted the log of the sensitivity vs. time for the test and control trials of Fig. 10 *A*. The greatest difference in Log (*S*) between low-Na^+ and normal-Na^+ curves in Fig. 10 *C* was 0.49; combining these data with other cells, we found this value to be 0.39 ± 0.12 (average ± SD; $n = 6$).

The result that rates of dark adaptation were slowed by Na^+ removal suggests that Na^+/Ca^{2+} exchange normally contributes to dark adaptation, speeding recovery from light-induced desensitization.

DISCUSSION

Our results support the idea that Na^+/Ca^{2+} exchange influences normal physiological function: specifically, (*a*) Na^+ removal lowered resting sensitivity in cells that were less than fully dark-adapted, but it had a minimal effect on fully dark-adapted cells; (*b*) the desensitization induced by Na^+ removal was reversible, and it was dependent on extracellular $[Ca^{2+}]$; (*c*) Na^+ reduction altered the receptor potentials evoked by brief flashes, causing a Ca_o^{2+}-dependent depolarizing afterpotential; (*d*) the degree of desensitization induced by bright adapting illumination was increased by Na_o^+ reduction; (*e*) the degree of desensitization induced by adapting light was decreased by Ca_o^{2+} reduction; (*f*) the rate and extent of recovery from desensitization induced by light were slowed by Na_o^+ reduction.

Resting sensitivity

Our observations that the reduction of $[Na^+]_o$ reduced light sensitivity in a Ca_o^{2+}- and Na_o^+-dependent manner suggests that Na^+/Ca^{2+} exchange normally contributes to maintaining high resting sensitivity by keeping $[Ca^{2+}]_i$ low.

The intensity dependence of the decline in resting sensitivity with Na^+ reduction suggests that the effects of Na^+/Ca^{2+} exchange are most noticeable when $[Ca^{2+}]_i$ is elevated. Dark-adapted photoreceptors did not lose sensitivity when bathed in low-Na^+ saline, where reverse exchange would likely be favored, but, when the sensitivity was reduced somewhat (and presumably $[Ca^{2+}]_i$ was elevated somewhat), Na^+ removal very quickly desensitized the cell. This observation suggests that reverse Na^+/Ca^{2+} exchange does not operate at very low levels of $[Ca^{2+}]_i$, and it is in agreement with our observations that suggest that the rate of Na^+/Ca^{2+} exchange decreases at low $[Ca^{2+}]_i$ (O'Day and Gray-Keller, 1989). This idea is similar to the results of DiPolo and Beaugé (1987) who suggest that Ca_i^{2+} modulates the rate of reverse exchange in squid axons.

On the other hand, it may be that, in a dark-adapted cell, $[Na^+]_i$ normally becomes extremely low (by the action of a Na^+/K^+ exchange pump [Brown and Lisman, 1972])

and that Na_o^+ removal cannot cause Ca^{2+} entry under these conditions, either because $[Na^+]_i$ is too small to activate reverse exchange or because the driving force for exchange (V-E_x) is not favorable for reverse exchange. $[Na^+]_i$ measurements made by Fein and Tsacopoulos (1988) suggest that $[Na^+]_i$ can be as low as 6 mM in a dark-adapted cell. $[Na^+]_i$ may be even lower in very dark-adapted cells that have not been impaled with a microelectrode.

The results of Fig. 3 *A* suggest that net inward Ca^{2+} leakage is normally low. In that experiment, we used a single, very sharp impaling microelectrode to minimize membrane damage, because impalement alone can cause serious inward Ca^{2+} leakage (O'Day and Gray-Keller, 1989). We observed that, in the presence of a large inward Ca^{2+} gradient and the absence of Na_o^+, there was no desensitization if sensitivity was initially high. This suggests that there was no significant rise in $[Ca^{2+}]_i$ in spite of the fact that there was no forward exchange to extrude Ca^{2+} against the large inward Ca^{2+} gradient. Thus, net Ca^{2+} leakage was probably very low, and if there was significant inward Ca^{2+} leakage, Ca^{2+} removal processes other than Na^+/Ca^{2+} exchange must be of sufficient capacity to maintain a very low $[Ca^{2+}]_i$.

Excitation

The observations that the waveshapes of individual responses to a test flash of light were affected by Na^+ reduction and Ca^{2+} reduction are consistent with the idea that Na^+/Ca^{2+} exchange can influence the receptor potential under normal conditions.

Because we observed variation in the receptor potential waveshapes at early times (< 300 ms), it is difficult to determine whether Na^+/Ca^{2+} exchange might contribute to shaping the receptor potential at those times. However, the Ca_o^{2+} dependence of the persistent depolarization in low-Na^+ salines (Fig. 5) suggests that it might be due to an elevation of $[Ca^{2+}]_i$ caused by Ca^{2+} entry during reverse Na^+/Ca^{2+} exchange. A rise in $[Ca^{2+}]_i$ might cause persistent membrane depolarization due to (*a*) opening of light-activated channels (Payne et al., 1986*a*), and/or (*b*) a reduction in the outward delayed rectifier K^+ currents (Lisman et al., 1982; Leonard and Lisman, 1981) that has also been associated with a rise in $[Ca^{2+}]_i$ (Chinn and Lisman, 1984). Other explanations are also possible; for example, entry of Li^+ in response to bright illumination might produce a Ca_o^{2+}-dependent effect on membrane potential. Li^+ is a blocker of phosphoinositide metabolism in other systems, and it has been suggested that phosphoinositide pathways may play a role in excitation in *Limulus* ventral photoreceptors. The result that quantum bump frequency was not affected by Na^+ reduction in dark-adapted photoreceptors suggests that substitution of Na^+ with Li^+ in the saline did not affect quantum efficiency. The fact that we did not observe a similar persistent depolarization in identical low-Ca^{2+} experiments in which Tris or choline was substituted for Na^+ suggests that the persistent effect was Li^+ specific, although Fein and Charlton (1978) did report a persistent depolarization using choline as the substituting ion in normal-Ca^{2+} saline.

The electrogenic nature of Na^+/Ca^{2+} exchange suggests a mechanism by which exchange might contribute a small component to the electrical response to light. Since light gives rise to a release of intracellular Ca^{2+} mediated by inositol (1,4,5) trisphosphate ($Ins(1,4,5)P_3$), and since a resulting rise in $[Ca^{2+}]_i$ should yield an

inward current owing to activation of Na^+/Ca^{2+} exchange, it is possible that a component of the light-induced inward current is due to the Na^+/Ca^{2+} exchanger. It is a simple matter to illustrate that the inward current cannot be due solely to Na^+/Ca^{2+} exchange, since a sizable Ins(1,4,5)P_3-induced inward current can be measured in the absence of extracellular Na^+ (Fig. 11). However, a brief calculation suggests that a component of inward current attributable to Na^+/Ca^{2+} exchange may be significant: From our previous study (O'Day and Gray-Keller, 1989), we might estimate that, in a dark-adapted cell, a 10 μM rise in $[Ca^{2+}]_i$ would result from a 10-ms light flash of intensity 6×10^{-7} W/cm^2, and that forward exchange would subsequently reduce $[Ca^{2+}]_i$ to 1 μM in ~9 s. If 0.5% of the change in total intracellular calcium appears as a change in free Ca^{2+} and if the cell volume is 5×10^{-10} liter, then $\sim 10^{-14}$ mol/s of Ca^{2+} would be transported. This corresponds to a

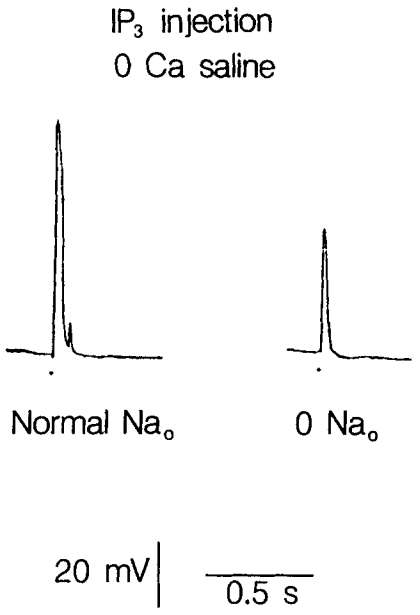

FIGURE 11. Ins(1,4,5)P_3 injection evoked a depolarizing response in the absence of Na_o^+. The photoreceptor was impaled with a single microelectrode filled with Ins(1,4,5)P_3 injection solution (100 μM Ins(1,4,5)P_3, 100 mM K-aspartate, 10 mM HEPES). A depolarizing response was elicited by injection of Ins(1,4,5)P_3 in 0-Ca^{2+} saline and in 0-Ca^{2+} 0-Na^+ saline.

membrane current of 1 nA if the Na^+/Ca^{2+} stoichiometry is 3:1 and no other ions are involved. This could be a small but noticeable component of the corresponding light-induced current, since a similar light (6×10^{-7} W/cm^2, 10 ms) could give rise to a 10–50-nA response in a similarly dark-adapted cell (e.g., Lisman and Brown, 1975).

Light-Adaptation

The enhancement of light-induced desensitization by Na^+ reduction (Fig. 7) suggests that in normal-Na^+ saline there is a Na_o^+-dependent process that reduces the effect of light-adaptation. Since this was observed under constant voltage clamp to −75 mV, voltage-dependent conductances were not involved (Lisman et al., 1982). Consistent with these two observations is the idea that Na^+/Ca^{2+} exchange operates during light

adaptation to reduce the desensitizing effects of light. This could occur if a significant portion of the intracellular Ca^{2+} that is mobilized by the adapting light from intracellular stores (Brown and Blinks, 1974) were quickly extruded from the cell by Na^+/Ca^{2+} exchange. In agreement with this concept is our observation that Na^+ reduction affected light-induced desensitization only at bright light intensities, when light-induced Ca^{2+} mobilization should have been large. This is true because Na^+/Ca^{2+} exchange appears to operate maximally at high $[Ca^{2+}]_i$ and negligibly at low $[Ca^{2+}]_i$ (O'Day and Gray-Keller, 1989). Consistent with these results are the observations of O'Day et al. (1982) and Stieve et al. (1984) that the intensity-response characteristics of *Limulus* ventral photoreceptors are dependent on Ca_o^{2+} and Na_o^+.

Dark-Adaptation

Our observation that the rate and degree of recovery from light adaptation in voltage-clamped and unclamped photoreceptors were reduced by reducing $[Na^+]_o$ suggests that Na^+/Ca^{2+} exchange normally contributes to dark adaptation. It may do so by reducing $[Ca^{2+}]_i$. If this is the case, it would suggest that Na^+/Ca^{2+} exchange is a principal factor in reducing Ca^{2+} that has been mobilized by light from intracellular stores. It would also indicate that reduction of $[Ca^{2+}]_i$ is a rate-limiting step at some point in dark adaptation.

The elevation of $[Ca^{2+}]_i$ induced by light is transient (Brown and Blinks, 1974; Levy and Fein, 1985), rising to a peak within 250 ms and falling with a time course that depends on the light intensity (O'Day and Gray-Keller, 1989). So, based on our previous results that suggest that Na^+/Ca^{2+} exchange is most effective at high $[Ca^{2+}]_i$ and ineffective at very low $[Ca^{2+}]_i$, we would expect that the major contribution of Na^+/Ca^{2+} exchange to dark adaptation would occur within a short period of time after an adapting flash, i.e., until the $[Ca^{2+}]_i$ level is decreased substantially. This is in agreement with the results of Classen-Linke and Stieve (1986) that suggest that dark adaptation has a Ca_o^{2+}-dependent phase.

We have used conventional electrophysiological protocols to quantify light adaptation and dark adaptation. In general these protocols have features that overlap conceptually. For example, in Figs. 6 and 8, one could argue that the effects of our manipulations on the rate of recovery are not truly separated from the effects on the degree of desensitization. This is correct and indeed it may not be possible to separate the effects of light adaptation from those of dark adaptation as we have presently defined the terms. We have chosen our definitions of light adaptation and dark adaptation phenomenologically, as readily quantifiable parameters. The physiological and biochemical processes underlying light adaptation and dark adaptation are not presently understood. Very likely they share some of the same intracellular biochemistry. Therefore, it may not be experimentally possible to separate the phenomena completely. Nonetheless the two parameters are valuable in describing some general emergent physiological effects of Na^+/Ca^{2+} exchange on the transducing photoreceptor.

Thus, our overall results suggest that, in addition to maintaining resting sensitivity by holding $[Ca^{2+}]_i$ low, Na^+/Ca^{2+} exchange also affects excitation and contributes to light adaptation and dark adaptation in the normally functioning photoreceptor.

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