# CALCIUM ACTIVATES AND INACTIVATES A PHOTORECEPTOR SOMA POTASSIUM CURRENT

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ABSTRACT Light-induced currents were measured with a two-microelectrode voltage clamp of type B photoreceptor somata, which had been isolated by axotomy from all synaptic interactions as well as from all membranes capable of generating impulse activity. In artificial seawater (ASW), light elicited a transient early inward current, $I_{Na^+}$, which depended on $Na_o^+$ and had a linear current-voltage relation and an extrapolated reversal potential of 30–40 mV (absolute). In 0-$Na^+$ ASW, light elicited a transient short-latency outward current that depended on $K_o^+$, increased exponentially with more positive voltages (greater than or equal to −40 mV), and reversed at −70 to −75 mV. This outward current was not blocked by $Ca^{++}$ channel blockers (e.g., $Cd^{++}$, $Co^{++}$) or substitution of $Ba_o^{++}$, for $Ca_o^{++}$, but was reduced by iontophoretic injection of EGTA. In both ASW and 0-$Na^+$ ASW, light also elicited a delayed, apparently inward current, which was associated with a decreased conductance, depended on $K_o^+$, increased exponentially with more positive voltages (greater than or equal to −40 mV), reversed at the equilibrium potential for $K^+$ flux in elevated $K_o^+$ was eliminated by substitution of $Ba_o^{++}$ for $Ca_o^{++}$, and was greatly reduced by $Cd_o^{++}$ or $Co_o^{++}$. Thus, light elicited an early $Ca^{++}$-dependent $K^+$ current, $I_C$, and a prolonged decrease of $I_C$. Iontophoretic injection of $Ca^{++}$ through a third microelectrode caused prolonged reduction of both $I_C$ and the light-induced decrease of $I_C$, but did not alter $I_{Ca^{++}}$ or the current-voltage relation of $I_C$. Ruthenium red (1 μM) in the external medium caused a prolongation of the light-induced decrease of $I_C$. Iontophoretic injection of EGTA often eliminated the light-induced $I_C$ decrease while decreasing peak $I_C$ (during depolarizing steps to −5 or 0 mV) by less than one-half. EGTA injection, on the average, did not affect steady state $I_C$ but reduced the light-induced decrease of steady state $I_C$ to approximately one-third of its original magnitude. The prolonged $I_C$ decrease, elicited by dim light in the absence of light-induced $I_C$ or $I_{Na^+}$, was more completely eliminated by EGTA injection. It was concluded that light, in addition to inducing a transient inward $Na^+$ current, causes both a transient increase and a prolonged decrease of $I_C$ via elevation of $Ca_i^{++}$.

## INTRODUCTION

Illumination of many invertebrate photoreceptors has been shown to cause an increased inward flux of Na ions (H. M. Brown et al., 1969, 1970; Millechia and Mauro, 1969*a*, *b*). Light-induced elevation of intracellular $Ca^{++}$ has been shown to contribute to adaptation of the photoreceptor response by reducing the light-evoked inward $Na^+$ current (J. E. Brown and Blinks, 1974; J. E. Brown et al., 1977; Fein and Lisman, 1975; Alkon et al., 1982*b*). The elevation of intracellular $Ca^{++}$ has also been shown to cause long-lasting inactivation of the early voltage-dependent $K^+$ current, $I_A$, and, to a lesser extent, the late $K^+$ current(s) for *Hermissenda* photoreceptors (Alkon et al., 1982*b*), and inactivation of a delayed rectifying $K^+$ current, $I_K$, for *Limulus* photoreceptors (Chinn and Lisman, 1984).

Recent measurements with differential absorption spectrophotometry of $Ca^{++}$ complexed with the dye arsenazo III indicated that light causes a prolonged rise of intracellular $Ca^{++}$ in photoreceptors of the nudibranch mollusk *Hermissenda crassicornis*, primarily as a secondary consequence of light-induced depolarization (Connor and Alkon, 1982, 1984). The light-induced rise of internal $Ca^{++}$ measured under current-clamp conditions was voltage dependent and arose to a significant degree as the result of transmembrane flux of $Ca^{++}$ (see also Thomas and Gorman, 1977; Ahmed and Connor, 1979). A light-induced release of $Ca_i^{++}$ from intracellular stores could not be ruled out in the Connor and Alkon study, although it was clear that such a release would necessarily be responsible for far less $Ca_i^{++}$ elevation than would transmembrane flux. The transmembrane flux was subsequently shown to be due to a voltage-dependent $Ca^{++}$ current across the soma membrane of the photoreceptors (Alkon et al., 1984). Also associated with this $Ca^{++}$ current (see also Adams and Gage, 1979; Connor, 1979; Hagiwara and Byerly, 1981) was a $Ca^{++}$-dependent $K^+$ current (see Meech, 1974, 1978; Heyer and Lux, 1976; Thompson, 1977; Eckert and Tillotson, 1978) or $I_C$. Because $I_C$ showed clear inactivation, whereas the voltage-dependent $Ca^{++}$ current did not inactivate, it was suggested that elevated internal $Ca^{++}$ might directly inactivate $I_C$ in a manner analogous to $Ca^{++}$-mediated inactivation of $I_A$ (Alkon et al., 1982*b*) and the light-induced inward $Na^+$ current (Alkon et al., 1983, Alkon and Sakakibara, 1984; Alkon et al., 1984).

In the present study, we undertook to define the separate ionic currents elicited by illumination of *Hermissenda* photoreceptors. The role of $Ca^{++}$, internal and external, in the activation and inactivation of light-elicited currents was also of interest. We report here that light causes, as previously described (Alkon, 1979), an inward $Na^+$ current whose activation and inactivation characteristics are not voltage dependent. Our observations are also consistent with a light-induced release of $Ca^{++}$ from intracellular stores, which is manifest under voltage clamp and which provides the basis for a second light-induced current: a $Ca^{++}$-dependent outward $K^+$ current (see also Alkon, 1979). This light-induced outward $K^+$ current has now been characterized at the single channel level with "cell-attached" and "inside-out" patch-clamp recordings from the Type B soma membrane (Sakakibara et al., manuscript submitted for publication). Evidence is also presented here that a light-induced rise of intracellular $Ca^{++}$ is responsible for a delayed but prolonged inactivation of $I_C$.

## METHODS

### Cell Preparation

All experiments were performed on type B cell somata, which had been isolated (as previously described in Alkon, 1979) by axotomy from all synaptic interaction as well as from any impulse-generating (i.e., active) membranes. Preparations were immobilized on a glass slide by the weight of stainless-steel pins whose ends were embedded in vaseline (cf. Alkon, 1975). Before impalement with microelectrodes, a thin connective tissue sheath was digested by incubation in protease (type VII; Sigma Chemical Co., St. Louis, MO) solution (1 mg/ml) for 1–10 min at 22°C.

### Voltage Clamp

Voltage clamp was effected by the insertion of two microelectrodes filled with 3 M KCl, made from thick-walled capillary glass (6020; A-M Systems, Everett, WA). The microelectrode used for the injection of current had a resistance of 10–15 MΩ. The microelectrode used for measuring the intracellular voltage had a resistance of 20–25 MΩ. A current-to-voltage converter was used to ground (via the Ag/AgCl wire) the perfusion chamber as well as to measure membrane current. The capacitative transient of the voltage-clamp current records settled within 5–12 ms. Command voltage steps occurred with a rise time of ~0.25 ms.

The resting potential measured after the insertion of either microelectrode tended to range between −40 and −55 mV. Cells with resting potentials found to be more positive than −35 mV by measurement with the first electrode were rejected. Cells with resting potentials found to be more positive than −30 mV by measurement with two microelectrodes were also rejected, as were cells with a >10 mV difference between the resting potentials recorded by the two microelectrodes. Holding currents for a resting potential of −60 mV were typically between −0.5 and −2.0 nA. Cells were not accepted when holding currents exceeded −5.0 nA with 10mM $K^+$ in the bathing medium.

### $Ca^{++}$ Injection

For these experiments, an additional microelectrode (separate from the two used for voltage clamp) was inserted into the cell. A separate amplifier and current-passing circuit permitted recording of intracellular voltage and injection of current. This electrode was filled with 0.8 M $CaCl_2$ and 0.5 M KCl. The 0.5 M KCl reduced the resistance of the electrode and thus minimized extraneous noise and improved stability. For $Mg^{++}$ injection, an electrode (separate from the two used for voltage clamp) filled with 1.0 M $MgCl_2$ was used.

### Experimental Conditions

Cells maintained at 20–22°C were bathed in artificial seawater (ASW) with the following composition: 430 mM $Na^+$, 10 mM $K^+$, 50 mM $Mg^{++}$, 10 mM $Ca^{++}$, 10mM Tris buffer (pH 7.4). In test solutions in which $Na^+$ was not included, tetramethylammonium ion ($TMA^+$) was added to effect approximately equal osmolarity (870–890 mOsmol). When external $K^+$ was elevated to 300 mM, no $TMA^+$ was added. When 10 mM $Ba^{++}$, 10 mM $Cd^{++}$, or 10 mM $Co^{++}$ was used, no $Ca^{++}$ was included. The solution containing 300 mM $K^+$, 100 mM $Ca^{++}$, and 100 mM tetraethylammonium ion ($TEA^+$) was somewhat hyperosmotic (~1,000 mOsmol). Solutions in the perfusion chamber (with a volume of ~1.0 ml) were exchanged by 7–10 sequential washes, using at least 10.0 ml of each test solution. EGTA electrodes were filled with 1.0 M $Na_4$ EGTA; the pH was adjusted to 7.4 with NaOH.

### Measurements

Data were quantitated from records made on a Brush chart recorder (Gould Instruments Inc., Cleveland, OH). The settling time was checked on a storage oscilloscope (Tektronix, Inc., Beaverton, OR). All potentials are given in absolute terms (i.e., the inside of the cell is negative with respect to ground); outward currents are represented as having positive value. Leak currents for each test potential were obtained by extrapolation using a current-voltage relation established with small positive and negative potential changes (±10, ±20 mV) from a holding potential of −60 mV. Currents recorded under voltage clamp at each test potential were then corrected by subtracting the appropriate extrapolated leak current value. The extrapolated leak current values for large (≥50 mV) positive voltage commands agreed well with the values measured for equal commands of opposite (i.e., negative) sign. All treatment conditions were repeated for type B cells from at least four different animals. The results presented are typical of those consistently obtained with such repetition.

### Illumination

Light stimuli were provided by a tungsten 6-V, 15-W bulb (N. V. Philips, Eindhoven, The Netherlands). The light source emitted only a small percentage of its light at or near 510 nm, at which a photosensitive peak had previously been identified for type B cells (Alkon, 1976). Thus, at higher light intensities ($>10^2$ ergs/cm$^{-2}$ · s$^{-1}$), progressively greater stimulation at 510 nm occurred, whereas at lower intensities ($10^1$–$10^2$ ergs/cm$^{-2}$ · s$^{-1}$), stimulation at longer wavelengths (≥630 nm) was effected. After 10 min of dark adaptation, light stimuli were repeated at 2-min intervals to maintain a constant state of light adaptation of the photoreceptors.

## RESULTS

### Light-induced $I_{Na^+}$

Illumination of the type B cell in ASW elicited an inward current (Fig.1), which occurred with a latency of 100–200 ms, reached its peak amplitude within 0.5–0.75 s, and inactivated to half its peak amplitude within 1–1.5 s. The amplitude of this inward current increased (Fig. 1) with progressively more negative holding potentials (less than or equal to ~20 mV), and decreased with lower external $Na^+$ concentrations in the bathing medium. Extrapolation of the current-voltage relation (Fig. 2 *A*) for this inward

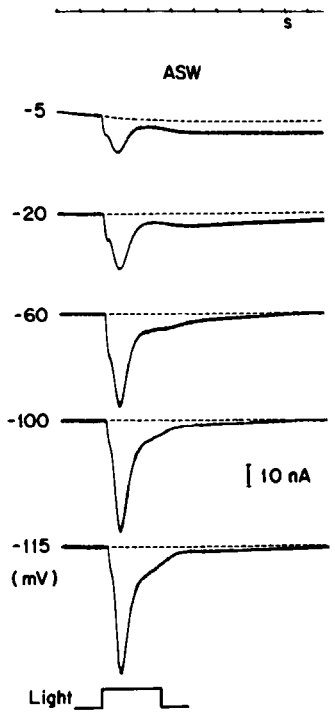


FIGURE 1 Light-induced inward $Na^+$ current. Light steps ($10^4$ ergs/$cm^2$ · s), presented at 2-min intervals after 10 min dark adaptation, elicited an inward current of constant amplitude at a holding potential of −60 mV. When the light occurred 5 s after the onset of a 20-s depolarizing command step, the peak amplitude of the large initial phase of the inward current ($I_{Na^+}$) became progressively smaller as the depolarizing command became more positive. A small, delayed (≥8 s from light onset) phase of the inward current, however, became larger with more depolarization. A delayed inward current at ≤8 s from light onset, apparent at more negative holding potentials, may represent residual $I_{Na^+}$. The biphasic nature of the $I_{Na^+}$ onset may be due to the presence of a second opposing light-induced current, $I_C$, described below.

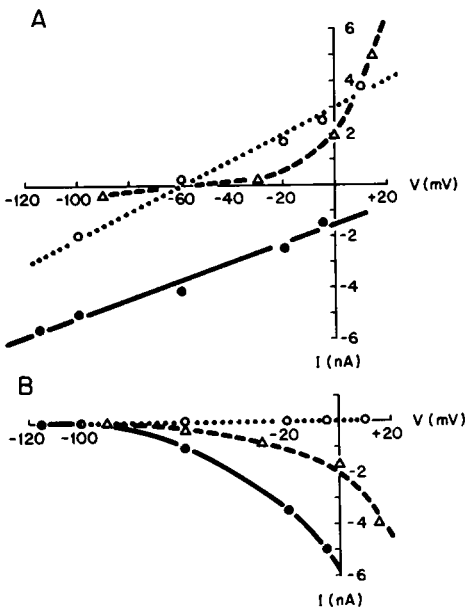


FIGURE 2 Current-voltage relations of light-induced currents of type B photoreceptor. (*A*) Early light-induced increase of inward $Na^+$ current and outward $K^+$ current (obtained for peak currents) in ASW (●), O $Na^+$-ASW (Δ), and $Ba^{++}$,-O $Na^+$-ASW (○). Note that the light-induced inward $Na^+$ current in ASW and the light-induced outward $K^+$ current in $Ba^{++}$, 0-$Na^+$ ASW are linear functions of voltage. In 0-$Na^+$ ASW, the light-induced outward $K^+$ current increases nonlinearly with increasingly positive voltage. (*B*) Delayed light-induced decrease of steady state, voltage-dependent $K^+$ current. In ASW (●) and 0-$Na^+$ ASW (Δ), the light-induced decrease (measured as isochronal values 9 s after light onset) is a nonlinear function of voltage. Replacement of $Ca^{++}$ with $Ba^{++}$ in the external medium (○) eliminates the light-induced reduction of $K^+$ current. Note that values for different conditions were not necessarily obtained with the same cell. Symbols are the same as in *A*.

current yielded an apparent reversal potential of +30–40 mV (absolute). Because another, usually much smaller, light-induced current also occurs while $I_{Na^+}$ reaches its maximum value (see below), this reversal potential for $I_{Na^+}$ must be considered a rough approximation.

## Light-induced $I_C$ ($Ca^{++}$-dependent $K^+$ Current)

In the absence of external $Na^+$ (i.e., after substitution in ASW with equimolar $TMA^+$), illumination of the type B cell elicited an outward current (Fig. 3), which occurred with a latency of 150–200 ms, reached its peak amplitude within 0.3–0.6 s, and inactivated to half its peak amplitude within 0.6–1.0 s. The amplitude of this outward current increased (Fig. 3) with progressively more positive holding potentials (greater than or equal to approximately −60 mV). The reversal potential of this light-induced outward current obtained from its current-voltage relation (Fig. 2 *A*) occurred at −70 to −75 mV (absolute).

The magnitude of this light-induced outward current depended on the concentration of $K^+$ ions in the external

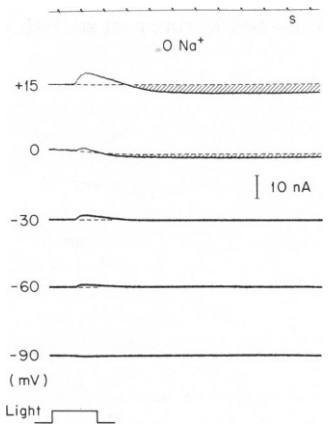


FIGURE 3 Light-induced $K^+$ currents. In 0-$Na^+$ ASW, light steps ($10^4$ ergs/$cm^2$ · s), presented at 2-min intervals after 10 min dark adaptation, elicited little or no current at holding potential of −60 mV. When the light occurred 5 s after the onset of a 20-s depolarizing command step, an initial outward current and a delayed inward current were elicited with commands more positive than −30 mV. With progressively more positive commands, both the outward and inward currents became more apparent. The dashed line indicates the current elicited by the command steps in the absence of light stimuli.

bathing medium. Elevation of external $K^+$ from 10 to 300 mM in 0-$Na^+$ ASW, for example, caused the light-induced current to be inward (Fig. 4) at all potentials $\leq 0$ mV (absolute). By measuring the reversal potential of the early transient $K^+$ current, $I_A$, in 300 mM $K^+$, 0-$Na^+$ ASW, it was possible to show (as previously, in Alkon and Sakakibara, 1984; Alkon et al., 1984) that the equilibrium potential for $K^+$ flux was 0 mV (which was also the level of resting membrane potential in 300 mM $K^+$). Thus, the major light-induced current in 300 mM $K^+$, 0-$Na^+$ ASW had the same reversal potential as did $I_A$, and the same dependence on the external $K^+$ concentration. At 0 mV in 300 mM $K^+$, 0-$Na^+$ ASW, the reversal potential for $K^+$ flux; there was no light-induced current observable (Fig. 4 *A*). These results were consistent with light causing an outward $K^+$ current.

Several experiments provided support for the hypothesis that the light-induced outward $K^+$ current arose (under voltage clamp) from the elevation of intracellular $Ca^{++}$ caused by the light-induced release from intracellular stores. First, since at 0 mV (in 300 mM $K^+$, 0-$Na^+$ ASW), the reversal potential for $K^+$ flux, there was no light-induced current observable (Fig. 4), a light-induced transmembrane flux of $Ca^{++}$ was considered unlikely. Furthermore, the addition of 2 mM 4-aminopyridine (4-AP) and 100 mM $TEA^+$ or the addition of 5–10 mM $Cd^{++}$ to the external bathing medium or the substitution of 10 mM $Ba^{++}$ (Fig. 5) for external $Ca^{++}$ did not eliminate or markedly affect the light-induced outward $K^+$ current. Since the substitution of $Ba^{++}$ for $Ca^{++}$ was previously shown to result in the elimination of the voltage-dependent $I_C$ (Alkon et al., 1984), a light-induced $Ba^{++}$ flux, which would liberate $Ca_i^{++}$, could also be ruled out. Iontophoretic injection of EGTA did, however, reduce this current. Thus, the light-induced outward $K^+$ current probably arose from

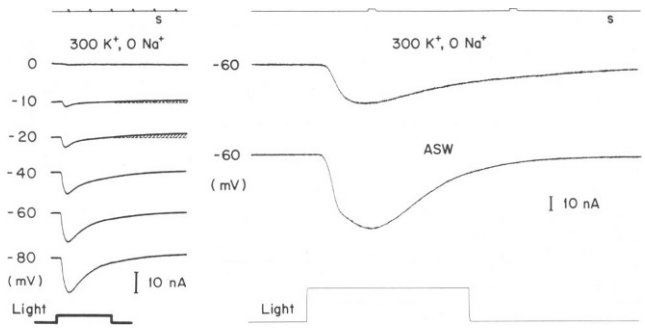


FIGURE 4 Reversed light-induced outward $K^+$ current. With 300 mM $K^+$ and 0 $Na^+$ in the external medium, the early light-induced outward $K^+$ current became inward for all command depolarizations (see Figs. 1 and 2 for conditions of light and command steps) of <0 mV absolute. 0 mV was previously determined to be the equilibrium potential for $K^+$ flux. Note that the delayed light-induced inward current has become outward in 0-$Na^+$, 300 mM $K^+$ ASW. The dashed line indicates the current elicited by the command steps in the absence of light stimuli. The lower record on the right shows the light-induced current, largely $I_{Na^+}$ in normal artificial sea water (ASW).

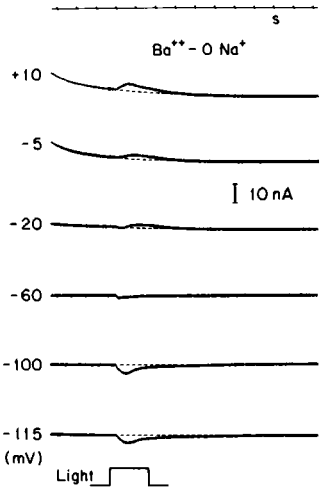


FIGURE 5 Light-induced outward $K^+$ current in $Ba^{++}$, 0-$Na^+$ ASW. Substitution of 10 mM $Ba^{++}$ for $Ca^{++}$ in 0-$Na^+$ ASW did not eliminate the light-induced outward current but did eliminate the delayed light induced inward current. See Figs. 1 and 2 for conditions of light and command steps. Other than the outward current (measured above the dashed lines), light caused no deviation from the current elicited by the command steps in the absence of light stimuli. At potentials $\leq -30$ mV a small residual $I_{Na^+}$ may be present.

the elevation of $Ca_i^{++}$ caused by the intracellular release of $Ca^{++}$ rather than a light-induced flux of $Ca^{++}$ across the membrane. It should be noted that the current-voltage relation (Fig. 2 *A*) of the light-induced outward current was linear over the voltage range examined in $Ba^{++}$, 0-$Na^+$ ASW, and was nonlinear in 0-$Na^+$ ASW with normal $Ca_o^{++}$. This nonlinearity may be due to the interaction of the light-induced release of $Ca^{++}$ with a voltage-dependent (as opposed to light-induced) flux of $Ca^{++}$ (Alkon et al., 1984) across the membrane (see below).

It should also be pointed out that the $I_{Na^+}$ and $I_C$ currents in Fig. 2 *A* were not measured for the same cell. Typically, $I_{Na^+}$ (see Fig. 1) was much larger than the light-induced $I_C$ (see Fig. 3) at potentials less than or equal to $-5$ mV, from which the $I_{Na^+}$ reversal potential was extrapolated.

## Light-induced Decrease of $K^+$ Current

In ASW, a delayed component of the inward current was apparent in the response of the type B photoreceptor to light (Fig. 1). This delayed inward current was sustained during a prolonged light step and persisted for many seconds after the cessation of light (for both brief and longer steps). The magnitude of the delayed light-induced inward current increased (Fig. 1) with depolarizing commands more positive than $-60$ mV (the holding potential). From the current-voltage relation (Fig. 2 *B*), this increase with more positive current steps was markedly nonlinear. The delayed light-induced current was still apparent in the absence of external $Na^+$ in the bathing medium (Fig. 3) and was altered by changes of external $K^+$ concentration. In 300 mM $K_o^+$, for example, at potentials more negative than 0 mV (the new $K^+$ equilibrium potential), the delayed

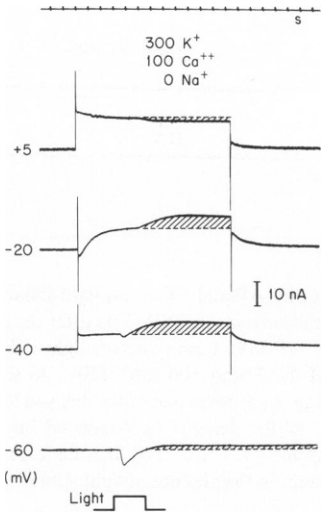


FIGURE 6 Light-induced currents in 100 mM $Ca^{++}$, 300 mM $K^+$, 0-$Na^+$ ASW. The delayed light-induced inward current in ASW is manifest as a delayed light-induced outward current for command depolarizations to potentials ≤0 mV in 100 mM $Ca^{++}$, 300 mM $K^+$, 0-$Na^+$ ASW. At potentials >0 mV, the $K^+$ equilibrium potential, the delayed light-induced current becomes inward. Note that the magnitude of the delayed light-induced current becomes greater relative to the magnitude of early light-induced $K^+$ current when external $Ca^{++}$ is elevated from 10 to 100 mM (compare Fig. 3 with records shown here). See Figs. 1 and 2 for conditions of light stimulation. The dashed lines indicate the current elicited by the command steps in the absence of light stimuli.

light-induced inward current became outward (Figs. 4 *A* and 6). At ~0 mV in 300 mM $K^+$, there was no delayed light-induced current (Fig. 4 *A*). At potentials more positive than 0 mV, the delayed light-induced current became inward. This reversal was particularly apparent when the delayed light-induced current in 300 mM $K_o^+$ was increased by elevating external $Ca^{++}$ from 10 to 100 mM (Fig. 6). Thus, in the absence of external $Na^+$, the reversal potential of the delayed light-induced inward current was that of the $K^+$ flux. Since in ASW $K^+$ currents tend to flow out of the type B cell through open $K^+$ channels at potentials greater than approximately −70 mV (the $K^+$ equilibrium potential), we reasoned that the delayed light-induced inward $K^+$ current must represent a decrease of $K^+$ current. A light-induced decrease of $K^+$ current is consistent with two previous observations: namely, that light reduces a late voltage-dependent $K^+$ current (see Fig. 7 and Alkon et al., 1982*a*, 1984), and that the input resistance remains markedly increased for many seconds after light stimulation of the type B photoreceptor (Alkon and Grossman, 1978). The persistence of the light-induced reduction is apparent in Fig. 7. This figure illustrates the reduction of the late current for at least 80 s after a light step presented in the absence of depolarization. Thus, the light-induced decrease of $K^+$ current does not require any interaction with the effects of prolonged membrane depolarization.

A comparison of the slopes of current-voltage relations determined by tail-current measurements immediately after a command depolarization, with and without a 2.0-s light step, showed that the delayed light-induced $K^+$ current is associated with an increased input resistance (Fig. 8). These measurements were made 15 ms after the depolarizing command because of the slow settling time of the voltage clamp used. The reversal potentials (approximately −80 mV absolute) for these tail currents were the same (Fig. 8) with or without a light step (whose offset preceded the depolarization offset by 3.0 s). Similar reversal potentials indicate that preceding light reduced the major current flowing during the command depolarization (i.e., outward $K^+$ current). The light-induced $I_{Na^+}$ was transient and was not appreciable during the tail current measurement. $I_{Ca^{++}}$ was small (1–2 nA peak amplitude at 0 mV absolute) compared with the voltage-dependent outward $K^+$ current (usually 10–15 nA), and for potentials of less than −20 mV absolute, did not measurably contribute to the tail currents.

## $I_C$ Is the $K^+$ Current Decreased by Light

To determine the nature of the voltage-dependent $K^+$ current, which was decreased by illumination, specific $K^+$ currents previously measured across the type B soma

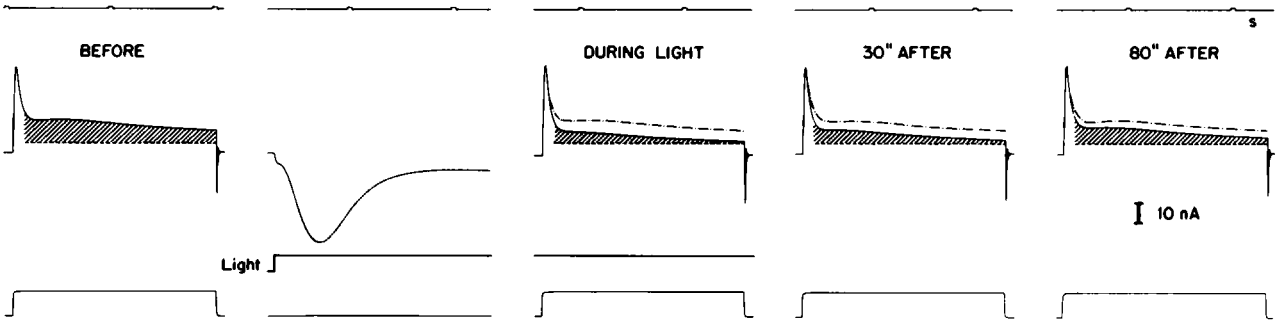


FIGURE 7 Effect of light on voltage-dependent outward $K^+$ currents. Depolarizing command steps occurring at intervals of ≥30 s elicit an early outward $K^+$ current ($I_A$) and a late $K^+$ current. A 5-s light step ($10^4$ ergs/cm$^2$ · s), presented while maintaining a holding potential of −60 mV, drastically reduces the late $K^+$ current (predominantly $I_C$), while only slightly affecting $I_A$. This reduction persists for more than 80s. The dashed lines indicate the level of late $K^+$ current preceding the light step.

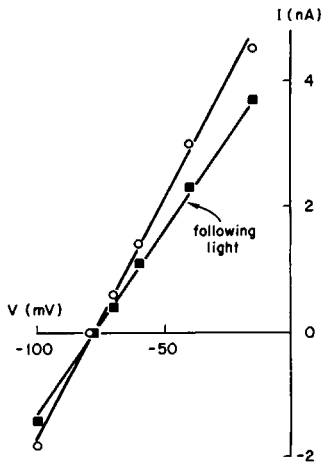


FIGURE 8 Current-voltage relations of tail currents with and without preceding light step. Command depolarizations (200 ms) to 0 mV were followed (with a 10-μs delay) by command steps to a range of potential levels. Tail currents were measured by taking isochronal values (15 ms after offset of the command depolarization). These same measurements were repeated with each command depolarization being preceded (by 4.0 s) by a 1.0-s light step (~$10^4$ ergs/cm$^2$ · s). Note that the slope difference between the two sets of measurements indicates a light-induced decrease of conductance to a common ion ($K^+$) with a reversal potential of approximately −80 mV.

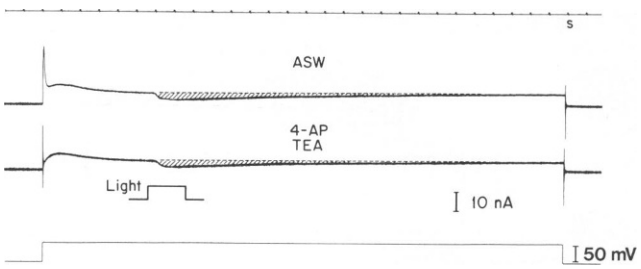


FIGURE 9 Effect of 4-AP and $TEA^+$ on light-induced currents. Upper record: light-induced currents in ASW 5.0 s after the onset of a command depolarization to −10 mV. Lower record: light-induced currents after addition of 2 mM 4-AP and 100 mM $TEA^+$ to the ASW. Note the elimination of $I_A$ and slight reduction of the delayed $K^+$ current with only a small reduction of the delayed light-induced inward current. Light intensity: $10^4$ ergs/cm$^2$ · s. The dashed lines indicate the current elicited by the command steps in the absence of light stimuli.

membrane (Alkon et al., 1982*b*, 1984) were eliminated or reduced by pharmacologic blocking agents or ion substitution. When, for example, $I_A$ was eliminated by 2 mM 4-AP, the delayed light-induced inward current was only slightly reduced. The elimination of the delayed rectifying $K^+$ current, which was shown to be only slightly activated at potentials ≤0 mV (Shoukimas, J. J., and D. L. Alkon, unpublished observations; Alkon et al., 1984), by the addition of 100 mM TEA also only slightly reduced the delayed light-induced inward current (Fig. 9). (It should be noted that some small blocking effect on $I_C$ caused by prolonged exposure to TEA cannot be ruled out.) The substitution of 10 mM $Ba^{++}$ for 10 mM $Ca^{++}$ in the external bathing medium, however, virtually eliminated the delayed light-induced current (Fig. 5). Because the substitution of $Ba^{++}$ for $Ca^{++}$ was previously found (Alkon et al., 1984) to eliminate the Ca-dependent $K^+$ current, $I_C$, and because the major component of the late $K^+$ current is $I_C$ (Alkon et al., 1984), we concluded that illumination of the type B photoreceptor is followed by a substantial and prolonged reduction of $I_C$ during the delayed light-induced "apparent" inward current. The effects of agents (e.g., $Cd^{++}$, $Co^{++}$) that block voltage-dependent $Ca^{++}$ channels were consistent with this conclusion. In the presence of these $Ca^{++}$ channel blockers, the delayed light-induced current was decreased. That it persisted at all, however, could be due to a steady state $I_C$ caused by intracellular $Ca^{++}$ levels present at the resting potential of the cell and/or to $K^+$ currents not affected by $Ca^{++}$ current blockers (such as $I_A$ and the delayed rectifier).

## Elevated $Ca_i^{++}$ Causes a Light-induced Decrease of $I_C$

Previous studies have shown that $I_{Ca^{++}}$, the voltage-dependent $Ca^{++}$ current, does not inactivate, whereas $I_C$, the voltage- and $Ca^{++}$-dependent $K^+$ current, does inactivate (Alkon et al., 1984). It was also previously shown that $I_C$ inactivation by light (Fig. 7) can occur in the absence of prolonged depolarization (Alkon et al., 1984). $I_C$ elicited by a brief command depolarization remains reduced for many seconds after a light step (e.g., 2 s) presented while the membrane potential is maintained at −60 mV (Fig. 7). However, light, as described above, does not affect $I_{Ca^{++}}$. An effect on the type B cell common to both light presentation and depolarization is elevation of $Ca_i^{++}$. Light-induced inactivation of $I_C$ therefore, might result from a light-induced rise of intracellular $Ca^{++}$. To demonstrate that in fact a light-induced rise of $Ca_i^{++}$ mediates prolonged $I_C$ inactivation, three different means of altering $Ca_i^{++}$ were used: $Ca^{++}$ injection, inhibition of mitochondrial uptake of $Ca_i^{++}$ by ruthenium red (Moore, 1971), and chelation of $Ca_i^{++}$ by EGTA. For each treatment, effects on the delayed light-induced current, the voltage-dependent $Ca^{++}$ current, $I_{Ca^{++}}$, and the voltage-dependent $I_C$ were measured.

*$Ca^{++}$ Injection.* $Ca^{++}$ was injected iontophoretically through one microelectrode under voltage-clamp control effected by two other microelectrodes. The injection of +2.0 nA for 1 min through the $Ca^{++}$ microelectrode caused a 20–50% reduction of $I_C$, lasting for several minutes or longer (Fig. 10; Table I). The injection of larger currents of longer duration (e.g., 2–4.0 nA for 2.5 min) caused a reduction of $I_C$ that often lasted for the remainder of the recording period (25–40 min).

$Ca^{++}$ injection also produced another clear effect. The light-induced reduction of $I_C$ was either diminished (Fig. 10) or eliminated after $Ca^{++}$ injection. $Ca^{++}$ injection also

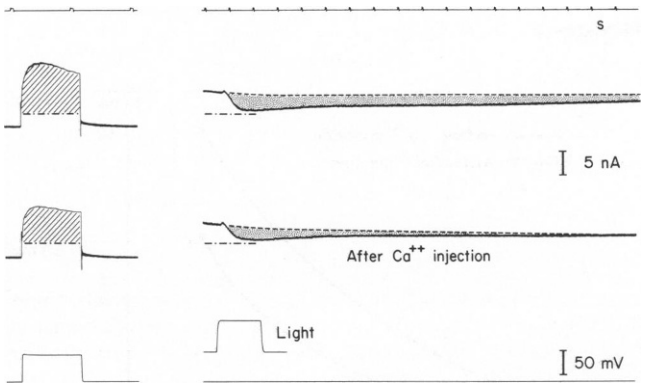


FIGURE 10 Effect of $Ca^{++}$ injection on voltage- and light-dependent currents. In 3 mM 4-AP, the delayed outward current is largely $I_C$ (see Alkon et al., 1984). Iontophoretic injection of $Ca^{++}$ (+2.0 nA, 2 min) under isopotential conditions reduces $I_C$ (records on left) and reduces the light-induced decrease of $I_C$ produced by a voltage step (records on right). The dot-dashed line indicates level of "leak" or non–voltage-dependent current. These effects entirely reversed within 7 min of the injection. Since $I_{Ca^{++}}$ across the type B soma membrane is insensitive to the same $Ca^{++}$ injection (Fig. 11), injected $Ca^{++}$ is affecting $I_C$ directly.

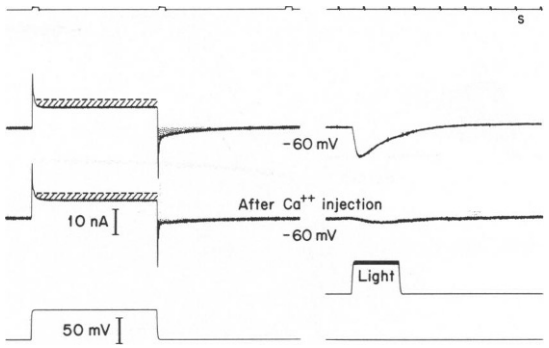


FIGURE 11 Effect of $Ca^{++}$ injection on voltage-dependent $Ca^{++}$ current, $I_{Ca^{++}}$. In 300 mM $K_0^+$ ASW, the small inward current, $I_{Ca^{++}}$, can be measured in isolation of $K^+$ currents at 0 mV absolute, the new reversal potential for $K^+$ flux. Voltage-dependent $K^+$ current is manifest as a tail current after offset of the command depolarization. This tail current, but not $I_{Ca^{++}}$, is reduced after iontophoretic injection of $Ca^{++}$ (+2.0 nA for 2 min) under isopotential conditions. The dashed line indicates level of "leak" or non–voltage-dependent current. Records on right show reduction of light-induced $I_{Na^+}$ after $Ca^{++}$ injection.

markedly shortened the duration of the light-induced decrease of $I_C$ (Table I). $Ca^{++}$ injection, however, had little or no effect on the voltage-dependent $I_{Ca^{++}}$ (Fig. 11; Table I), nor did it change the current-voltage relations of $I_C$ (Fig. 12). Thus, the effects (on $I_C$ and light-induced $I_C$ reduction) of an elevated $Ca^{++}$ caused by injection cannot be accounted for by an effect on $I_{Ca^{++}}$, as has been well documented for other neurons (Tillotson and Horn, 1978; Tillotson, 1979; Brehm et al., 1980; Eckert and Ewald, 1981; Eckert et al., 1981; Eckert and Tillotson, 1981). These results are consistent with the previous observation (Alkon et al., 1982*b*) that intracellular iontophoretic injection of $Ca^{++}$ under voltage clamp causes a prolonged reduction of the late voltage-dependent $K^+$ current (as well as $I_A$). It is important to point out that injection of $Mg^{++}$ under voltage clamp did not affect the voltage-dependent $K^+$ currents, although it did markedly and irreversibly reduce the light-induced $I_{Na^+}$ (Alkon, 1982*b*). Finally, it should be noted that an initial increase of $I_C$ shortly after the onset of $Ca^{++}$ injection (as was indicated by the results of a previous experiment by Grossman et al., 1981) was not examined in the present study. A brief injection period (e.g., 0.5–1.0 s) rather than the long period (1–2 min) used here would probably be necessary.

TABLE I
EFFECTS OF $Ca^{++}$ INJECTION

| Cell | $I_C$ at −5 mV* | | Light-induced $I_C$ ($t_{1/2}$)‡ | |
|---|---|---|---|---|
| | Before | After | Before | After |
| | *nA* | *nA* | | |
| 1 | 11.5 | 6.8 | 14.5 | 0 |
| 2 | 10.6 | 8.8 | 14.6 | 4.7 |
| 3 | 10.3 | 6.5 | 11.2 | 5.1 |
| 4 | 12.0 | 8.0 | | |
| 5 | 10.0 | 5.5 | | |
| Mean | 10.9 | 7.1§ | 13.4 | 3.3‖ |
| SE | 0.75 | 1.16 | 1.58 | 2.32 |
| Cell | $I_{Ca^{++}}$ at −5 mV¶ | | | |
| | Before | After | | |
| 6 | −3.0 | −3.0 | | |
| 7 | −3.0 | −2.9 | | |
| 8 | −2.8 | −2.8 | | |
| 9 | −2.2 | −2.0 | | |
| Mean | −2.75 | −2.67 | | |
| SE | 0.4 | 0.5 | | |

*Peak current values (*nA*) in 3 mM 4-AP, 100 mM TEA ASW.
‡Time in seconds for light-induced decrease of $I_C$ to recover from one-half maximum value.
‖Significantly different from pre-$Ca^{++}$ injection value at $p < 0.01$ by one tailed $t$ test.
§At $p < 0.001$.
¶Peak current values (*nA*) in 0-$Na^+$, 300-$K^+$ ASW.

*Ruthenium Red.* Perfusion of the type B cell with ruthenium red (10 $\mu$M) in ASW for at least 5 min caused, on the average, a slight increase (Table II) of $I_C$. Prolongation of the light-induced reduction of $I_C$, however, was a more reliable and clear effect of the ruthenium treatment (Fig. 13; Table II). These results are consistent with the interpretation that light-induced $Ca_i^{++}$ elevation, enhanced by block of mitochondrial uptake, causes a transiently increased activation of $I_C$ and a more prolonged inactivation of $I_C$. No ruthenium effects on $I_{Ca^{++}}$ were observed.

*EGTA Injection.* EGTA was iontophoresed with negative current injection (−2.0 nA for 2 min) through an electrode (filled with 1.0 M EGTA) while

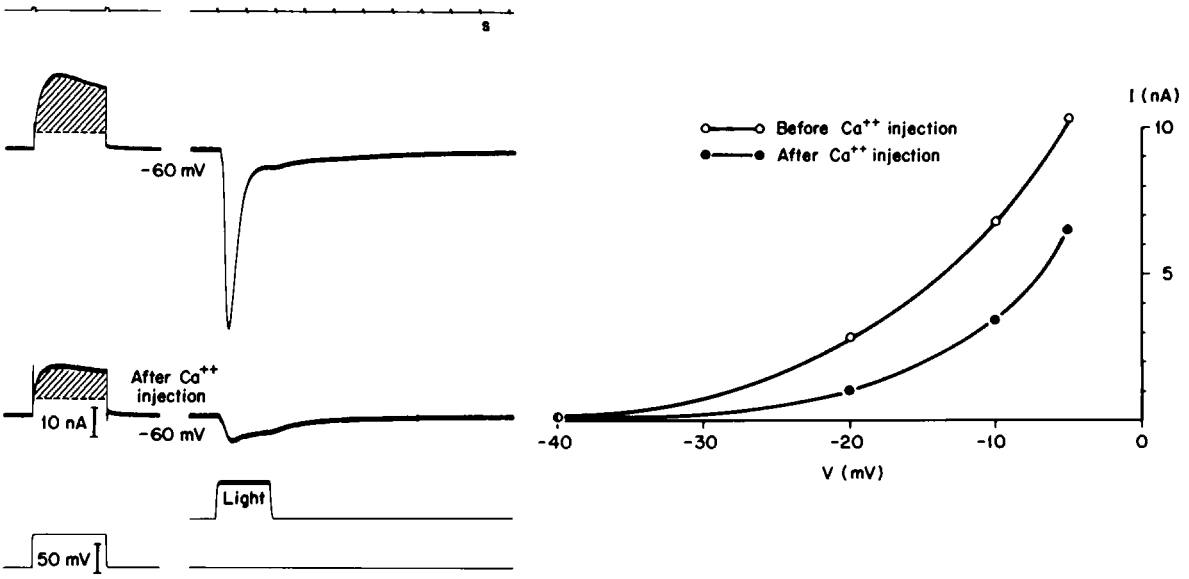


FIGURE 12 Voltage dependence of $I_C$ before and after $Ca^{++}$ injection. Records (on left) show reduction of voltage-dependent outward $K^+$ current (largely $I_C$) as well as the reduction of the light-induced inward $Na^+$ current. Note that (from the *I-V* relations depicted on the right) although the magnitude of $I_C$ (measured in 4-AP ASW) is reduced, its voltage dependence is unchanged after iontophoretic injection of $Ca^{++}$ (+2.0 nA for 2 min) under isopotential conditions.

maintaining isopotentiality by injecting equal but opposite current through a second electrode in the type B soma. EGTA injection consistently reduced the magnitude of the delayed light-induced current (Fig. 14; Table III). This effect of EGTA injection on this light-induced current occurred in ASW or 0-$Na^+$ ASW and thus was independent of $Na^+$ flux across the membrane. For 6 of 13 cells studied (cells 15–20, Table III), EGTA injection reduced peak $I_C$ (at −5 or 0 mV) by ~50% or less. However, for these same six cells, EGTA injection entirely eliminated the light-induced reduction of $I_C$. For these six cells, a single EGTA injection had a far greater effect on the light-induced decrease of $I_C$ than on the voltage- (and thus $Ca^{++}$-) dependent increase of $I_C$.

TABLE II
EFFECTS OF RUTHENIUM RED

| Cell | $I_C$ at −5 mV* | | Light-induced $I_C$ ($t_{1/2}$)‡ | |
|---|---|---|---|---|
| | Before | After | Before | After |
| | *nA* | *nA* | | |
| 10 | 9.0 | 8.6 | 9.0 | 12.1 |
| 11 | 9.3 | 12.8 | 6.0 | 7.8 |
| 12 | 15.5 | 15.1 | 7.7 | 10.1 |
| 13 | 6.4 | 9.8 | 5.7 | 10.6 |
| 14 | 6.3 | 7.3 | 5.2 | 7.2 |
| Mean | 9.3 | 10.7 | 6.7 | 9.6§ |
| SE | 3.34 | 2.85 | 1.42 | 1.82 |

*Peak current values (*nA*).
‡Time in seconds for light-induced decrease of $I_C$ to recover from one-half maximum value.
§Significantly different from pre-ruthenium value at $p < 0.025$ by one tailed *t* test.

The difference between EGTA effects on voltage-dependent $I_C$ activation and light-induced decrease of $I_C$ is even more apparent when steady state currents are considered. Steady state $I_C$ (measured 5.0 s after onset of a depolarizing command) was often not significantly reduced by EGTA injection (cf. Table III and Fig. 17), but light caused substantially less reduction of this steady state $I_C$ after EGTA injection. Thus, for almost the same steady state $I_C$, light caused a much smaller decrease after EGTA injection.

The ratio of steady state $I_C$ to peak $I_C$ provides an index of the degree of inactivation that occurs during the command depolarization. This ratio increased, i.e., the degree of inactivation decreased, after EGTA injection. (This effect is implicit in the comparison of EGTA's clear reduction of peak $I_C$ with no significant reduction of steady

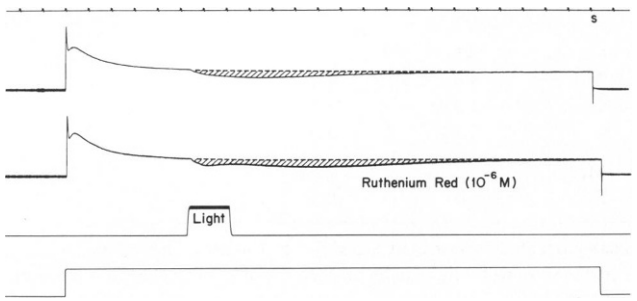


FIGURE 13 Effect of perfusion with ruthenium red on light-induced decrease of $I_C$. Addition of ruthenium red ($10^{-5}$ M) to external ASW causes a prolongation of light-induced decrease of $I_C$ (indicated by shaded area) with little effect on $I_C$ magnitude. See Figs. 1 and 2 for conditions of light stimulation.

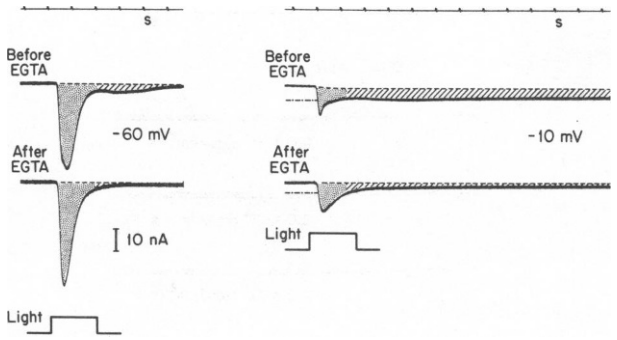


FIGURE 14 EGTA injection reduces the delayed light-induced current. (*A*) Light-induced current at −60 mV before (upper record) and after (lower record) intracellular injection of EGTA (−2.0 nA for 2 minutes). Note that the light-induced inward $Na^+$ current is increased after injection, whereas the delayed inward component is reduced. (*B*) Light-induced current at −10 mV before (upper record) and after (lower record) intracellular injection of EGTA. Note that the light-induced inward $Na^+$ current is slightly increased and prolonged, whereas the delayed inward component is reduced after EGTA injection. All light steps ($10^4$ ergs/cm$^2$ · s) occurred at 2-min intervals after 10 min of dark adaptation. The light steps in *B* occurred at 5.0 s after the onset of command depolarization to −10 mV. The dashed lines indicate the current elicited by the command steps in the absence of light stimuli. The dot-dashed lines indicate level of non–voltage-dependent (or "leak") current.

state $I_C$.) This last result suggests that EGTA reduces inactivation of $I_C$ due to $Ca_i^{++}$ elevation resulting from an inward flux of $Ca^{++}$ through voltage-dependent $Ca^{++}$ channels.

With the light source used here (see Methods), dim illumination ($10\text{-}10^2$ ergs/cm$^2$ · s) caused a delayed decrease of $K^+$ current in the absence of any observable increase of $K^+$ or $Na^+$ current (Figs. 15 and 16). Again, this observation is consistent with light-released $Ca_i^{++}$, at lower concentrations, only causing reduction of $I_C$, and with greater light intensities, and thus higher concentrations of $Ca_i^{++}$, causing some initial activation of $I_C$. During steady depolarization (e.g., to −10 or −5 mV absolute) $I_C$ activation appears to be less sensitive to small increments of $Ca_i^{++}$ than $I_C$ inactivation. As might be expected, EGTA elimination of light-induced decrease of $I_C$ was always more complete when lower light intensities were used (Figs. 17 and 18).

## DISCUSSION

### Light-induced Currents

Our results are consistent with the interpretation that illumination of the *Hermissenda* photoreceptor has at least two primary biophysical effects: an increased inward flux of $Na^+$ and elevation of intracellular $Ca^{++}$ caused by release from intracellular stores. The light-induced release of intracellular $Ca^{++}$ can, in turn, be responsible for a number of secondary effects, including an increased outward flux of $K^+$ and a delayed, prolonged decrease of outward $K^+$ flux. Another secondary effect of light-induced $Ca^{++}$ elevation, the reduction of light-induced inward $Na^+$ flux, has been extensively treated by other

TABLE III
EFFECTS OF EGTA INJECTION

| Cell | $I_C$ at −5 mV* | | ($C_1$) $I_C$ at 0 mV* | | ($C_2$) $I_C$ steady-state at 0 mV | | Light-induced decrease $I_C$¶ | | $C_2/C_1$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After | Before | After | Before | After |
| | *nA* | *nA* | *nA* | *nA* | *nA* | *nA* | *nA* | *nA* | | |
| 15 | 14.6 | 8.2 | 30.2 | 16.0 | 11.0 | 6.1 | 3.5 | 0 | 0.36 | 0.38 |
| 16 | 9.0 | 5.1 | 20.5 | 17.0 | 0 | 0 | 3.5 | 0 | — | — |
| 17 | 7.7 | 4.0 | — | — | 0 | 0 | 2.0 | 0 | — | — |
| 18 | 15.1 | 6.0 | 37.5 | 18.4 | 0 | 0 | 3.0 | 0 | — | — |
| 19 | — | — | 6.5 | 3.5 | 1.5 | 1.3 | 1.2 | 0 | 0.23 | 0.37 |
| 20 | — | — | 20.0 | 9.0 | 6.4 | 4.5 | 3.8 | 0 | 0.32 | 0.50 |
| 21 | 9.5 | 7.2 | 23.0 | — | 3.0 | 3.7 | 6.0 | 3.6 | 0.32 | 0.51 |
| 22 | 10.0 | 4.7 | 22.0 | 9.6 | 6.0 | 4.5 | 4.6 | 2.3 | 0.6 | 0.96 |
| 23 | 3.5 | 1.4 | 9.7 | 6.7 | — | — | — | — | — | — |
| 24 | 7.0 | 5.0 | 26.0 | 24.0 | 7.8 | 7.3 | 6.3 | 2.5 | 0.30 | 0.31 |
| 25 | 3.5 | 1.2 | 9.8 | 4.6 | 2.2 | 2.2 | 1.8 | 1.0 | 0.22 | 0.48 |
| 26 | 11.8 | 7.0 | — | — | 3.5 | 3.0 | 3.5 | 2.0 | 0.30 | 0.42 |
| 27 | 6.5 | 2.0 | — | — | 2.8 | 1.0 | 2.5 | 1.3 | 0.43 | 0.50 |
| Mean | 8.9 | 4.7‡ | 20.5 | 12.1§ | 3.7 | 2.8 | 3.5 | 1.1‖ | 0.34 | 0.49‡ |
| SE | 3.69 | 2.23 | 9.22 | 6.63 | 3.32 | 2.37 | 1.50 | 1.22 | 0.11 | 0.18 |

*Peak current values (*nA*).
‡Significantly different from pre-EGTA injection value at $p < 0.01$.
§$p < 0.002$.
‖$p < 0.001$.
¶Maximum light-induced decrease of $I_C$.

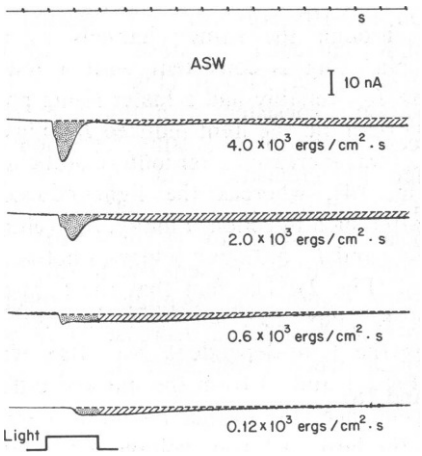


FIGURE 15 Effect of stimulus intensity on light-induced currents in ASW. With dim illumination (lower records), only a delayed inward current is elicited. With greater light intensities, the early light-induced inward $Na^+$ current becomes apparent. Light intensities are below and on the right of each record. Each light stimulus occurred 5 s after the onset of a 20-s command depolarization to −10 mV (holding potential, −60 mV). The interval between the dim light stimuli (≤50 ergs/cm² · s) was 1 min, and that between brighter stimuli was 2 min. The dashed lines indicate the current elicited by the command steps in the absence of light stimuli.

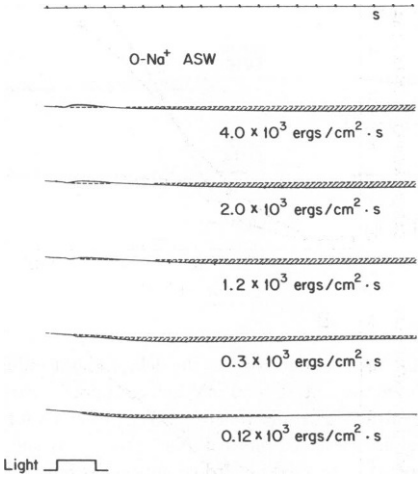


FIGURE 16 Effect of stimulus intensity on light-induced currents in 0-$Na^+$ ASW. With dim illumination (lower records), only a delayed inward current is elicited. With greater light intensities, the early light-induced outward $K^+$ current becomes apparent. Light intensities are below and on the right of each record. Each light stimulus occurred 5 s after the onset of a 20-s command depolarization to −10 mV. The interval between the dim light stimuli (≤50 ergs/cm² · s) was 1 min, and that between brighter stimuli was 2 min. The dashed lines indicate the current elicited by the command steps in the absence of light stimuli.

investigators (J. E. Brown and Blinks, 1974; J. E. Brown et al., 1977).

### $I_C$ Inactivation

Our experiments indicated that elevated intracellular $Ca^{++}$ is responsible not only for activation of outward $K^+$ flux (see also Grossman et al., 1981) but also for prolonged inactivation. Treatments (e.g., $Ca^{++}$, $Cd^{++}$, and $Ba^{++}$ substitution for $Ca^{++}$) that reduced the magnitude of the voltage-dependent flux of $Ca^{++}$ across the type B membrane, and thus reduced the voltage-dependent activation of $I_C$, also reduced the delayed light-induced decrease of $K^+$ current. In the presence of 2–5 mM 4-AP and 100 mM TEA, concentrations sufficient to eliminate or drastically reduce $I_A$ and the delayed rectifier, when $I_C$ is the major current (if not the only remaining voltage-dependent $K^+$ current), light still causes delayed, long-lasting reduction of $K^+$ current. These results indicate that $I_C$ undergoes prolonged inactivation by light. The results do not exclude the possibility, however, that other $K^+$ currents, such as $I_A$ (Alkon et al., 1982*b*) and the delayed rectifier (see Chinn and Lisman, 1984), are also inactivated by illumination. However, past experiments (Alkon et al, 1982*a*) indicate that $I_A$ inactivation, unlike inactivation of the late voltage-dependent $K^+$ current, occurs most clearly with depolarization paired with light rather than light alone (i.e., light during voltage clamp at −60 mV).

A number of observations indicate that light-induced $I_C$ reduction is mediated by elevated $Ca_i^{++}$. $Ca^{++}$ injection caused a prolonged reduction of $I_C$ and a decrease of light-induced $I_C$ reduction. $Ca^{++}$ injection did not significantly affect $I_{Ca^{++}}$. Ruthenium red caused a slight increase of $I_C$ and a consistent prolongation of light-induced $I_C$ reduction. EGTA injection entirely eliminated the light-induced $I_C$ reduction when it had a much smaller effect on $I_C$ activation. This was particularly true for steady state $I_C$. The light-induced $I_C$ reduction with dim illumination

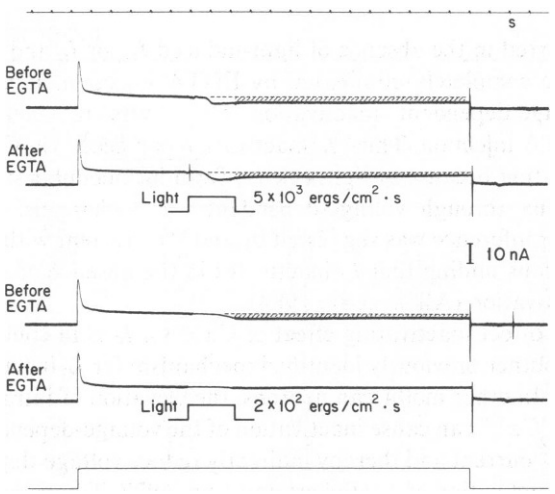


FIGURE 17 Effect of EGTA injection on light-induced currents in ASW. Light-induced currents at −10 mV before (upper records) and after (lower records) intracellular injection of EGTA (−2.0 nA for 5 min). The stimulus intensity is indicated below and on the right of each record. The delayed light-induced inward current with dim light is eliminated after EGTA injection. The delayed light-induced inward current with brighter illumination is reduced, whereas the early inward current is slightly increased after EGTA injection. Conditions of light stimulus frequency and command depolarizations are as described for Fig. 15. The dashed lines indicate the current elicited by the command steps in the absence of light stimuli.

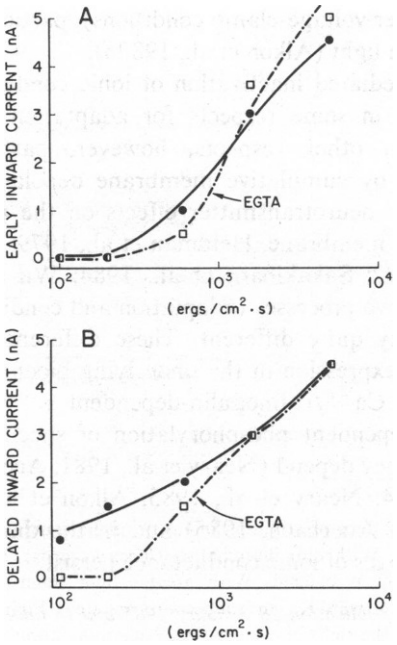


FIGURE 18 Graphical representation of EGTA effects on light-induced currents as a function of light intensity. The peak amplitudes of early (*A*) and delayed (*B*) light-induced currents are plotted as a function of light intensity before (solid line) and after (dashed line) EGTA injection. All values were taken at −10 mV. Conditions of light stimulus frequency and command depolarizations are as described for Fig. 14. Note that EGTA injection eliminates the delayed light-induced inward current for lower light intensities, while only slightly affecting the early inward current. With brighter light intensities, EGTA causes a slight enhancement of the early light-induced inward current, while causing only slight reduction of the delayed light-induced current. Values from records of Fig. 17 are included here.

occurred in the absence of light-induced $I_{Na}$ or $I_C$ and was more completely eliminated by EGTA injection. Finally, voltage-dependent inactivation of $I_C$ was reduced by EGTA injection. Thus, $I_C$ inactivation can occur via $Ca_i^{++}$ elevation because of light release from intracellular stores or flux through voltage-dependent $Ca^{++}$ channels. The latter inference was suggested by and is consistent with the previous finding that $I_C$ inactivates in the absence of $I_{Ca^{++}}$ inactivation (Alkon et al., 1984).

A direct inactivating effect of $Ca^{++}$ on $I_C$ is in contrast to another previously identified mechanism for $I_C$ inactivation. In other molluscan neurons, the elevation of intracellular $Ca^{++}$ can cause inactivation of the voltage-dependent $Ca^{++}$ current and thereby indirectly reduce voltage-dependent activation of $I_C$ (Eckert and Lux, 1977; Tillotson and Horn, 1978; Tillotson, 1979; Brehm et al., 1980; Eckert and Ewald, 1981; Eckert et al., 1981; Eckert and Tillotson, 1981). For the type B cell, the voltage-dependent $Ca^{++}$ current does not inactivate in 10 mM $Ca_o^{++}$, but does inactivate in elevated external $Ca^{++}$ (e.g., 50–100 mM). Other *Hermissenda* neurons, however, do have voltage-dependent $Ca^{++}$ currents that inactivate in 10 mM $Ca_o^{++}$ (Acosta-Urquidi, J., and D. L. Alkon, unpublished observations).

The fact that the light-dependent increased $K^+$ flux does not occur through the same channels as the light-dependent $Na^+$ flux is consistent with a few types of observations. $I_{Na^+}$ reliably had a faster rising phase (Figs. 1, 2, and 4) than did the light-induced $I_C$ (Figs. 2–5). In addition, $I_{Na^+}$ was increased after iontophoretic injection of EGTA (Fig. 14), whereas the light-induced $I_C$ was decreased after such injection. Finally, the voltage dependences of $I_{Na^+}$ and $I_C$, although linear in both cases, were not identical (Fig. 2). The fact that the light-dependent decrease of $K^+$ flux does not occur through the same channels as the light-dependent $Na^+$ flux was clearly apparent (Figs. 1 and 2) from the marked differences of voltage dependence (the former having a markedly nonlinear, and the latter a linear, voltage dependence). Furthermore, $Ca^{++}$ channel blockers and substitution of $Ba^{++}$ for external $Ca^{++}$ eliminated the light-induced decrease of $I_C$ but did not affect $I_{Na^+}$.

## $Ca_i^{++}$ and $I_C$ Inactivation

The relationship of $Ca_i^{++}$ to $I_C$ inactivation will be better understood when the measurement of $Ca_i^{++}$ (e.g., by the arsenazo III technique) is made simultaneously with voltage-clamp measurements. $Ca_i^{++}$ was previously shown to undergo marked and prolonged (minutes) elevation (by the arsenazo III technique) during the type B cell's depolarizing response to light (Connor and Alkon, 1984). Presentation of the light stimuli used here ($\geq 10^3$ ergs/cm$^+$ · s) during a depolarizing command to −10 or −5 mV can be assumed to be accompanied by similar prolonged $Ca^{++}$ elevation. It is interesting, however, that presentation of the light in the absence of depolarization (i.e., while the cell is voltage-clamped at −60 mV) still produced a considerable long-lasting reduction of $I_C$ (Fig. 7). This was usually not the case for $I_A$ (Alkon et al., 1982*a*, *b*). It may be that $I_C$ inactivation requires somewhat less $Ca_i^{++}$ elevation than does $I_A$ inactivation. Since light-induced $Ca_i^{++}$ elevation is very much less than light and depolarization-induced $Ca_i^{++}$ elevation (Connor and Alkon, 1984), the persistence of light-induced $I_C$ reduction may not be due simply to the persistence of higher $Ca_i^{++}$ concentrations. $Ca^{++}$-activated biochemical transformations such as $Ca^{++}$/calmodulin-dependent phosphorylation and $Ca^{++}$ phospholipid-dependent phosphorylation of specific proteins (Neary et al., 1981; Neary and Alkon, 1983; Acosta-Urquidi et al., 1984 Neary et al., 1985; Farley and Auerbach, 1985; Alkon et al., In press) might be important for explaining the slow recovery of $I_C$ inactivation.

## Adaptation

The levels of intracellular $Ca^{++}$ to which different ionic conductances are sensitive will clearly affect the sensitivity of the photoreceptor to light stimuli. The persistence of $Ca^{++}$-mediated inactivation of individual conductances is important for determining the photoreceptor's state of

adaptation. Inactivation of $K^+$ conductances (e.g., $I_A$ and $I_C$) will make the photoreceptor more sensitive to illumination, thus at least partially offsetting the effect of $I_{Na^+}$ inactivation, which reduces light sensitivity. Light stimuli immediately after prolonged exposure of the type B photoreceptor to adapting illumination of high intensities ($\geq 10^4$ ergs/cm$^2$ · s) elicit brief hyperpolarizing responses to light stimuli (Alkon and Fuortes, 1972; Alkon, 1976; Detwiler, 1976). After such light adaptation, $Na^+$ current elicited by light is greatly reduced because of both rhodopsin bleaching and reduction of the driving force for $I_{Na^+}$ by the cell's persistent depolarization, and $Ca^{++}$-mediated inactivation of $I_{Na^+}$. Sustained voltage-dependent flux of $Ca^{++}$ across the type B membrane, as well as $Ca^{++}$-mediated inactivation of $I_A$ and $I_C$, are probably responsible for the persistent depolarization of the type B cell in a light-adapted state. After a brief interval following the adapting light, the release of intracellular $Ca^{++}$ elicited by additional light stimuli is presumably sufficient to elicit $I_C$ (for which the driving force has been increased by the persistent depolarization) and thus cause transient hyperpolarization of the photoreceptor in spite of prolonged $I_C$ inactivation produced by the preceding adapting light. Thus, the completeness as well as the persistence of conductance inactivation by $Ca^{++}$ will also determine which ionic conductance will predominate and control the direction of light-elicited changes of membrane potential.

## Conditioning

The relative completeness of inactivation of the type B ionic conductances during light adaptation may be different from that on days after conditioning (Alkon, 1974; Crow and Alkon, 1978) the animal with repeated pairing of light with a second distinct sensory stimulus, rotation. On days after classical conditioning voltage-dependent activation at $I_A$ and $I_C$ are substantially reduced (Alkon et al., 1982*a*, 1985; Forman et al., 1984). This reduction of $K^+$ currents makes the type B cell more excitable and helps account for the storage and recall of associatively learned information (Alkon, 1980*a*; Crow and Alkon, 1980; Farley and Alkon, 1982; West et al., 1982; Alkon, 1983; Alkon, 1984). This new balance of membrane currents may depend on differences of recovery from $Ca^{++}$-mediated inactivation over a much longer period of time than was the case for adaptation. Furthermore, conditioning-induced conductance modification involves intracellular $Ca^{++}$ elevation of greater magnitudes and durations than occurs with light alone (Farley and Alkon, 1982; Connor and Alkon, 1984). During acquisition of the conditioned behavior, $Ca^{++}$ elevation accompanies prolonged accumulative depolarization of the type B membrane (Alkon, 1980*b*), a depolarization that cannot be duplicated or approximated by any pattern or regimen of adapting light alone, although long-lasting inactivation (many minutes) of $I_A$ and $I_C$ can be produced by sufficient depolarization of the type B soma (under voltage-clamp conditions), particularly when paired with light (Alkon et al., 1982*b*).

$Ca^{++}$-mediated inactivation of ionic conductance may be similar in some respects for adaptation and conditioning. In other respects, however, particularly as influenced by cumulative membrane depolarization and possibly by neurotransmitter effects on the postsynaptic type B cell membrane, Heldman et al., 1979; Tabata and Alkon, 1982; Sakakibara et al., 1984; Wu and Farley, 1984, the two processes (adaptation and conditioning) are undoubtedly quite different. These differences, in turn, may have expression in the underlying biochemical reactions (e.g., $Ca^{++}$/calmodulin-dependent and $Ca^{++}$/phospholipid-dependent phosphorylation of specific proteins) on which they depend (Neary et al., 1981; Acosta-Urquidi et al., 1984; Neary et al., 1985; Alkon et al.; In press; Farley and Auerbach, 1985) and in the durations with which changes of ionic conductance persist.

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