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21. The coefficient of variation was calculated from more than 150 EPP's measured at the same (± 2 mv) resting membrane potential. These observations were made at resting membrane potentials ranging from 36 to 51 mv.
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Ionic Basis of the Photoresponse of Aplysia Giant Neuron: K^+ Permeability Increase

Abstract. *The giant neuron of the* Aplysia *abdominal ganglion hyperpolarizes during illumination. The light-initiated potential change is associated with an increase of membrane conductance. It reverses sign at the potassium equilibrium potential (about −83 millivolts), which was determined from direct measurements of internal potassium activity. The membrane hyperpolarization is produced entirely by a light-induced increase in potassium permeability.*

The ionic basis of the depolarizing receptor potential has been examined in detail in photoreceptors of *Balanus, Limulus*, and *Apis* (*1*). However, the ionic basis of hyperpolarizing receptor potentials in vertebrate and invertebrate photoreceptors has been more difficult to determine (*2, 3*). We studied the light-induced membrane hyperpolarization in the giant neuron of the abdominal ganglion of *Aplysia californica* (*4*), and found that it was due exclusively to an increase in membrane permeability to potassium ions.

The giant neuron was illuminated with white light from a tungsten-halide source or with monochromatic light from a mercury-xenon source. Techniques of voltage and current clamp and the measurement of membrane potential and internal K^+ activity have been described (*1, 5*).

About 400 msec after the giant cell was illuminated with a pulse of white light, the membrane gradually hyperpolarized, reaching its most negative value in about 20 seconds (Fig. 1A). There was usually a slight decline in the potential change during sustained illumination. When the light pulse was terminated, the membrane repolarized, requiring about 40 seconds to attain the resting level prior to illumination. Occasionally there was an even slower decay phase (Fig. 1B). The membrane potential change is smaller and slower than the potential change to light that has been described for other photoreceptors. Spectral studies indicated that the potential change is elicited most effectively at 490 nm. The membrane potential changed when constant-current pulses were passed across the membrane prior to, during, and after illumination (Fig. 1B). Light produced a membrane hyperpolarization of about 5 mv. The input resistance of the membrane decreased from 1.58 megohms in darkness to 1.38 megohms during illumination. This represented an increase of membrane conductance of 10^{-7} ohm^{-1}, or 13 percent over that in darkness. Figure 1C shows a record of light-elicited membrane current when the membrane potential was voltage-clamped to the resting potential. Light elicited a slowly changing outward membrane current with about the same time course as the membrane potential change (see Fig. 1A). The similarity in waveforms is to be expected since the time constant of the membrane (about 180 msec) is much shorter than the change in membrane current.

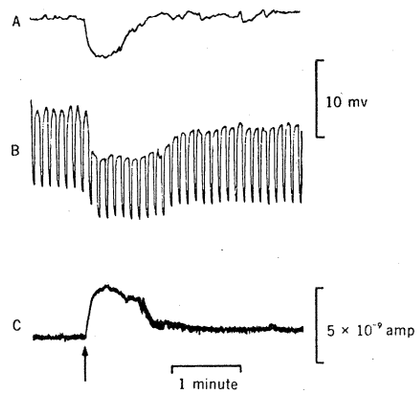

Fig. 1. (A) Membrane potential change produced by illumination of the *Aplysia* giant neuron. Resting potential was −45 mv. (B) Potential changes produced by passing 6-na current pulses (inward) across the giant neuron prior to, during, and after illumination. Resting potential was −45 mv. (C) Membrane current produced by light when the giant cell membrane was voltage-clamped to the resting potential (−54 mv). The onset of illumination is indicated by the arrow at the bottom of the traces. The periods of illumination were (A) 30 seconds, (B) 1 minute, and (C) 50 seconds.

The possibility that these changes in the giant cell are produced by synaptic action due to light stimulation of a presynaptic cell was ruled out. Low Ca^{2+} (0.5 m*M*) solutions that abolish or greatly reduce synaptic action in this cell augmented, rather than diminished, the membrane potential change to light. Furthermore, the hyperpolarization to light was maintained in two cells that were ligated between the soma and the axon with a fine thread and completely removed from the ganglion. The possibility that the change is produced by light activation of an electrogenic pump was also ruled out. Ouabain or K^+-free solutions, which depolarized the cell immediately on application, presumably due to blockage of an electrogenic Na^+ pump (*6*), had little effect on the potential change to light at the time the pump was manifestly blocked.

The potential changes elicited by light at different membrane potential levels produced by passing steps of current of different intensities across the cell membrane was recorded (Fig. 2B). In each case, sufficient time was allowed during the passage of current for the membrane to attain a relatively steady potential level before light was applied. In control solutions (Fig. 2B), the light-induced potential change became smaller as the membrane was hyperpolarized, and at −81 mv there was a very small negative deflection to light. The sign of the potential change to light was reversed at membrane potential levels more negative than −84 mv. The relation between the maximum potential change elicited by light, ΔV_L, and the membrane potential, V_m, prior to illumination is shown by the solid line in Fig. 2A. The line intersects the membrane potential axis at about −84 mv. The potassium equilibrium potential (E_K) determined at the same time from measurements of the internal K^+ activity (186 m*M*) was −83.6 mv (*7*). In 11 other cells, the differences between the mean value of the reversal potentials and the mean value of the simultaneously determined E_K's was 1.3 mv. The potential changes to light at different membrane potential levels were measured when the external K^+ activity was raised to 21 m*M* (concentration, 30 m*M*). Immediately after the solution was changed, the resting potential became more negative and the

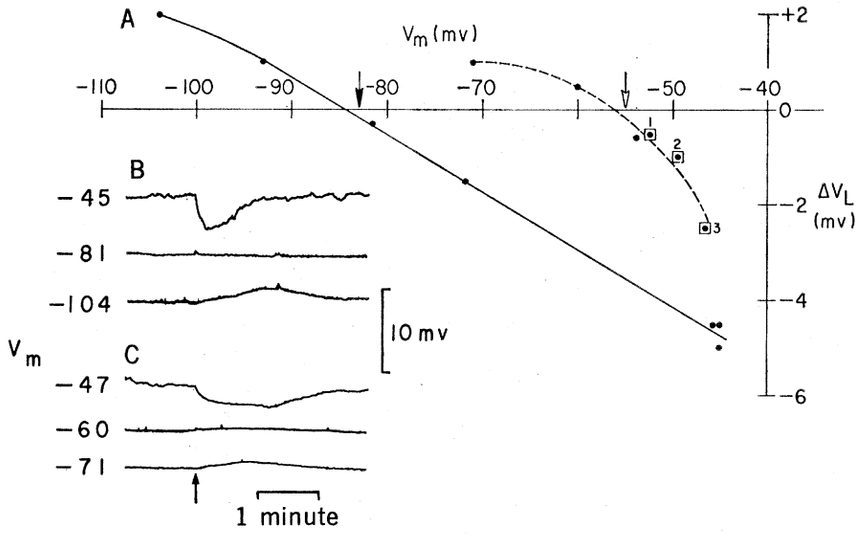

Fig. 2. Membrane potential changes elicited by light at different membrane potentials prior to illumination (indicated to the left of each trace) with the ganglion in solutions of (B) 7 m*M* external K^+ activity (control) and (C) 21 m*M* external K^+ activity. (A) The relation between the light-induced membrane potential change, ΔV_L, and the membrane potential, V_m, prior to illumination with the ganglion in 7 m*M* external K^+-activity saline (solid line) and 21 m*M* external K^+-activity saline (broken line). The arrows indicate E_K's in control and high K^+ solutions, respectively.

membrane conductance increased about tenfold. The light-induced hyperpolarization was reduced to about one-tenth of the value in normal saline. Over a period of about 10 minutes, the membrane potential in darkness gradually attained the same level (−45 mv) as that in normal saline, and the light-induced potential changes became greater (Fig. 2A, square data points). The potential change to light reversed sign at −60 mv (Fig. 2C). The relation between the ΔV_L's and V_m's in the 21 m*M* K^+ solution is shown by the dashed curve in Fig. 2A. The reversal potential in this case was about −56 mv. Internal K^+ activity had not changed during this time, and the E_K was therefore stable at a value of −55 mv.

Our results indicate that the hyperpolarization produced by light in the *Aplysia* giant cell is due largely to a K^+ permeability increase since there is almost perfect correspondence between E_K and the reversal potential of the light-induced potential change. The conclusion seems further strengthened by the close correspondence (about 10 percent) between the membrane current elicited by light during voltage clamp and that calculated from the measured change of membrane conductance (Δg_M) during illumination. Thus, $\Delta I_K = \Delta g_M (E_m - E_K)$, where ΔI_K is the change in K^+ current, E_m is the membrane potential, and Δg_M is attributed entirely to an increase in K^+ conductance (*8*).

For the cell shown in Fig. 1, the conductance change due to illumination was 10^{-7} ohm^{-1}, E_K was −82 to −83 mv, and the membrane potential is assigned the value of the holding potential used in the voltage clamp experiment (−54 mv). If the change in conductance was solely due to a K^+ conductance increase, the light-induced current should be about 2.9 na. The directly measured light-induced current was 3.2 na when the membrane potential was clamped to −54 mv.

A membrane hyperpolarization in the giant neuron of *Aplysia fasciata* was elicited by a 490-nm light; the same cell was depolarized by a 578-nm light (*4*). By contrast, the 578-nm light of the same energy as the 490-nm light was merely less effective in eliciting the membrane hyperpolarization in the giant neuron of *Aplysia californica*.

The membrane hyperpolarization associated with an increase of membrane conductance produced by illumination of the *Aplysia* giant neuron is not unlike the membrane changes produced by illumination of photoreceptors in the primitive chordate *Salpa* (*9*) and the distal photoreceptor of *Pecten* (*3*). These cells bear specialized membrane structures, namely, microvilli and cilia, respectively, where the photopigment is thought to be located. These structures are not present in the *Aplysia* giant neuron, although lipochondria granules may play a similar role (*4*).

In *Salpa* and *Pecten*, the ionic mechanism of the membrane potential change is not known. Thus, it is not possible to conclude with certainty that the ionic mechanism in these cells is identical to that of the *Aplysia* giant neuron. The mechanism we described here is clearly different than that for the hyperpolarization produced by light in vertebrate photoreceptors (*2*). In these cells, there is also a membrane hyperpolarization during illumination, but the membrane conductance decreases rather than increases. A decrease of permeability to Na^+ is thought to occur rather than an increase in permeability to K^+.

Calcium may be the link between light absorption by the photopigment and the alteration of membrane permeabilities in photoreceptors (*10*). It was reported that internal injection of Ca^{2+} increases K^+ permeability in the *Aplysia* giant neuron (*11*). If the action of light on a photopigment in the *Aplysia* giant neuron produces a release of internal Ca^{2+}, the response to light that we have reported could be the consequence.

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8. Although the slope conductance was measured in the present experiments, this equation estimated ΔI_K, since the current-voltage relation of the light-induced current was reasonably linear in this cell.
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