Light Reduces the Voltage-dependent Inward Current in *Limulus* Ventral Photoreceptors

KEVIN CHINN and JOHN LISMAN

From the Department of Biology, Brandeis University, Waltham, Massachusetts 02254

**ABSTRACT** In *Limulus* ventral photoreceptors, illumination not only increases a specialized light-activated sodium conductance but also modulates voltage-dependent conductances. Previous work has demonstrated that the delayed rectifier current is reduced by light; we report here that the early voltage-dependent inward current is also reduced by light. Furthermore, by analyzing tail currents, we show that voltage-dependent inward current is maintained during continuous depolarization and that this maintained inward current can be reduced by light. EGTA injection was found to increase the maintained inward current.

**INTRODUCTION**

*Limulus* ventral photoreceptors contain both light-activated and voltage-dependent conductances. The principal effect of light is to increase the light-activated sodium conductance, causing the cell to depolarize (Millecchia and Mauro, 1969; Brown and Mote, 1974). In addition, light can modulate one of the voltage-dependent conductances of the cell, the delayed rectifier (i_k) (Lisman and Brown, 1971; Leonard and Lisman, 1981). The decrease in this potassium current produced by light is functionally important in stabilizing the plateau voltage of the receptor potential (Leonard and Lisman, 1981). Ventral photoreceptors contain two other voltage-dependent conductances: one responsible for a transient outward potassium current (i_A) (Pepose and Lisman, 1978), the other for an inward current carried by sodium and calcium. It is not known whether the sodium and calcium pass through the same or different channels (Lisman et al., 1982). We report here that in addition to reducing i_k, light also reduces the voltage-dependent inward current. Furthermore, we have found that the voltage-dependent inward current is maintained during long depolarizations and that this maintained component is modulated by light. Preliminary reports of some of these results have appeared (Chinn and Lisman, 1983a, b).

**METHODS**

Voltage clamping and recording methods were similar to those described by Lisman and Brown (1971). All current records were compensated for leakage conductance as described.
RESULTS

Effect of Light on the Voltage-dependent Inward Current

The effect of light on the voltage-dependent inward current was first studied at potentials where a net early inward current ($i_n$) could be measured. Cells were depolarized from a holding potential of $-70$ to $-35$ mV in the dark, producing a net inward current followed by a net outward current (Fig. 1A). To examine the effect of light on $i_n$, we presented the cell with a 30-s flash of the brightest white light obtainable in our system. Several seconds after turning off the light, the cell was depolarized as before. Both the peak inward current and the outward current were reduced by light (Fig. 1B). The peak inward current was reduced by 31–42% ($N = 3$) and returned to dark control values within 5–10 min. With the very bright light used in this experiment, cells usually did not survive more than several repetitions of the protocol. Thus, unless otherwise indicated, the light intensity in subsequent experiments was reduced using a narrow bandpass filter (530 nm). The final intensity was $7 \times 10^{14}$ photons s$^{-1}$ cm$^{-2}$. We examined the effects of this lower-intensity light on the net $i_n$. In these experiments, 1-s light flashes were presented at a frequency of one every 15 s, alternated with depolarizing pulses given 8 s after each flash. As before, both the peak inward current and the outward current were reversibly reduced by light, but the reduction was smaller. In eight cells, the average reduction of peak inward

![Figure 1](image_url)

**FIGURE 1.** Effect of light on early net inward and early net outward current. For both traces, the photoreceptor was depolarized from a holding potential of $-70$ to $-35$ mV. Arrows indicate the onset of a 1-s depolarizing voltage pulse. Current recordings have been juxtaposed for ease of comparison. The baseline current level is indicated by the dashed line. The photoreceptor was depolarized in the dark, producing the current trace shown in A. After this, a 30-s flash of white light was presented to the photoreceptor. (B) Current trace obtained 5 s after the light was turned off. Note that both the peak inward current and the outward current are smaller after the light. Recovery occurred within 6 min (not shown).
current was $10 \pm 4\%$ (SD). In cells used for this study, the inward currents activated by consecutive pulses varied by no more than 2%. In a few cells that were unsuitable for these studies, inward currents showed an unexpected 5–10% variation from pulse to pulse.

Existence of a Maintained Inward Current

We sought to determine whether the inward current was maintained during long depolarizations and if the maintained inward current could be affected by light. Because long depolarization activates both inward and outward currents, we needed to find a way of separating these currents. One way of doing this was to activate voltage-sensitive currents by giving a depolarizing voltage pulse and then to examine the time-varying current (termed the tail current) during repolarization. We found that the inward current turned off faster than the outward current, making it possible to study a maintained inward current, as described below.

The membrane was depolarized from a holding potential of $-70$ to $0$ or $+15$ mV for $2.5$ s and then repolarized by $20$ mV. The resulting tail current consisted of several components. During the first $20–50$ ms after repolarization (depending on pulse protocol), the current increased with time (Fig. 2, upward arrow), initially because the capacitative current declined and subsequently because of the turning off of an inward tail current. After reaching a peak, the current declined as a single exponential (Fig. 2, downward arrow). This last component is the outward tail current caused by a decrease in $i_K$ (Pepose and Lisman, 1978; Leonard and Lisman, 1981). The magnitude of the inward tail current (Fig. 3B) was computed by subtracting out both the capacitative current and the outward

![Figure 2. Tail current record. The photoreceptor was depolarized from $-70$ to $0$ mV for $2.5$ s. The star indicates the steady state outward current at the end of this period. After this, the cell was repolarized by $20$ mV, producing a “tail current,” which consisted of several components. During the initial period following repolarization (upward arrow), the current contained a capacitative transient and an inward tail current (seen in greater detail in another cell in Fig. 3). After this, an outward tail current was evident (downward arrow) that decayed as a single exponential. The size of the outward tail current component at the onset of the voltage pulse was found by extrapolating this exponential back in time (upper dashed line). The baseline current level is indicated by the lower dashed line. The current trace was filtered ($\tau = 1$ ms).](image-url)
FIGURE 3. Inward tail current. (A) The photoreceptor was depolarized from -70 to +15 mV for 2.5 s (not shown). The tail current in trace 1 occurred when the cell was repolarized by 20 mV. The size of the capacitative transient was determined by examining the current in response to a 20-mV hyperpolarization from a holding potential of -70 mV (trace 2). The current at -70 mV is zero, and marks the zero-current baseline for trace 1. Trace 1 was corrected for capacitative current, as shown schematically by the dashed line. The dotted line above trace 1 is the extrapolated outward tail current. The difference between the outward tail current and corrected total current is the inward tail current ($\Delta \text{i}_{\text{in}}$). This is marked 10 ms after repolarization by the double arrow. Current traces were filtered ($\tau = 0.2$ ms). (B) $\Delta \text{i}_{\text{in}}$ determined from the same cell as in A is plotted before (x) and after (○) exposure to 10 mM Ni²⁺. The dashed line connecting the x’s is an exponential curve fit to the points ($\tau = 15$ ms).
tail currents, as illustrated schematically in Fig. 3A. The initial 50 ms of the tail current of Fig. 2 is shown at high time resolution in Fig. 3A, trace 1. The component due to the capacitative transient alone (Fig. 3A, trace 2) was subtracted from the upper trace, yielding a “corrected” total current shown schematically in Fig. 3A, trace 1, by the dashed line. The dotted line is the outward tail current extrapolated back to the onset of the repolarizing pulse. The inward tail current ($\Delta i_m$) was taken to be the difference between the “corrected” total current and the outward tail current, and it is plotted ($\times$) in Fig. 3B. For all cells tested ($N = 13$), the decay of $\Delta i_m$ could be approximated by a single exponential. For cells that had been depolarized to $+15$ mV and then repolarized to $-5$ mV, the time constant of decay was $10.0 \pm 2.5$ ms (SD) ($N = 13$) and the extrapolated amplitude of the inward tail current at the onset of the repolarizing pulse was $10.0 \pm 2.0$ nA (SD). For cells ($N = 4$) depolarized to $0$ mV and repolarized to $-20$ mV, the time constant of decay was $5.0 \pm 0.5$ ms (SD) and the amplitude of the inward tail current was $4.0 \pm 1.5$ nA (SD). We did not examine tail currents at more negative potentials since the maintained inward current ($i_m$) turned off too rapidly to resolve. The open circles in Fig. 3B show that the inward tail current, like the early inward current (Lisman et al., 1982), could be blocked by 10 mM Ni$^{2+}$. It is therefore extremely likely that the inward tail current that follows a hyperpolarizing step is the turning off of a maintained inward current rather than the activation of an anomalous outward current.

As shown in Fig. 4, the inward tail current was reduced by light. The experimental protocol was as follows. Cells were depolarized in the dark from $-70$ to $+15$ mV for 3 s, followed by repolarization to $-5$ mV. The effect of light on the inward tail current was examined by presenting 1-s light flashes (1/15 s) 8 s before each voltage pulse. In the four cells tested, the amplitude of the inward tail current was reduced by 25%, from $10.0 \pm 1.5$ nA (SE) to $7.5 \pm 1.0$ nA (SE). For all the cells tested, the time constant of decay ($\sim 9$ ms) was unaffected by light and recovery occurred within 5–10 min.

**Light Decreases $i_m$ in EGTA-injected Cells**

In the course of experiments designed to test whether the light-induced reduction of $i_k$ is mediated by a rise in intracellular free calcium ($C_{a0}$) (Chinn and Lisman, 1984), we pressure-injected EGTA into photoreceptors. Whereas the net outward current during a depolarization is normally reduced after illumination (Lisman and Brown, 1971), we found that light led to an increase in the net outward current in cells injected with EGTA. We describe this effect of EGTA below, and show that the increase in net outward current is due to a decrease in a voltage-dependent inward current rather than to an increase in a true outward current.

Fig. 5 shows how the net voltage-dependent outward current is modulated by light under normal conditions and after EGTA injection. For all current traces, the outward current was evoked by depolarizing the photoreceptor from $-70$ to $0$ mV for 2.5 s. The current traces show a transient outward current ($i_o$) which decays, leaving the maintained outward current. The upper traces, which were given before EGTA injection, show that after a bright 1-s light was turned off,
the net maintained outward current evoked by a depolarizing voltage pulse was smaller than during a similar voltage pulse given before illumination. Recovery occurred within 6 min. This light-induced decrease in the outward current has been previously shown to be due to a light-induced decrease in the maintained voltage-dependent outward potassium current ($I_K$) (Leonard and Lisman, 1981). The effects of EGTA injection were then examined. Pressure injection of EGTA (pCa 7.1, pH 7.2) to intracellular concentrations of 40–70 mM itself produced a moderate reduction ($30 \pm 11\%\;[SE];\;N = 4$) in the net maintained outward current (compare the top left and bottom left traces in Fig. 5). This is due to an EGTA-induced increase in the maintained voltage-dependent inward current (see below). Under these conditions, illumination of the cell led to an increase in the net outward current (dashed line). This effect of light was partly or completely reversible in three out of five cells.

In a separate set of experiments, we found that EGTA injection also had curious effects on tail currents. In uninjected cells, the peak current during the tail current was always less than the steady state maintained outward current before repolarization (Fig. 2). However, in cells injected with EGTA to intracellular concentrations of 40–70 mM, the peak amplitude of the tail current (Fig. 6A at upward arrow) could be as large as or greater than the steady state current.
**Figure 5.** Effect of light on net outward current before (top row of traces) and after (bottom row of traces) injecting EGTA. For all current traces, the photoreceptor was depolarized from a holding potential of −70 to 0 mV. Before injection, the photoreceptor was depolarized in the dark and again 1 min after a 1-s flash of light (530 nm). Note that light reduced the net outward current (indicated at 2.5 s after the onset of the voltage pulse by the dashed line). 6 min after the light, the outward current had almost fully recovered. After pressure-injecting EGTA to an intracellular concentration of 60 mM, the outward current was reduced (bottom left trace). 1 min after a 1-s flash of light, the net outward current was increased (see dashed line). Recovery occurred 6 min after the light. Current traces were filtered (τ = 1 ms).

**Figure 6.** Effects of EGTA and light on tail currents. (A) The photoreceptor was pressure-injected with EGTA to an intracellular concentration of 55 mM. Outward current was evoked by a voltage pulse from −70 to 0 mV for 2.5 s. The steady state outward current at the end of this period is indicated by the downward arrow. After this, the photoreceptor was repolarized by 20 mV. The peak amplitude of the tail current (upward arrow) was almost the same as the steady state outward current. (B) After light, the steady state outward current was increased. Also, the peak amplitude of the tail current was much smaller than the steady state outward current. Current traces were filtered (τ = 0.2 ms).
FIGURE 7. Plot of inward tail current from an EGTA-injected cell before (×) and after (○) light. Light abolished the inward tail current. After injecting the cell to an intracellular EGTA concentration of 55 mM, the cell was depolarized from -70 to 0 mV for 2.5 s and then repolarized by 20 mV. Δᵢₓ was determined as in Fig. 3 before and after a 1-s flash of light. The dashed line connecting the X's is the exponential curve fit to the points (τ = 11.0 ms).

FIGURE 8. Effect of EGTA and Ni²⁺ on tail currents. (A) The cell was injected with EGTA to an intracellular concentration of 67 mM. After this, it was depolarized from -70 to 0 mV for 2.5 s. The steady state outward current at 2.5 s is indicated by the arrow. The cell was then repolarized by 20 mV. Note that the peak amplitude of the tail current was greater than the steady state outward current. (B) After exposure to 10 mM Ni²⁺, the steady state outward current (at arrow) was increased. Also, the peak amplitude of the tail current was much smaller than the steady state outward current. Current traces were filtered (τ = 0.2 ms).

just before repolarization (Fig. 6A, downward arrow). This effect could occur if EGTA injection increased a maintained inward current and if repolarization initially turned off more inward than outward current. These curious tail currents in EGTA-injected cells were strongly affected by light, as shown in Fig. 6B. Following illumination, the peak current after repolarization was always smaller than the steady state outward current.

The effect of light on the inward component of tail currents in EGTA-injected cells is shown in Fig. 7. Cells were depolarized from -70 to 0 mV and then repolarized by 20 mV. When we examined inward tail currents in EGTA-
injected cells before light, we found that for the five cells tested, the decay time constant of the inward tail current followed a single exponential (Fig. 7). The time constant was $10.0 \pm 2.0 \text{ ms (SD)}$ as compared with $5.0 \pm 0.5 \text{ ms (SD)}$ in uninjected cells. The extrapolated amplitude of the inward tail current at the onset of the repolarizing voltage pulse was $4.0 \pm 1.0 \text{ nA (SD)}$. This is not very different from that obtained in uninjected cells ($4.0 \pm 1.5 \text{ nA (SD)}$), but no measurements of this kind were made on the same cell before and after EGTA injection. Illumination of EGTA-injected cells reduced inward tail currents by at least 95% (Fig. 7), thus demonstrating that the maintained inward currents induced by EGTA injection are more sensitive to light than those in uninjected cells. Three of the five cells survived the treatment with EGTA/light long enough for us to determine if the inward tail currents would recover after light. For reasons that are unclear, we did not observe recovery of the inward tail currents in any of these cells.

The results described above could be explained if EGTA injection increased a voltage-dependent maintained inward current and if this maintained inward current was decreased by light. To further test this hypothesis, we exposed EGTA-injected cells to Ni$^{2+}$ in order to block inward current (Lisman et al., 1982). In EGTA-injected cells, the steady state outward current was often increased after exposure to Ni$^{2+}$ (compare Fig. 8, A and B, at arrows), as expected if a maintained inward current was blocked by Ni$^{2+}$. Furthermore, after exposure to Ni$^{2+}$, the peak amplitude of the tail current was always much smaller than the steady state outward current (Fig. 8B), as expected if the current was now due primarily to $i_k$. Finally, after exposing EGTA-injected cells to Ni$^{2+}$, light no longer increased the net outward current. These results support the hypothesis that EGTA induces a maintained inward current that can be reduced by light, and that this effect accounts for the light-induced increase in the maintained net outward current seen in EGTA-injected cells.

**DISCUSSION**

**Light Reduces Inward Current**

In a previous study (Leonard and Lisman, 1981), it was found that light can reduce the voltage-dependent maintained potassium current, $i_k$. In this paper, we present evidence that light can also reduce the voltage-dependent early inward current (Fig. 1). This decrease in the early inward current is not due to an increase in the voltage-dependent outward current, because the outward current is also reduced by light (Fig. 1).

We have also found that a component of voltage-dependent inward current is maintained for several seconds during a depolarizing voltage pulse. Indirect evidence for this was previously obtained by O'Day et al. (1982). In their experiments, the sensitivity of the photoreceptor to light was measured before and after depolarizing voltage pulses of varying duration. As the pulse duration was increased (up to 10 s), the sensitivity of the cell to light decreased, provided Ca$^{2+}$ was present in the external solution. Because the light-dependent conductance is known to be reduced by increases in intracellular Ca$^{2+}$ (Lisman and
Brown, 1975), O'Day et al. postulated that calcium channels stayed open during these long depolarizations, allowing calcium to enter the cell continuously and reduce the sensitivity to light. Our experiments demonstrate more directly that the inward current is maintained during long depolarization (Fig. 3A). Like the early inward current, the maintained inward current is blocked by Ni$^{2+}$ (Fig. 3B) and reduced by light (Fig. 4). The relative contribution of Na$^+$ and Ca$^{2+}$ to the maintained inward current is not known.

*EGTA-induced Inward Current and Its Modulation by Light*

EGTA injection appears to increase a maintained inward current, as evidenced by the reduction of the net outward maintained current after EGTA injection (Fig. 5). Exposing such cells to Ni$^{2+}$ increased the net outward current, as expected if Ni$^{2+}$ blocks a maintained inward current (Fig. 3B). A maintained inward current which, on repolarization, inactivated faster than the maintained outward current would also explain the unusual tail currents observed in EGTA-injected cells (Figs. 6A and 8A). Exposing EGTA-injected cells to Ni$^{2+}$ abolished these unusual tail currents. Interestingly, whereas in uninjected cells, light only reduced the inward tail current by ~30%, in EGTA-injected cells, light reduced the inward tail current by >95% (Fig. 7). Why this should be so is unclear. It is also unclear why the amplitudes of the inward tail current in EGTA-injected cells were similar to those in uninjected cells. If EGTA increases the inward current, one would expect an increase in the size of the inward tail current. However, it is known that some calcium currents turn off with multiple time constants, the fastest of which are below the resolution of our system (Fenwick et al., 1982; Tsuda et al., 1982). Therefore, it is possible that we could not detect the enlarged inward tail current in EGTA-injected cells.

*Mechanism of the Action of Light on Inward Current*

Work on calcium channels in other systems indicates that a component of inactivation of these channels is dependent on a rise of Ca$\text{_{i}}$ (Tillotson, 1979; Eckert and Ewald, 1982), and a similar mechanism may explain our results in *Limulus*. Thus, the reduction of both the early and the maintained inward current by light may be due to the elevation of Ca$\text{_{i}}$ by light (Brown and Blinks, 1974; Brown et al., 1977). The increase of maintained inward current produced by EGTA injection would be expected if EGTA blocks the inactivation that is dependent on the voltage-dependent rise in Ca$\text{_{i}}$. The fact that the maintained inward current seen in EGTA-injected cells can be reduced by light is less easy to explain. One would think that the high intracellular concentration of EGTA would block the light-induced rise in Ca$\text{_{i}}$. If this is the case, then light must block the EGTA-induced inward current by a calcium-independent mechanism (see, for example, Cachelin et al., 1983). Alternatively, the light-induced release of internal free calcium in these cells may be great enough to overwhelm the buffer and allow a light-induced rise in Ca$\text{_{i}}$ sufficient to reduce the inward current.

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