Light Modulates Voltage-Dependent Potassium Channels in *Limulus* Ventral Photoreceptors

Abstract. *Voltage-dependent outward current measured in the dark is reduced after illumination. This reduction can be blocked by tetraethylammonium and is associated with a decrease in total membrane conductance. The voltage dependence of the current reduced by light is the same as that of the delayed rectifier. These results indicate that light modulates the delayed rectifier. This modulation serves to maintain a stable voltage response to constant illumination.*

Voltage-dependent K^+ channels (termed the delayed rectifier) were first characterized in squid axon (*1*) and have subsequently been found in a wide variety of nerve cells. Modulation (*2*) of the delayed rectifier could have important consequences because these channels participate in the formation of the action potential (*1*), in the formation of the receptor potential in sensory cells (*3*), and in transmitter release (*4*). We now report that the delayed rectifier in *Limulus* ventral photoreceptors is modulated by illumination and that this modulation is functionally important.

To measure the voltage-dependent properties of the membrane, we used a two-microelectrode voltage clamp technique. The currents superimposed (*5*) in Fig. 1a were evoked by voltage pulses of duration 1.6 seconds, given in 10-mV increments. Depolarization to voltages more positive than −30 mV resulted in a large initial outward current that decayed to a steady state within 1 second. The transient nature of the outward current is due to a rapidly inactivating channel that strongly resembles (*6*) the A current of snail neurons (*7*). The steady-state currents remaining after this inactivation are carried by the delayed rectifier. The steady-state currents in Fig. 1a were measured after the cell had been in the dark for more than 30 minutes. Similar measurements (Fig. 1b) were made shortly after terminating a bright light (*8*). Under these conditions, the steady-state outward currents were reduced, but the shape of the normalized steady-state current-voltage relation (Fig. 1c) was the same before and after illumination (*9*). Light therefore reduces the steady-state outward current without substantially affecting its voltage dependence.

This reduction by light of the net outward currents could be due either to a decrease of voltage-dependent outward current or to an increase of voltage-dependent inward current. A decrease of the outward K^+ current would be associated with a reduction in total membrane conductance, whereas an increase of inward current would be associated with an increase in conductance. To distinguish between these alternatives, we examined the aftereffect of light on the instantaneous current-voltage curve, the slope of which is a measure of total membrane conductance (*10*). The cell was depolarized to zero voltage to activate the K^+ conductance and then was rapidly repolarized to a series of potentials (Fig. 2a, inset). The currents immediately following the repolarization steps were calculated as described in the legend to Fig. 2 and were used to construct instantaneous current-voltage curves (Fig. 2a). These plots show that the total conductance activated by depolarization was reduced when light preceded the depolarization; the reversal potential of the currents was unchanged. These results are in agreement with the notion that light decreases the voltage-dependent K^+ conductance.

Direct evidence that the conductance decreased by light is actually a K^+ conductance was obtained by raising the extracellular K^+ concentration. This caused the reversal potential to become more positive (*11*) (Fig. 2a). Furthermore, iontophoretic injection of tetraethylammonium (TEA), a substance known to block the voltage-dependent K^+ channels in *Limulus* (*3*), greatly reduced the effect of light on the steady-state outward current (Fig. 2b). In contrast, 10 m*M* $NiCl_2$, which completely blocks voltage-dependent inward currents (*6*), did not significantly reduce the effect of light on the steady-state outward currents. These results further indicate that the effect of light on maintained outward current is due to a reduction of the voltage-dependent K^+ conductance and is not related to an effect of light on voltage-dependent inward currents.

The kinetics of the modulation of the voltage-dependent K^+ conductance can be deduced from previous experiments (*12*) in which the light-induced decrease in outward current was measured. The decrease occurred slowly (in seconds) during illumination, and the recovery in the dark took up to 10 minutes (*13*). These changes in outward current were termed the "slow process" because of their sluggishness in comparison with the light-activated increase in Na^+ conductance (*14*), which responds to changes in illumination within a few hundred milliseconds.

The amplitude of the receptor potential depends on both the Na^+ and K^+ conductances. Modulation of the K^+ conductance functions to stabilize the plateau phase of the receptor potential (Fig. 3). In contrast to the stability of the plateau potential (V_p), the plateau value of the Na^+ current (I_{Na}) progressively decreased with each flash. At the same time, however, there was a progressive decrease in K^+ current (I_K). Thus the

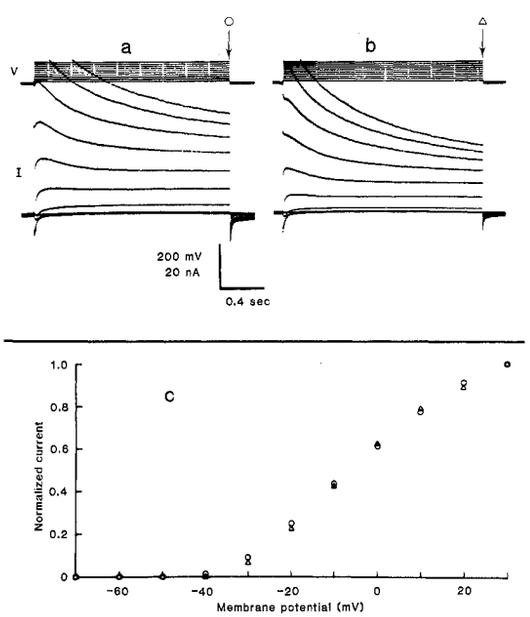

Fig. 1. An aftereffect of light is a reduction of the steady-state voltage-dependent outward current measured in the dark. (a) Superimposed current responses to a family of depolarizing voltage pulses. The cell was clamped to a holding potential of −70 mV and kept in the dark for 30 minutes. Voltage pulses had a duration of 1.6 seconds and were given in increments of 10 mV. The steady-state current was measured at the end of the depolarizing voltage pulse at the time indicated by the arrows. (b) The same voltage pulses were applied as in (a), but a bright light (10^{-5} W/cm^2, ~ 1 second) preceded each pulse. The flash was terminated ~ 3 seconds before the onset of the pulse, so that only the voltage-dependent currents were recorded. (c) The steady-state current measured at the end of each voltage pulse in (a) and (b) is normalized and plotted as a function of membrane potential.

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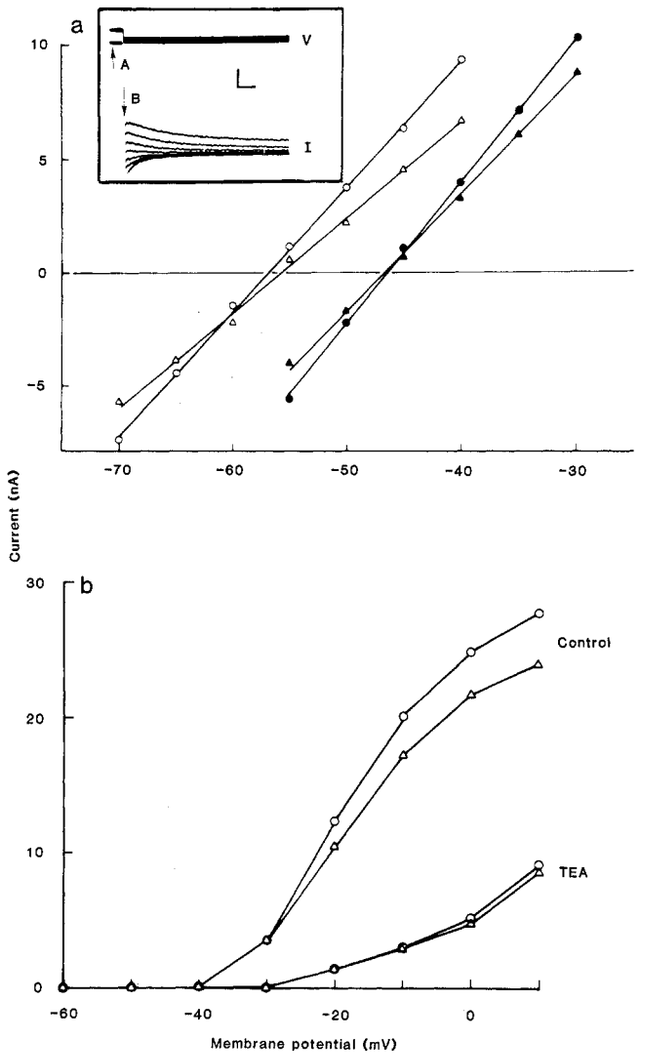

Fig. 2. The voltage-dependent current affected by light is K^+ current. (a) Inset: The photoreceptor was depolarized from a holding potential of −70 mV to 0 mV for 1.5 seconds to activate the voltage-dependent K^+ conductance. The membrane was then repolarized to a family of more negative potentials in increments of 5 mV. The superimposed tail currents elicited by this protocol are shown. The arrow at *A* marks the steady-state current activated by the depolarization. The arrow at *B* marks the time at which the voltage reached its new value following repolarization (inset calibration: vertical, 100 mV, 5 nA; horizontal, 20 msec). The current at *B* was obscured by a capacitative transient after which the current declined exponentially as the channels closed (*22*). The instantaneous values of the currents at *B*, which were extrapolated from the exponentials, were plotted as a function of membrane potential in (a). The results from the same cell under four different conditions are plotted together. (○) The cell was dark-adapted for 30 minutes in the normal seawater containing 10 m*M* K^+. (△) Next, a light flash of the same intensity and duration as that described in Fig. 1 was presented before the depolarization from holding potential to 0 mV. (●) The external solution was then changed to one containing 30 m*M* K^+ (substituted for Na^+), and the cell was dark-adapted for 30 minutes. (▲) Finally, the cell was light-adapted as before. Light reduced the slope of the instantaneous current-voltage relation without significantly changing the reversal potential. The reversal potential was affected by changes in the external K^+ concentration. (b) Intracellular TEA blocked the effect of light on the steady-state outward currents. The steady-state current-voltage curves for the same cell are shown before and after iontophoretic injection of ~ 10^{-6} coulombs from a third microelectrode containing 100 m*M* TEA. The points were obtained with the protocol described in Fig. 1. (○) Dark-adapted; (△) light-adapted.

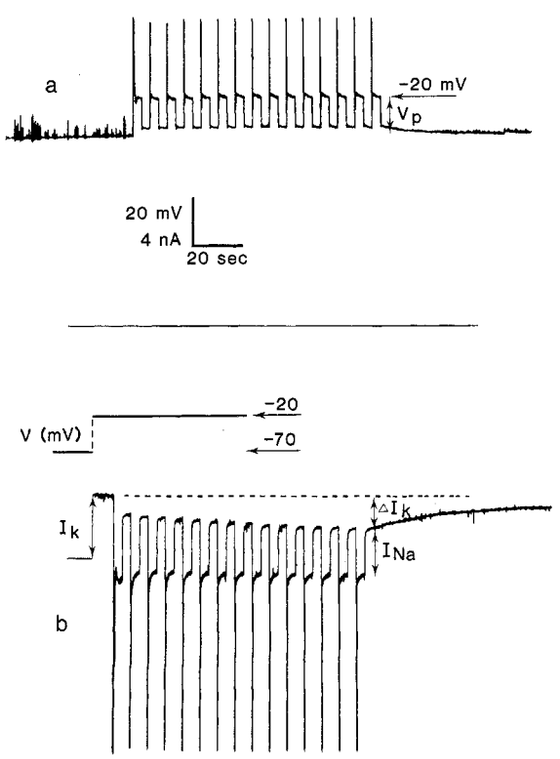

Fig. 3. A physiological role of the light-mediated reduction of the voltage-dependent K^+ conductance. (a) The voltage response to each of a series of 4-second repetitive flashes (10^{-5} W/cm^2) consisted of a transient depolarization, which rapidly decayed to a steady plateau voltage (V_p). The absolute value of V_p did not change with repeated illumination. (b) Under voltage clamp, the photoreceptor was depolarized to the value of the plateau voltage response (−20 mV). The magnitude of outward current activated by this depolarization is shown by the arrow labeled I_K. As each flash was presented, there was a large transient inward current that decayed to a plateau. When the flash was terminated, the light-activated current (I_{Na}) turned off rapidly, but the current did not return to the resting value because of a reduction (ΔI_K) in the magnitude of I_K. With repeated illumination, I_{Na} decreased and ΔI_K increased. The increase in ΔI_K implies a decrease in I_K; thus the constant V_p occurred because a reduction in I_{Na} was compensated for by a reduction in I_K.

stable plateau voltage (*15*) was achieved because a gradual decrease in the light-activated Na^+ conductance was compensated for by a gradual decrease in the voltage-dependent K^+ conductance. If this compensation did not occur, the plateau voltage would slowly droop, and a steady illumination might be falsely perceived as a slowly dimming one (*16*).

One mechanism by which light might lower the K^+ conductance is reduction of intracellular *p*H (pH_i). Potassium channels in squid axon (*17*) are blocked by protonation of a site with an apparent *p*K of 6.9. A large light-induced reduction of pH_i occurs in the barnacle photoreceptor (*18*). In *Limulus*, however, measurements of pH_i made with phenol red indicate that the average pH_i of the cell is not significantly affected by light (*19*). Light does cause a large increase (*20*) in Ca_i^{2+}, and injection of Ca^{2+} causes a decrease in net outward current (*3*). This evidence suggests that Ca^{2+} might reduce the K^+ conductance, though there is little precedent for this in other preparations (*21*). Further work will be required to determine whether the K^+ conductance in *Limulus* is modulated by pH_i, Ca_i^{2+}, or some other cytoplasmic factor.

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References and Notes

1. A. L. Hodgkin and A. F. Huxley, *J. Physiol. (London)* **116**, 449 (1952).
2. "Modulation" here refers to a change in the maximum K^+ conductance (G_K) that can be activated by depolarization. There have been previous reports of voltage-dependent K^+ currents that undergo changes. R. W. Tsien [*J. Physiol. (London)* **64**, 293 (1974)] showed that epinephrine causes a shift in the voltage dependence of a K^+ current in cardiac Purkinje fibers. D. A. Brown and P. R. Adams [*Nature (London)* **283**, 673 (1980)] reported suppression of a voltage-sensitive K^+ current by muscarinic agonists. They point out, however, that this current is distinct from the delayed rectifier, which also exists in their preparation and which more strongly resembles the classic Hodgkin and Huxley K^+ current (*1*).
3. J. Pepose and J. Lisman [*J. Gen. Physiol.* **71**, 101 (1978)] showed that the steady-state outward current in *Limulus* is carried by a single class of K^+ channels that appears to be directly gated by voltage, rather than activated by Ca^{2+}. (Even though there is no direct evidence of a Ca-activated K^+ current in *Limulus*, it is impossible to rule out a minor contribution from such a component.) They further showed that the K^+ conductance is similar to that of the squid K^+ channel pharmacologically. Additional evidence bearing on this similarity is that, as in the squid channel, there is an *e*-fold change in conductance for each ~ 5-mV change in voltage (data not shown). Furthermore, the closing of the channels, as revealed by tail currents, follows a single exponential that decreases with increasing negativity, and the instantaneous current-voltage curve is linear (Fig. 2a).
4. K. Kusano, D. R. Livengood, R. Werman, *J. Gen. Physiol.* **50**, 2579 (1967).
5. The voltage-independent leakage currents have been subtracted out, so that only voltage-dependent currents are recorded.
6. J. Lisman, G. Fain, M. Swan, *Biol. Bull. (Woods Hole, Mass.)* **155**, 453 (1978).
7. J. A. Connor and C. F. Stevens, *J. Physiol. (London)* **213**, 31 (1971).
8. The photoreceptors were light-adapted by presenting a bright light (10^{-5} W/cm^2) for 1 second before the beginning of each voltage pulse. The light was terminated at least 3 seconds before the onset of the voltage pulse; the intervening time was sufficient to allow the light-activated I_{Na} to subside, so that only the currents activated by voltage were recorded.
9. In four of ten cells, there was no change in the voltage of half-maximal activation of outward current ($V_{1/2}$). In one cell, $V_{1/2}$ was made more negative, and in five others, more positive. The average shift for ten cells was +4.3 ± 3.9 mV.
10. Conductances do not change during a rapid (instantaneous) change in voltage. Thus, an instantaneous current-voltage curve measured just after repolarization provides a measure of the conductances activated (at steady state) by depolarization.
11. The observed change in the reversal potential was +11 mV. The change predicted by the Nernst equation is +28 mV. This discrepancy can be explained if the channel is not perfectly selective for K^+, and has a permeability ratio of 16 : 1 for K^+ to Na^+.
12. J. E. Lisman and J. E. Brown, *J. Gen. Physiol.* **58**, 544 (1971).
13. J. Lisman, thesis, Massachusetts Institute of Technology, Cambridge (1971).
14. We refer to this value as the Na^+ conductance because it has been shown by R. Millecchia and A. Mauro [*J. Gen. Physiol.* **54**, 310 (1969)] to be dependent on the external Na^+ concentration in a Nernstian way. J. E. Brown and M. Mote [*J. Gen. Physiol.* **63**, 337 (1974)] showed, however, that there is appreciable permeability to K^+ through the light-activated channel.
15. We used interrupted flashes so that the changes in I_K and I_{Na} could both be observed. Experiments reported by G. Fain and J. Lisman (*Prog. Biophys. Mol. Biol.*, in press) in which the Na^+ current was observed during a long uninterrupted flash also show a progressive decline in the magnitude of I_{Na} with no decline in the plateau voltage response.
16. The mechanism by which the ventral photoreceptors send signals to the brain is not understood. In the lateral eye, which has many of the properties of the ventral eye, signaling is accomplished by changes in action potential frequency. The frequency of spikes is sensitive to voltage [0.77 spike per second per millivolt was measured by M. Fuortes, *J. Physiol. (London)* **148**, 14 (1959)].
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18. H. M. Brown and R. W. Meech, *J. Physiol. (London)* **297**, 73 (1979).
19. J. E. Brown, P. K. Brown, L. H. Pinto, *ibid.* **267**, 299 (1977).
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21. Such an interaction was not observed by T. Begenisich and C. Lynch [*ibid.* **63**, 675 (1974)] in squid. There is, however, some positive evidence for direct interaction of Ca^{2+} with voltage-dependent K^+ channels. C. Miller [*J. Membr. Biol.* **40**, 1 (1978)] studied the voltage-dependent K^+ conductance in fragmented sarcoplasmic reticulum fused to planar phospholipid bilayer and found that addition of 3.5 m*M* Ca^{2+} to the bilayer caused a reduction (60 percent) of the voltage-dependent K^+ conductance.
22. The conductance turns off as a single exponential. The τ for a given repolarization is not changed by illumination.
23. We thank B. Meech, G. Fain, and A. Szent-Györgyi for commenting on the manuscript.
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Absence of Correlation Between Base-Pair Sequence and RNA Conformation

Abstract. *A survey of all available double-stranded RNA crystal structures shows that there is a considerable range of variation in local conformation of a given base-pair doublet, but that there is no significant correlation between base-pair sequence and RNA local conformation.*

The presence or absence of correlation between base-pair sequence and DNA-RNA conformation at the local level is important for understanding protein–nucleic acid interaction, one of the most central processes in all cells. Until now it was not possible to answer the question owing to lack of a large enough data base for statistical studies. In past years there has been an effort to experimentally determine the conformational variation of double helical nucleic acids by various optical spectroscopic techniques such as ultraviolet circular dichroism and infrared linear dichroism. Such effort resulted in several indirect pieces of evidence that the conformation of double-stranded DNA (dsDNA) depends on solution conditions and base sequence (*1–4*). However, these techniques suffer from the fact that they measure average properties of entire molecules rather than local properties such as local conformation. Thus no structural details can be derived.

Until recently, structural details of double-stranded polynucleotides have been derived exclusively from x-ray fiber diffraction data, the interpretation of which depends on the assumption that all nucleotides along a polymer chain have the same conformation. Like the optical techniques mentioned above, this technique gives information about the average structure only. Thus the three-dimensional structural model derived from this technique leaves the impression of extremely regular structures of dsDNA and dsRNA. Local heterogeneity in conformation which must exist is lost in these artificially uniform structures.

Recent determination of three-dimensional structures of dsDNA model compounds (*5–9*) provides the most specific and detailed support to the hypothesis that dsDNA structures do not have regular conformation throughout their entire length, but that the local conformation may be significantly different from the DNA-B form depending on the particular base sequence in that region and on the solution conditions. These self-complementary structures, dCGCGCG (C, cytosine; G, guanine) (*5*), dCGCG (*6, 7*), dATAT (A, adenine; T, thymine) (*8*),

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