Calcium Mediates the Light-induced Decrease in Maintained K^+ Current in *Limulus* Ventral Photoreceptors

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ABSTRACT In addition to increasing the conductance to sodium, light reduces the maintained voltage-dependent potassium current (i_K) in *Limulus* ventral photoreceptors. We have investigated the mechanism underlying this long-lasting decrease in i_K. Intracellular injection of calcium produced a similar reduction of the voltage-dependent outward current. This reduction was not due to an activation of the voltage-dependent inward current (i_{in}) because calcium injection reduced the outward current even under conditions where i_{in} was blocked with Ni^{2+}, and because calcium injection produced a decrease in conductance, as measured from the slope of the instantaneous *i-V* curve. The effect of light on i_K could be blocked by injection of the calcium buffer EGTA (pCa 7.1) to an intracellular concentration of 50–70 mM. Even larger injections of the pH buffer MOPS (100–200 mM) did not reduce the effect of light on i_K. These experiments show that intracellular free calcium (Ca_i^{2+}) can reduce i_K. Furthermore, since Ca_i^{2+} is known to increase in light, our results are consistent with the hypothesis that calcium is the internal transmitter for the light-induced decrease in i_K.

INTRODUCTION

Limulus ventral photoreceptors contain both light-activated and voltage-sensitive conductances (Fain and Lisman, 1981). The principal effect of light is to increase membrane permeability to sodium ions (Millecchia and Mauro, 1969; Brown and Mote, 1974). However, in addition, light modulates one of the voltage-dependent conductances: the maintained potassium conductance (delayed rectifier) (Leonard and Lisman, 1981). This light-induced decrease in the voltage-dependent potassium conductance has been termed the "slow process" because its recovery after illumination is slow (minutes) in comparison with the recovery (100 ms) of the light-activated sodium conductance (Lisman and Brown, 1971). Modulation of the delayed rectifier by light is functionally important because it helps to stabilize the plateau voltage of the receptor potential during constant illumination (Leonard and Lisman, 1981). The delayed rectifier in *Limulus* is similar in its voltage dependence and pharmacology (Pepose and Lisman, 1978;

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J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/84/09/0447/16$1.00 447
Volume 84 September 1984 447–462

Leonard and Lisman, 1981) to the delayed rectifier in other preparations (Hodgkin and Huxley, 1952; Kusano et al., 1967).

It was our aim to investigate the mechanism by which the delayed rectifier (i_K) is modulated by light. Since illumination is known to increase both the intracellular free calcium (Ca_i^{2+}) and free proton concentration, we sought to determine if either of these changes mediated the modulation of i_K. Light-induced increases in Ca_i^{2+} have been measured using the calcium-sensitive luminescent protein aequorin (Brown and Blinks, 1974), the dye arsenazo III (Brown et al., 1977; Nagy and Stieve, 1983), and calcium-sensitive microelectrodes (Levy, 1983; Levy and Fein, 1983). Ca_i^{2+} in the dark is 3.5 ± 2.8 μM (Levy, 1983) and increases during bright light to 230 ± 150 μM (Brown et al., 1977). Small light-induced increases in intracellular proton concentration (<0.1 pH unit) have also been demonstrated in these photoreceptors using phenol red (Brown et al., 1977; Bolsover et al., 1983).

In other preparations, there is little evidence for blockade of the delayed rectifier by calcium. The delayed rectifier is not affected by increasing internal calcium concentration in either squid axon (Begenisich and Lynch, 1974) or *Hermissenda* photoreceptors (Alkon et al., 1982). Calcium increases potassium conductance in many cells, but this is due to the activation of another type of potassium channel, distinct from the delayed rectifier (Meech, 1978; Adams et al., 1980). There is, however, evidence for a blocking action of protons in other systems. Protons block the delayed rectifier in squid (Wanke et al., 1979) and block the inwardly rectifying potassium channel in starfish oocytes (Moody and Hagiwara, 1982).

To investigate whether calcium or protons have a role in the modulation of the maintained potassium current in *Limulus*, we injected calcium, the calcium buffer EGTA, or the pH buffer MOPS into ventral photoreceptors. Injection of calcium in darkness reversibly reduced i_K. Injecting EGTA inhibited the light-induced decrease in i_K, whereas MOPS did not. These findings suggest that calcium can block the delayed rectifier in *Limulus* and that changes in Ca_i^{2+} mediate the light-induced decrease in i_K. Preliminary reports of some of these results have appeared (Chinn and Lisman, 1983*a*, *b*).

METHODS

Voltage-clamping and recording methods were similar to those described in Lisman and Brown (1971). Membrane current was measured with a current-to-voltage transducer. The voltage signal representing clamp current was smoothed with a single-stage filter that had a time constant of 1 ms. Unless otherwise indicated, all records were compensated for leakage current as described previously (Lisman et al., 1982). The holding voltage was −65 or −70 mV. Since the current through voltage-dependent conductances is zero at these holding potentials (Lisman et al., 1982), and since leakage current is substracted out, the baseline current in the figures is at zero current.

In most experiments, the bathing media was artificial seawater (ASW) (424 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 22 mM $MgCl_2$, 26 mM $MgSO_4$, 15 mM Tris-Cl). In some experiments, the bathing medium contained Ni^{2+}. In these experiments, the control ASW solution contained an extra 10 mM $MgCl_2$, which was replaced by 10 mM $NiCl_2$ in the test solution. In some experiments, 0.3 mM 4-aminopyridine (4-AP) (Sigma Chemical Co.,

St. Louis, MO) was added to normal ASW to block the fast, transient, voltage-dependent outward current (Lisman et al., 1982). All bathing solutions used in this study had a pH of 7.8.

In some experiments, the calcium buffer EGTA (Eastman Kodak Co., Rochester, NY) or the pH buffer MOPS (Sigma Chemical Co.) was pressure-injected into the cell through the voltage-sensing electrode. The pH buffer injection solution contained 3 M MOPS and was set to pH 7.2 with KOH. This solution also contained 15 mM of a dye, either phenol red or arsenazo III (both from Sigma Chemical Co.). The dye was used to monitor the amount of solution injected as described below. The calcium buffer solution (pCa 7.1) contained 1.3 M EGTA and 0.65 M $Ca(OH)_2$ and was adjusted to pH 7.2 with KOH. Affinity constants of EGTA for calcium and protons, used in the determination of the pCa, were obtained from Sillen and Martell (1971). The effective affinity constant (pK_a) of EGTA for calcium in the injection solution was 7.1. The solution also contained 15 mM phenol red.

The amount of solution injected into the cell was determined by measuring the reduction in the light transmitted through the cell caused by the entry of the dye contained in the injection solution. Transmitted light was measured using a photodiode in a manner similar to that of Lisman and Strong (1979). These measurements were compared with a calibration curve that we made by determining the absorbance of several different dilute solutions of the dye in a 50-μm-pathlength microcuvette (Vitro Dynamics, Rockaway, NJ). By measuring the cell diameter, which we assumed to be equal to the pathlength, and the percent change in light transmitted through the cell following injection, we could calculate the concentration of buffer injected into the cell. For most cells, the absorption of light by the dye was 7–10%. In control experiments, we found that the dye itself did not affect the light-induced decrease in i_K.

RESULTS

Light Reduces the Maintained Outward Current

Depolarizing *Limulus* ventral photoreceptors in the dark to 0 mV from a holding potential of −70 mV activates several types of voltage-dependent channels (Lisman et al., 1982; O'Day et al., 1982). These channels are responsible for an inward current (i_{in}) carried by sodium and calcium, a fast transient outward current (i_A) similar to the A-current seen in molluscan neurons (Thompson, 1977; Adams et al., 1980), and a maintained steady state outward potassium current (i_K), which is pharmacologically distinct from i_A (Lisman et al., 1982). There is no evidence for a calcium-activated potassium channel in these photoreceptors (Pepose and Lisman, 1978; Lisman et al., 1982).

Fig. 1 illustrates the effect of light on maintained outward current. The potassium conductance was activated in the dark by stepping from a holding potential of −70 to 0 mV for 5 s. The current record shows the fast transient outward current and the maintained outward current. The voltage-dependent inward current is not seen because of the simultaneous presence of a large outward current at this stimulus voltage. After turning the voltage clamp off, the photoreceptor was exposed for 30 s to the brightest white light obtainable in our system. The clamp was turned off because cells are sometimes destroyed by the large clamp currents that flow during very bright illumination. The effect of light on K^+ currents described below can also be observed if the voltage is

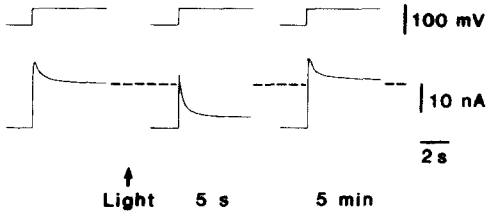

FIGURE 1. Light-induced reduction in outward current. A voltage-clamped photoreceptor was depolarized from −70 to 0 mV in the dark. The photoreceptor was then exposed for 30 s (onset indicated by the arrow) to the brightest light obtainable from the optical system. The effect of light on g_K was monitored by examining the outward current evoked by the same voltage step 5 s after the light was turned off. 5 min after illumination, the outward current had returned to preillumination levels.

clamped during illumination (Lisman and Brown, 1971; Leonard and Lisman, 1981), and is therefore a direct effect of light rather than a secondary result of light-induced depolarization. After the light was turned off, the membrane voltage returned to its preflash level. The voltage clamp was then turned on and a voltage pulse from −70 to 0 mV was again given. Under these conditions, the maintained outward current was greatly reduced (by ~75%). This effect was reversible: within 5 min after the light, outward currents had returned to control levels. Using this protocol, light-induced decreases in the maintained outward current ranged from 50 to 75% (n = 4). With very bright lights of this kind, cells usually did not survive for more than several repetitions of the protocol. Thus, in subsequent experiments, the stimulus duration was shortened to 1 s and the light was made dimmer by a narrow bandpass filter (530 nm) such that the intensity was 7×10^{14} photons s^{-1} cm^{-2}. Using such stimuli, the light-induced decrease in the outward current was less pronounced (10–30%), but cells survived many repetitions of the protocol.

Since i_A can be reduced by light in *Hermissenda* photoreceptors (Alkon et al., 1982), we tested to see whether part of the light-induced decrease in the maintained outward current might be due to a modulation of a component of i_A that had not inactivated by the end of our pulses. For this purpose, we examined the light-induced decrease in the maintained outward current under conditions where i_A was severely reduced. The experimental protocol was as follows. The potassium conductance was activated either by stepping from a holding potential of −70 to 0 mV (Fig. 2*A*) or by stepping from a holding potential of −30 to 0 mV (Fig. 2*B*). In the first case, i_A was large, while in the second case, i_A was largely inactivated (Pepose and Lisman, 1978). Fig. 2 shows that the percent decrease in the maintained outward current by light, measured between 2 and 6.5 s after the onset of the voltage pulse, was only minimally dependent on the holding potential. In five cells, light reduced the maintained outward current by 20 ± 5% (SD) in the presence of i_A and by 22 ± 4% (SD) in its absence. We also examined the light-induced decrease in the maintained outward current in the presence of 0.3 mM 4-AP in the bathing media. 4-AP blocks i_A much more strongly than i_K (Lisman et al., 1982). The light-induced decrease in the main-

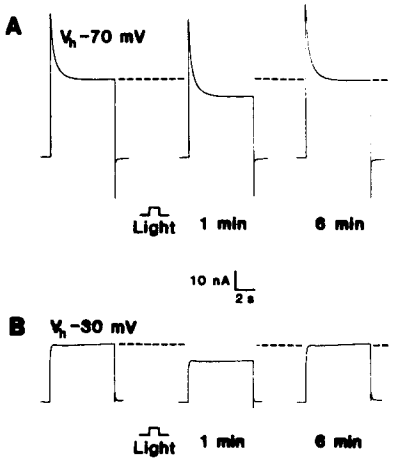

FIGURE 2. Effect of holding potential (V_h) on the light-induced decrease in outward current. (*A*) A voltage-clamped photoreceptor was depolarized in the dark to 0 mV from a V_h of −70 mV. It was then exposed to a 1-s flash of bright light. The effect of light on i_K was monitored by examining the outward current evoked by the same voltage step depolarization 1 min and again 6 min after the light. Light reduced i_K by 22%. Note that the fast transient outward current (i_A) was much larger than in the cell of Fig. 1. (*B*) The experimental protocol was the same as in *A* except that V_h was −30 mV. Light reduced i_K by 23%. In this and the other figures, the light intensity was reduced from that used in Fig. 1 to an intensity of 7×10^{14} photons s^{-1} cm^{-2} (530 nm).

tained outward current, measured 5.5 s after the onset of a voltage pulse from −70 to 0 mV, was 33 ± 4% (SD) in the absence of 4-AP and 61 ± 11% (SD) (N = 2) in the presence of 4-AP. It is not clear why the percentage decrease in the maintained outward current was greater in the presence of 4-AP. In any case, the 4-AP experiments confirm that the light-induced decrease in the maintained outward current involves a modulation of i_K rather than i_A.

Effect of Calcium Injection on i_K

To determine if Ca_i^{2+} affects i_K, we examined the maintained outward current before and after iontophoretic injection of calcium in the dark. The cell was voltage-clamped with two microelectrodes filled with 3 M KCl. A third intracellular microelectrode containing 100 mM $CaCl_2$ was used to inject calcium. A bucking current of 2–4 nA served to prevent leakage of calcium into the cell and to prevent clogging of the electrode tip. For all experiments, the electrodes were determined to be isopotential. All calcium injections were done under voltage clamp. There was therefore no change in the membrane potential during the calcium injections.

Fig. 3*A* shows that the maintained outward current was reduced by calcium injection. 13-nA injection pulses of 6 s duration were given once every 20 s. To monitor the outward current, voltage-clamp pulses were given 3 s after each calcium injection. After five to six calcium injections, the maintained outward

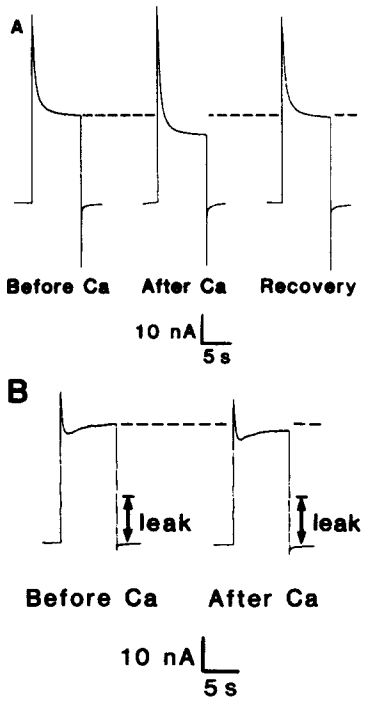

FIGURE 3. Outward current was reduced by iontophoretic calcium injection. (*A*) Reduction of outward current by injection of calcium when the cell was bathed in ASW. The potassium conductance was activated by stepping from a holding potential of −70 to 0 mV. The middle current trace is after six injections of calcium (13 nA, 6 s). Recovery occurred within 10 min. (*B*) Reduction of outward current by iontophoretic injection of calcium when the photoreceptor was bathed in ASW containing 10 mM Ni^{2+}. The potassium conductance was activated and calcium was injected as in *A*. To shorten the time of the experiment, the outward current was examined after only two calcium injections. In this experiment, leakage current was not corrected. The amount of leakage current is indicated in the figure. To determine the leakage current, we measured the current evoked by a 20-mV hyperpolarizing voltage pulse from the holding potential and then multiplied this value by 3.5 to find the amount of leakage current associated with a 70-mV depolarizing voltage pulse.

current was reduced to a new, steady level. The current returned to the preinjection level within 10 min after terminating the injections. In five cells, the average decrease was 18 ± 7% (SD). In one other cell, no change in the outward current occurred. In all cells, the leakage resistance was monitored using 10- or 20-mV hyperpolarizing pulses from a holding potential of −70 mV and was unaffected by the calcium injections.

Calcium injection never reduced i_K by as much as our brightest lights (compare Figs. 1 and 3*A*). One explanation for this is that diffusion of calcium away from the electrode tip was restricted. Evidence for calcium gradients during injection of calcium into *Limulus* ventral photoreceptors has been previously reported

(Fein and Lisman, 1975). It is therefore likely that at sites distant from the electrode, Ca_i^{2+} did not rise by as much as it did near the injection site, thereby limiting the number of channels affected.

The decrease in the net outward current produced by calcium injection might be due to an activation of a voltage-dependent inward current rather than to a true decrease in the voltage-dependent outward current. To test this possibility, we injected calcium in the presence of 10 mM Ni^{2+} in the bathing media (Fig. 3*B*). Ni^{2+} has previously been shown to block i_{in} (Lisman et al., 1982). We found that Ni^{2+} by itself slowly reduced i_K. Since it was therefore necessary to minimize the time of the experiment, we examined the outward current after only two calcium injections rather than the five to six injections used in the experiment in Fig. 3*A*. While this meant that less calcium was injected, we found that calcium injection still reduced the maintained outward current. Since the current traces in Fig. 3*B* were not compensated for leakage, the leakage current (estimated from hyperpolarizing pulses) is indicated in the figure. Again, the leakage resistance was unaffected by calcium injection. In the cell of Fig. 3*B*, the rate of reduction of i_K by Ni^{2+} before calcium injection was only 0.4 nA/min (~2%/min), whereas following the initial calcium injection, i_K was reduced by 2.0 nA within 40 s (~14%/min). Thus, the reduction in i_K over time was greatly increased by calcium injection and cannot be attributed to Ni^{2+}. These results therefore show that the decrease in the net outward current caused by calcium injection is not due to activation of i_{in}, since reduction of the outward current by calcium occurs under conditions where i_{in} is blocked.

To further test whether calcium affected an inward or outward current, instantaneous current-voltage (*i-V*) curves were constructed from tail current data obtained before and after calcium injection. If calcium mediates a decrease in g_K, calcium injections should produce a decrease in the slope of the instantaneous *i-V* curves but not change the reversal voltage. For these experiments, the photoreceptor was depolarized to 0 mV for 3.5 s to activate i_K. The membrane was then repolarized to a family of more negative potentials. During the first 20 ms after repolarization, the change in current was due to the decline of the capacitative transient and to a small, rapidly inactivating maintained inward current that is described in the companion paper (Chinn and Lisman, 1984). After approximately the first 20 ms, the current decayed over the next 180 ms with a single-exponential time course (Fig. 4, inset). This tail current is due to the decline of the voltage-dependent K^+ conductance (Pepose and Lisman, 1978). The coefficient of determination of the linear least-squares fit of the points between 20 and 200 ms to an exponential was better than 0.95. The instantaneous values of the potassium current immediately after repolarization were obtained by extrapolating the exponentials back to the time of the onset of the repolarizing pulse. Tail currents after calcium injection were obtained in a manner identical to that before calcium injection except that a calcium injection pulse (13 nA, 6 s) preceded (by 3 s) each depolarizing voltage pulse. Tail currents were measured only after the repetitive calcium injections had reduced the maintained outward current to a steady level. Instantaneous current-voltage curves are plotted before (×) and after (○) calcium injection in Fig. 4. Calcium

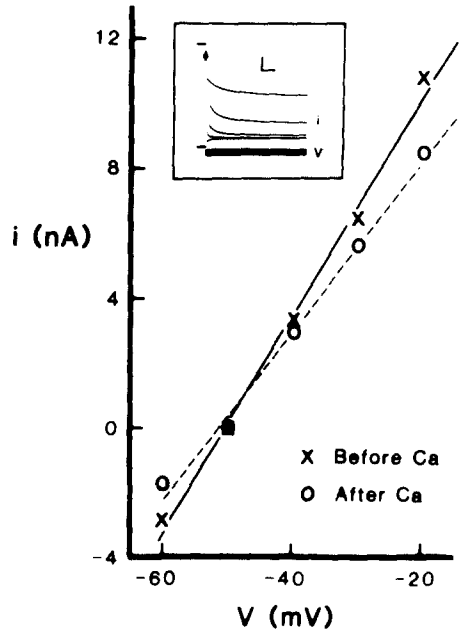

FIGURE 4. Effect of calcium on the instantaneous *i-V* curve. The cell was depolarized from a holding potential of −70 to 0 mV for 3.5 s and then repolarized to a family of more negative potentials. Instantaneous *i-V* curves were constructed from tail currents as detailed in the text. The curves are shown before (×) and after (○) iontophoretic injection of calcium. After calcium injection, the slope conductance is reduced, but the reversal potential is virtually the same. Data here are from a different cell than in the inset. The inset shows a family of superimposed tail currents obtained before calcium injection. The arrow points to the steady state outward current just before the change in potential occurred. The vertical calibration bar indicates 5 nA and 100 mV, while the horizontal calibration bar indicates 50 ms.

injection reduced the slope conductance (from 0.33 to 0.26 μS), but did not affect the reversal potential. In four experiments, the average change in reversal potential was −2.0 ± 1.4 mV (SE). In all cases, partial or complete recovery was obtained. Furthermore, even though the potassium tail currents were smaller immediately after calcium injection, the decay time constant was not significantly affected (changed by <5%), which indicates that calcium reduced g_K without strongly affecting the kinetics of potassium channel closure. These experiments, like the experiments in Ni^{2+}, indicate that the calcium-induced decrease in outward current is due to a decrease in i_K rather than to activation of an inward current.

The reduction in potassium conductance by calcium might be due to a positive shift in the voltage range at which potassium channels become active. This would be manifested by a shift of the g_K-*V* curve toward more positive potentials. This is not the case, however, as seen in Fig. 5. Currents in this experiment were evoked by voltage pulses from a holding potential of −70 mV, given in ~18-mV

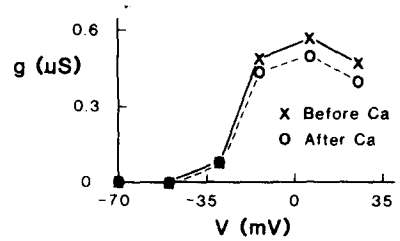

FIGURE 5. Effect of calcium on the g_K-V curve. The potassium current was activated at different potentials by depolarizing voltage pulses given in 18-mV increments, both before and after calcium injection. A g_K-V curve was constructed from the data before (×) and after (○) calcium injection. For the calculation of g_K, the reversal potential of i_K was taken to be −55 mV.

increments. Calcium injection reduced g_K at all voltages without shifting its voltage dependence. These results were obtained in two cells and are in agreement with experiments by Pepose and Lisman (1978) which show that i-V curves do not undergo shifts after calcium injection. Thus, calcium acts by reducing $\bar{g}_K$, not by altering its voltage dependence.

Effects of Buffering Internal Free Calcium on the Light-induced Decrease in i_K

Because our experiments indicated that raising Ca_i^{2+} can reduce i_K, it was of interest to determine whether the light-induced decrease in i_K could be blocked by pressure-injecting EGTA into the photoreceptor. EGTA has previously been shown to inhibit the light-induced rise in Ca_i^{2+} measured with aequorin (Brown and Blinks, 1974). The protocol was as follows. Before injection, the light-induced decrease in i_K was first examined using two 3 M KCl electrodes to voltage-clamp the photoreceptor (Fig. 6*A*; top traces). The KCl voltage-sensing electrode was then removed and replaced with an electrode containing EGTA set to pCa 7.1 at pH 7.2. This protocol was necessary because EGTA electrodes often became plugged over time, making injection impossible. Cells were then injected with EGTA. After the injection, we again voltage-clamped the cells and, for reasons described below, changed the bathing media to one containing 10 mM Ni^{2+}. 8 min after application of Ni^{2+}, the effect of light on outward current was measured. Under these conditions, the light-induced decrease in i_K was greatly inhibited, as illustrated in the bottom traces of Fig. 6*A*. In three cells with intracellular EGTA concentrations ranging from 30 to 50 mM, the light-induced decrease in i_K was inhibited by 74 ± 6% (SD). In control experiments, we found that Ni^{2+} itself did not affect the percent decrease in i_K produced by light, confirming similar experiments reported by Leonard and Lisman (1981). In three other cells injected to somewhat higher EGTA concentrations (50–70 mM), the light-induced decrease in i_K was completely inhibited. Thus, the EGTA experiments indicate that intracellular EGTA can reduce or block the light-induced decrease of i_K. Similarly high concentrations of EGTA are required to inhibit light adaptation in ventral photoreceptors, a process that is due to an increase in Ca_i (Lisman and Brown, 1975).

In the EGTA experiments described above, Ni^{2+} was added to the bathing

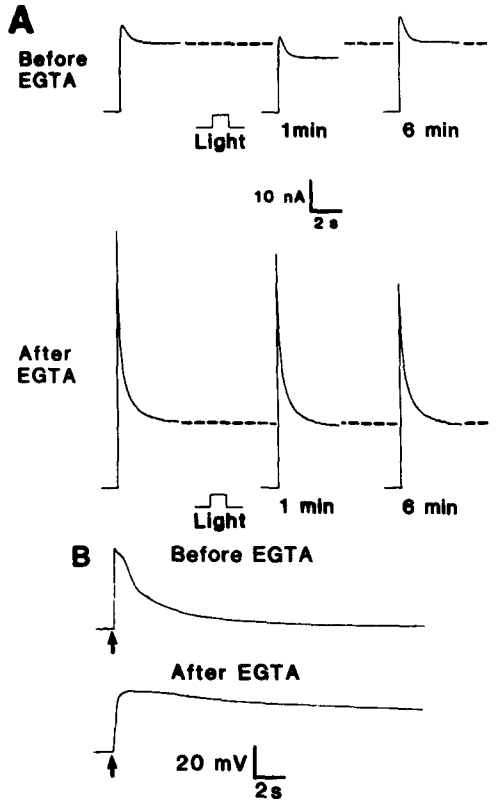

FIGURE 6. (*A*) Effect of intracellular calcium buffer (EGTA) on the light-induced decrease in i_K. Potassium conductance was activated by stepping from a holding potential of −65 to 0 mV. The light-induced reduction in i_K before EGTA injection is shown in the top traces. After pressure-injecting EGTA to an intracellular concentration of 50 mM, the ASW bathing media was changed to one containing 10 mM Ni^{2+}. The effect of light on i_K was greatly reduced (bottom traces). Pulses were given 8 min after the addition of Ni^{2+}. (*B*) The receptor potential was prolonged following EGTA injection. Data here are from the same cell as in *A*.

media after EGTA injection in order to block i_{in}. Without Ni^{2+}, the effect of light was to increase rather than to decrease the net outward current in EGTA-injected cells. As described in detail in the companion paper (Chinn and Lisman, 1984), this paradoxical effect of light was due to a light-induced decrease in a maintained, voltage-dependent inward current, which became substantial in EGTA-injected cells.

In all three cells in which the outward currents were examined before and after EGTA injection, i_A was larger following injection (Fig. 6*A*). The cause of the enlarged A-currents after EGTA injection is unknown, but may be related to the effects of calcium on i_A described by Alkon et al. (1982). We also found that after injecting EGTA, the receptor potential became prolonged (Fig. 6*B*), as previously reported by Lisman and Brown (1975).

Effects of Buffering H^+ on the Light-induced Decrease in i_K

A very small decrease in pH (<0.1 pH unit) occurs in ventral photoreceptors after exposure to bright light (Brown et al., 1977). We investigated whether protons have a role in the light-induced decrease in i_K by pressure-injecting the pH buffer MOPS (pH 7.2) into the cell. Fig. 7*A* shows the light-induced decrease in i_K before (top traces) and after (bottom traces) injecting MOPS to an intracellular concentration of 180 mM. The light-induced decrease in i_K was the same in both cases. In five cells injected with MOPS to intracellular concentrations of 100–200 mM, the light-induced decrease in i_K was not affected. In three of these cells, the buffer injection itself reduced i_K by 27 ± 4% (SD). However, the percent decrease in i_K produced by light was unaffected. These results indicate that changes in pH are not important in the light-induced decrease in i_K. The late

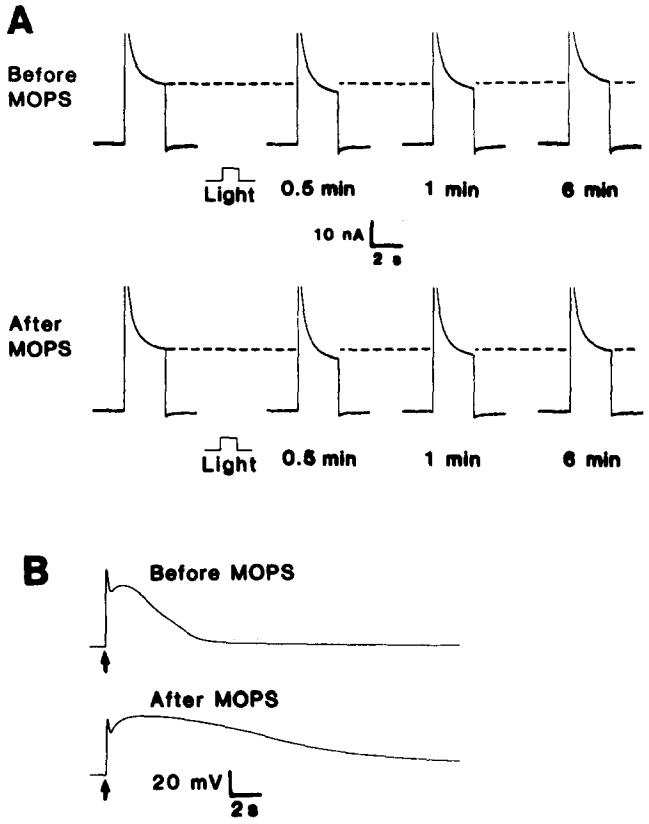

FIGURE 7. Effects of intracellular injection of the pH buffer MOPS. (*A*) Lack of effect of the pH buffer MOPS on the light-induced decrease in i_K. The potassium conductance was activated by stepping from a holding potential of −70 to 0 mV. Before buffer injection (top traces), i_K was reduced by 15% at 0.5 min and 10% at 1 min after the light. After injecting MOPS to an intracellular concentration of 180 mM, light reduced i_K by the same amount (bottom traces). (*B*) The receptor potential was prolonged following injection of MOPS. Data here are from a different cell than in *A*, in which the prolonged receptor potential was not as dramatic.

receptor potential was prolonged after injecting these large concentrations of pH buffer (Fig. 7*B*). A similar effect of large buffer injections on the receptor potential has been reported previously (Coles and Brown, 1976), but the underlying cause is unknown.

DISCUSSION

i_K Is Reduced by Calcium

In this paper, we present evidence that raising the intracellular free calcium concentration reduces the maintained voltage-dependent K^+ conductance (delayed rectifier). When calcium was injected, a clear, reversible decrease in net outward current was observed (Fig. 3*A*). This calcium-induced decrease was not due to an increase in i_{in} since similar results were obtained in the presence of 10 mM Ni^{2+} (Fig. 3*B*), which blocks i_{in} (Lisman et al., 1982; O'Day et al., 1982). Furthermore, calcium injection reduced the slope conductance of the instantaneous *i-V* curve with minimal changes in the reversal potential (Fig. 4). In these experiments, calcium did not significantly affect the time constant of decay of the tail currents, which indicates that the kinetics of potassium channel closure were not significantly affected by calcium. Furthermore, the reduction of i_K by calcium was not caused by shifting the voltage range at which the potassium channels were active to more positive potentials (Fig. 5). The results therefore indicate that calcium reduces $\bar{g}_K$. Although it seems clear that Ca can reduce $\bar{g}_K$, it is not known whether Ca_i^{2+} interacts directly with the channel or whether there are intervening chemical events.

The results presented here are the first indication that the delayed rectifier can be blocked by calcium. In squid axon (Begenisich and Lynch, 1974) and *Hermissenda* photoreceptors (Alkon et al., 1982), the potassium channels responsible for the maintained outward current were found to be largely unaffected by calcium. Lux et al. (1976) and Hofmeier and Lux (1981) reported that calcium injection depressed the later outward current in snail neurons. However, this has since been found to be due to the calcium inhibition of an inward calcium current, which in turn reduced the calcium-activated potassium current (Eckert and Ewald, 1982). Studies in *Limulus* do not present such complications since the calcium-activated potassium current does not seem to exist in this preparation (Pepose and Lisman, 1978; Lisman et al., 1982). In fact, in preparations that have a calcium-activated potassium current, the activation of this current by calcium may obscure the effects of calcium on i_K.

Calcium Is Likely to Be the Internal Transmitter for the Light-induced Decrease in i_K

Our results suggest that calcium is the internal transmitter that mediates the light-induced decrease in i_K. If a substance is an internal transmitter, it should be possible to: (*a*) mimic the response to the normal stimulus by changing the concentration of the presumed transmitter in the absence of the stimulus; (*b*) detect changes in the concentration of the transmitter in response to the stimulus; (*c*) inhibit the response to the normal stimulus by preventing changes in concen-

tration of the transmitter. Calcium qualitatively fulfills these criteria: the effect of light on i_K is mimicked by injecting calcium (Figs. 3 and 4); Ca_i^{2+} increases in response to light (Brown et al., 1977); the light-induced decrease in i_K is inhibited by buffering Ca_i^{2+} (Fig. 6*A*).

While calcium is likely to be the internal transmitter controlling i_K, several important questions about its function remain unanswered. We know that the light-induced increase in Ca_i^{2+} is largely due to release from internal stores (Brown and Blinks, 1974), although entry from outside the photoreceptor also contributes to this increase (O'Day and Lisman, 1979; O'Day et al., 1982). However, the nature of the signal between the absorption of light in the plasma membrane and the release of calcium from internal stores is not known (Lisman and Strong, 1979). Furthermore, as mentioned above, it is unclear whether calcium reduces i_K directly or indirectly. It would be of interest in this regard to compare the time course of Ca_i during light with the time course of the modulation of i_K. At this point, no detailed comparison is possible, but the available evidence indicates that the kinetics are not grossly different. The decrease in i_K during light has a rapid initial component that occurs within a few hundred milliseconds (Lisman and Brown, 1971), on the same time scale as the rise in Ca_i^{2+} (Brown et al., 1977; Nagy and Stieve, 1983). There follows a slower (tens of seconds) further reduction in i_K (Leonard and Lisman, 1981), possibly related to the slow rises in Ca_i measured by Levy (1983). After illumination is terminated, both Ca_i (Levy, 1983) and i_K (Lisman, 1971) return to the dark-adapted level within minutes.

Effects of Buffering Intracellular pH

A small light-induced change in pH (<0.1 pH unit) has been found in *Limulus* ventral photoreceptors (Brown et al., 1977; Bolsover et al., 1983). In other invertebrate neurons, the intracellular pH buffering capacity has been found to be ~20 mM of a buffer at its pK (Thomas, 1974; Bolsover and Brown, 1982). If this is the case in *Limulus*, then the pH buffering capacity would have been increased 6–11 times by our pH buffer injections. Since the light-induced decrease in i_K was not blocked by these injections (Fig. 7*A*), it seems unlikely that pH changes are involved in the light-induced decrease in i_K. Moreover, since we have shown that internal free calcium seems to mediate the light-induced decrease in i_K, the lack of effect of pH buffer implies that pH changes are not important in stimulating the increase in Ca_i^{2+} or in mediating the effect of calcium on i_K.

Functional Significance of Calcium Modulation of i_K

In *Limulus*, a maintained light stimulus results in a transient depolarization that decays to a plateau value. The plateau voltage is constant (Leonard and Lisman, 1981), as it should be for steady illumination to be perceived as such (Fuortes, 1959; Leonard and Lisman, 1981). In contrast to the constant value of plateau voltage, the value of the light-dependent sodium current (i_{Na}) decreases with time during the plateau (Leonard and Lisman, 1981). This reduction in i_{Na} can presumably be attributed to progressive light-induced increases in internal free calcium, the transmitter of adaptation in these cells. It is only because the light-

induced decrease in i_{Na} is offset by the light-induced decrease in i_K that the plateau voltage remains constant (Leonard and Lisman, 1981). To achieve such coordination of i_{Na} and i_K, it would be desirable to have the decline in the potassium conductance and the decline in the light-dependent sodium conductance controlled by the same substance. Thus, the finding that intracellular calcium can decrease i_K is sensible from a functional viewpoint.

We thank Gordon Fain, Jonathan Shoukimas, Judy Strong, Juan Bacigalupo, and Phyllis Robinson for commenting on the manuscript, Dave Eaton for his excellent machine work, and Barbara Yeaton for secretarial assistance.

This work was supported by National Science Foundation grant BNS 8105746 and National Institutes of Health grant EY04249.

Received for publication 6 December 1983 and in revised form 15 May 1984.

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