Ion Channels Activated by Light in *Limulus* Ventral Photoreceptors

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ABSTRACT   The light-activated conductance of *Limulus* ventral photoreceptors was studied using the patch-clamp technique. Channels (40 pS) were observed whose probability of opening was greatly increased by light. In some cells the latency of channel activation was nearly the same as that of the macroscopic response, while in other cells the channel latency was much greater. Like the macroscopic conductance, channel activity was reduced by light adaptation but enhanced by the intracellular injection of the calcium chelator EGTA. The latter observation indicates that channel activation was not a secondary result of the light-induced rise in intracellular calcium. A two-microelectrode voltage-clamp method was used to measure the voltage dependence of the light-activated macroscopic conductance. It was found that this conductance is constant over a wide voltage range more negative than zero, but it increases markedly at positive voltages. The single channel currents measured over this same voltage range show that the single channel conductance is independent of voltage,-but that channel gating properties are dependent on voltage. Both the mean channel open time and the opening rate increase at positive voltages. These properties change in a manner consistent with the voltage dependence of the macroscopic conductance. The broad range of similarities between the macroscopic and single channel currents supports the conclusion that the 40-pS channel that we have observed is the principal channel underlying the response to light in these photoreceptors.

INTRODUCTION

Although substantial progress has been made in understanding the channels that underlie electrical events in neurons, little is known about the channels that underlie receptor potentials in sensory neurons. We address this problem here in a well-studied sensory neuron, the photoreceptor of the *Limulus* ventral eye. Illumination of these cells causes a depolarization that is due to an increase in membrane conductance for cations (Millecchia and Mauro, 1969; J. E. Brown and Mote, 1974). Because of the large size of the current flow (~1 nA) evoked by a single photon (Cone, 1973) and because a single photon can produce a

conductance change in a significant fraction of the cell membrane (J. E. Brown and Coles, 1979), it is thought that an intracellular messenger couples rhodopsin to the light-activated channels and that many channels open during the response to a single photon. The first evidence for such channels came from analysis of light-induced noise (Wong, 1978). More recently, single channel currents activated by light have been observed directly using the patch-clamp technique (Bacigalupo and Lisman, 1983, 1984). These channels have a conductance of ~40 pS, a lifetime in the millisecond range, and a reversal potential similar to that of the macroscopic light-induced current. Moreover, the opening of these channels is a "direct" effect of light rather than a secondary effect of voltage changes in the cell (Bacigalupo and Lisman, 1983). In this paper, we further describe these channels and compare their properties with those of the macroscopic conductance. Such a comparison is of particular importance because previous work has indicated that the latency of channel activity was often much greater than the latency of the macroscopic current; this raises the possibility that the observed channels might underlie some secondary or minor process in transduction, perhaps related to cell metabolism (Auerbach and Sachs, 1984). The experiments presented here, however, show several further areas of correspondence between the properties of the macroscopic current and the channel properties, which makes it quite likely that these channels underlie the light response. Preliminary reports of some of these results have appeared (Bacigalupo and Lisman, 1984; Chinn et al., 1984).

METHODS

Preparation

Limulus were obtained from the Marine Biological Laboratory, Woods Hole, MA. Ventral nerves were dissected out of the animals, desheathed, and pinned onto the bottom of the experimental chamber. The nerves were then treated with 2% Pronase (Calbiochem-Behring Corp., La Jolla, CA) for ~1 min, a procedure that is standard for the ventral eye preparation. The glial cells surrounding the individual photoreceptor were then removed by teasing the glia away with a suction pipette of 20 μm tip diameter. This is termed the denuding procedure and is described more fully in Stern et al. (1982). In order to optimally orient the cell for recording, it was necessary to separate several millimeters of the photoreceptor axon from the ventral nerve.

The cell body of the ventral photoreceptor is subdivided into lobes that are distinct in structure and function. One type of lobe (the A-lobe) has a smooth, unfolded membrane; the other type (the R-lobe) contains microvilli on its plasma membrane (Stern et al., 1982; Calman and Chamberlain, 1982). Such microvilli are typical of invertebrate photoreceptors and contain the rhodopsin molecules responsible for the absorption of light (Langer and Thorell, 1966; Goldsmith et al., 1968). The response to light is initiated in the R-lobe (Stern et al., 1982). We were able to obtain patch-clamp current recordings from the smooth A-lobe in 80% of our attempts (n = 44). However, none of the channels in the A-lobe were directly affected by light. Using the same approach successfully applied to the A-lobe, we obtained seals on the R-lobe rarely and then only after applying considerable suction. The currents measured from these patches (n = 28) contained a sporadic noise that was independent of light and voltage and was probably due to damage to the patch membrane caused by the suction. In order to obtain better seals on the R-lobe, further methods of treating the cells were explored. We found that mild treatment

of the cell in a sonicator bath (as described below) enabled us to obtain seals on the R-lobe with less suction and to make recordings in which the sporadic noise was usually absent. On the R-lobe of sonicated cells, 43% of the attempts ($n = 420$) to obtain high-resistance seals were successful. Of these patches ($n = 180$), 17 contained observable light-activated channels. Toward the end of this project, we found that the opening probability of the light-activated channel is voltage dependent and that the observability of these channels can be greatly increased by depolarizing the patch. We therefore suspect that many patches that showed no obvious channel activity at resting potential may have actually had light-activated channels in them.

The sonication procedure was as follows. The chamber containing the ventral nerve was covered with a microscope slide and wrapped with parafilm to prevent leakage of water from the sonicator bath into the chamber. The chamber was then suspended in the sonicator (model G112SPIT, Lab. Supply Co. Inc, Hicksville, NY), which was set at 80 V for 2 min. After sonication, photoreceptors were denuded as described above. The function of the photoreceptors was not markedly affected by the sonication. Their resting potential was -48.2 ± 14.7 mV (mean $\pm$ SD, $n = 67$), which is within the normal range (-40 to -70 mV; Fain and Lisman, 1981), they produced normal light responses, and they maintained their ability to respond to single photons. Although the cells' general appearance in the light microscope was not affected by sonication, there were some noticeable effects. First, small vesicles appeared in the bath after sonication that were never observed in unsonicated preparations. Second, photoreceptors of preparations that were exposed to slightly longer sonication (≥ 4 min) occasionally had their axons sectioned into several pieces. Finally, it is our impression that it was easier to remove the glial cells from sonicated photoreceptors. It is not clear how sonication affected the ability to obtain good seals, but it probably altered the R-lobe surface so that areas of it became appropriate for obtaining seals. Preliminary studies of a few sonicated photoreceptors in the scanning electron microscope indicated that the cells did not have external microvilli; however, this was also sometimes the case for nonsonicated denuded photoreceptors (Stern et al., 1982). Further work will be necessary in order to understand why sonication is helpful for seal formation.

Experimental Apparatus

Experiments were done under a compound microscope (MicroZoom, Bausch & Lomb, Inc., Rochester, NY). The denuding of the photoreceptors was done under red light at a magnification of 115; the positioning of the patch pipette was done at a magnification of 230. The denuding pipette was mounted on a sliding micromanipulator (Stern et al., 1982). This pipette was sometimes replaced by a microelectrode after the denuding procedure was completed. The patch pipette was mounted on a second micromanipulator (Line Tool Co, Allentown, PA), which was driven by miniature motors (motor mike, Ardel Kinamatic Corp., College Point, NY).

Light Stimuli

The unattenuated stimulating light (520 nm) used in patch-clamp experiments had a maximal intensity (I_0) of $\sim 1.4 \times 10^{14}$ photons cm^{-2} s^{-1} at the level of the preparation. The intensity of the light (I) was controlled with neutral density filters and is expressed as relative log light intensity (log I/I_0).

Patch-Clamp Recording

The techniques used for patch-clamping were standard (Hamill et al., 1981). Pipettes with ~ 0.5-μm tips were coated with Sylgard, fire-polished, and filled with artificial seawater (ASW). The bath also contained ASW. The composition of the ASW was (mM): 425

NaCl, 10 KCl, 10 CaCl$_2$, 22 MgCl$_2$, 26 MgSO$_4$, 15 Tris-Cl, pH 7.8. Recordings were stored on magnetic tape. The frequency response was determined by the time constant (0.8 ms) of our patch-clamp amplifier. Recordings were played back on chart paper at a slower tape speed. Channel open times were measured as the time between the half-maximal single channel current during the opening and closing transitions. The voltage dependence of channel properties was studied by changing the command voltage of the patch amplifier. All experiments were done at room temperature.

Microelectrodes

In some of the patch-clamp experiments, an intracellular microelectrode was used to record the membrane potential. The microelectrodes were filled with 3 M KCl and had resistances of ~10 MΩ, except where otherwise indicated. In other experiments, a microelectrode was used to inject EGTA (Eastman Kodak Co., Rochester, NY). EGTA was injected by pressure (2–5 psi) through a large-tipped microelectrode (~3 MΩ when filled with 3 M KCl) filled with 1.5 M EGTA that had been adjusted to pH 7.2 with KOH. In some of the experiments, 7 mM HEPES was added to the EGTA solution.

For measuring the voltage dependence of the macroscopic current, a two-microelectrode voltage-clamp method similar to that described by Lisman and Brown (1972) was used. Membrane current was measured with a current-to-voltage transducer. The voltage signal representing clamp current was smoothed with a single-stage RC filter that had a time constant of 1 ms.

RESULTS

Latency of Channel Activity

The latent period is defined as the time after a flash before there is a detectable response. Macroscopic recordings indicate that latency depends on light intensity, decreasing as light intensity is raised. In no case, however, is the latency of the macroscopic response longer than 150 ms under normal conditions. In contrast, Bacigalupo and Lisman (1983) described patch-clamp recordings in which light-induced channel activation occurred with a latency of as much as 5 s. However, as shown in Fig. 1, this kind of discrepancy represents an extreme case. The figure shows patch-clamp records from three cells (A–C) in which the latency of channel activity was very different. In Fig. 1 C, the latency was >2 s (see also Fig. 3). The activity in this cell rose to a maintained level; there was no sign of an initial burst of activity as would be expected from the macroscopic response (not shown), which has a large initial transient phase (Millecchia and Mauro, 1969). In such cells, channel activity also ended slowly (2–10 s) when the light was turned off (not shown), which again differs from the macroscopic response, which turns off in <200 ms. Fig. 1 A shows recordings from a patch on another cell in which the difference between the latency of the macroscopic response and the channel currents was small (<30 ms). In 2 of the 17 patches studied, the envelope of channel activity had roughly the same kinetics as the macroscopic response. In such patches, channel activity was initially high but then declined to a lower maintained level (see Fig. 2) in a manner similar to that of the macroscopic response. Fig. 1 B shows an intermediate case in which there was a clear discrepancy of channel and macroscopic latencies, but it was not as large as that in the bottom trace. Why the discrepancies between macroscopic and channel currents

should vary so much from cell to cell is difficult to assess since the biochemical basis for the latent period is not known. Possible explanations are given in the Discussion. In all cells studied, the latency of channel activation decreased when the light intensity was raised, in agreement with properties of the macroscopic response.

Effect of Light Adaptation on Channel Activation

Photoreceptors adapt to light: the conductance increase evoked by a light stimulus of given intensity is reduced in amplitude if the cell has been previously
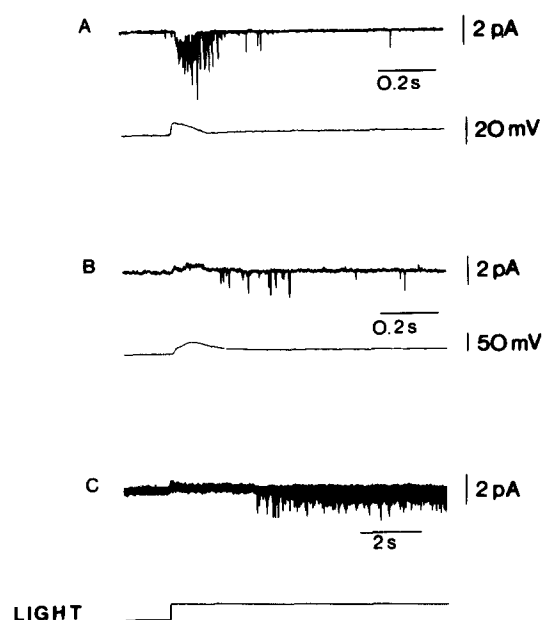

FIGURE 1. Latency of channel activity evoked by step of light (bottommost trace) in three different cells. (A) Channel activity (top trace) and macroscopic response (second trace) have similar latencies. The macroscopic response was monitored with a microelectrode, which, in this case, had almost come out of the cell. The amplitude of the voltage is therefore attenuated. (B) Channel activity (top trace) in this cell lags the macroscopic response (second trace) by ~200 ms. (C) Channel activity in this cell lags the macroscopic response by several seconds. The macroscopic response was not measured directly in this cell, but its onset is indicated by the small, positive deflection in the current trace.

exposed to an adapting light (Lisman and Brown, 1975a). The response to light recovers in magnitude if the cell is allowed to dark-adapt. The experiment in Fig. 2 shows that light adaptation can also be observed at the channel level. Channel activity was first evoked by stimulating a dark-adapted photoreceptor with a step of light (upper trace). This stimulus both excited and adapted the cell. From work at the macroscopic level, it is known that adaptation begins during the response to an adapting light and accounts for the decline in the response during the stimulus (Lisman and Brown, 1975a). This decline is also

evident in the channel response as a reduction in the opening rate during adapting stimuli (Fig. 2, top and bottom traces). 10 s after the first adapting light was turned off, a second presentation of the same light produced channel activity (middle trace) that was much smaller than the response of a dark-adapted cell. After the cell was left in the dark for 3 min, the response (bottom trace) recovered to the dark-adapted level. Similar results were obtained in the six patches studied using this protocol.

Effect of Intracellular EGTA

Previous work on the macroscopic properties of the light-activated conductance in *Limulus* ventral photoreceptors has shown that intracellular free Ca^{2+} plays an important role in the adaptation process. Ca_i^{2+} goes up during light (J. E. Brown
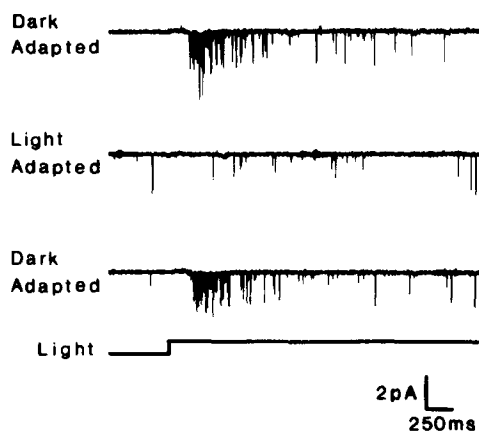

FIGURE 2. Light adaptation observed at the single channel level. The top trace is the light response of the dark-adapted photoreceptor. 10 s after turning off the light, the stimulus was repeated, and the channel response evoked by the stimulation is shown in the middle trace. The bottom trace is the response of the cell to the stimulus after the cell was dark-adapted for 3 min. Stimulus intensity, −1.0.

and Blinks, 1974; J. E. Brown et al., 1977; Levy and Fein, 1985), and this elevation leads to a reduction in the conductance activated by light (Lisman and Brown, 1972). It is not known whether Ca^{2+} acts directly on channels themselves or at some earlier stage in the transduction process. One way of reducing the normal light-induced rise in Ca_i^{2+} is by injecting the calcium chelator EGTA into the cytoplasm (J. E. Brown and Blinks, 1974). This leads to a dramatic increase in the maintained conductance evoked by a steady light (Lisman and Brown, 1975b). It follows that if the channels we have observed underlie the macroscopic light-activated conductance, the total charge carried by these channels in response to a given stimulus should be increased by EGTA injection. On the other hand, if the channels we have observed open as a result of a rise in Ca_i^{2+}, as occurs for some types of channels observed in other systems (Marty, 1981; Yellen, 1982; Latorre et al., 1984), then the activation of the channels should be inhibited by EGTA.

The effects of EGTA injections on light-induced channel activity are shown in Fig. 3. Channel currents were observed using a patch pipette. At the same time, the cell was impaled with a microelectrode that could be used for injection of EGTA. Before injection, three sequential, identical stimuli were given at intervals

Before Injection

1st Injection

2nd Injection
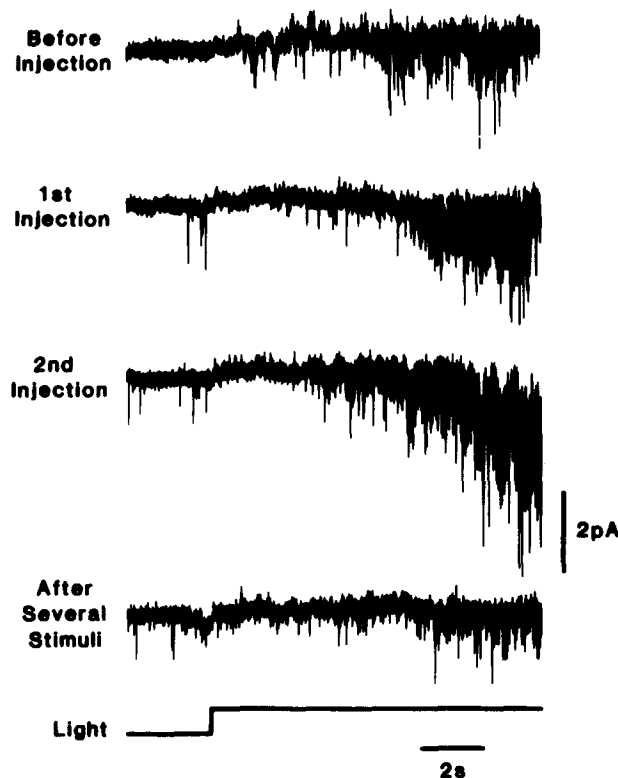
2pA

After Several Stimuli

Light

2s

FIGURE 3.   Effect of EGTA on channel activation. Patch current measured before and after successive EGTA injections. EGTA was contained in the voltage-measuring microelectrode and pressure-injected into the photoreceptor by applying 3–5 psi for ~5 s to the back of the microelectrode. Identical light stimuli of intensity −1.0 were applied at 1-min intervals. Approximately 5 s before each stimulus, the patch was hyperpolarized to 50 mV below resting potential. The purpose of this pulse was both to compensate for the drop in the driving force during the large maintained light-induced depolarization that occurs in EGTA-injected cells (Lisman and Brown, 1975b) and to prevent activation of the voltage-dependent channels in this patch. Each successive EGTA injection progressively increased channel activity. Light-induced channel activity was reduced after subsequent repetitive light stimuli (bottom trace), probably because of saturation of the injected EGTA.

of ~1 min. Each stimulus evoked a similar response, one of which is shown in the top trace of Fig. 3. EGTA was then injected (see Methods), which caused a large increase in the plateau of the macroscopic voltage response to light. This effect of EGTA injection has been previously described and shown to be due to

the Ca^{2+}-buffering capacity of EGTA (Lisman and Brown, 1975b; J. E. Brown and Blinks, 1974; J. E. Brown et al., 1977). After EGTA injection, the light-induced channel activation was greater than before injection, as shown in the second trace of Fig. 3. After further EGTA injection, the channel activity became even greater (third trace of Fig. 3). The response decreased somewhat after several further light stimuli, a phenomenon also seen at the macroscopic level and thought to be due to saturation of EGTA with Ca^{2+} (Lisman and Brown, 1975b). The basic conclusion from experiments like those in Fig. 3 is that channel activation is not a consequence of the light-induced rise in Ca_i^{2+}, because it is not inhibited by EGTA. Rather, channel activation is enhanced by EGTA, as is the macroscopic response. In EGTA-injected cells, the rate of channel opening during a steady light gradually falls, probably because of saturation of EGTA. Under these conditions, the conductance and mean open time of the light-activated channels could be measured and was found to be in the same range as those in uninjected cells.

Voltage Dependence of Light-activated Channels

Channel currents were measured during continuous illumination at a series of different patch voltages. The reversal potential for the single channel currents was obtained from the current-voltage (I-V) plot of these currents and was +10 mV for the cell shown in Fig. 4. In five experiments, the reversal potential for the light-activated single currents ranged from +2 to +19 mV (8.6 ± 3.2 mV; mean ± SEM), which is within the range previously reported for the macroscopic light-activated current: 0 to +20 mV (Millecchia and Mauro, 1969; J. E. Brown and Mote, 1974). From the slope of the I-V curve in Fig. 4, the conductance of the channel was calculated to be 45 pS. The average value for the single channel conductance was 39.2 ± 2.8 pS (mean ± SEM, n = 5). The I-V relationship of the light-activated channel was linear within the voltage range we examined, which indicates that the conductance of the open channel did not depend on the membrane potential. In making these I-V curves, we ignored the infrequent, smaller channel events (Fig. 3 of Bacigalupo and Lisman, 1983) that occur during light. Although a systematic study of a single patch was not done, measurements in different patches at different light intensities (−3.0–0) indicated that neither the single channel conductance nor the mean open time has any obvious dependence on light intensity.

Previous work (Bacigalupo and Lisman, 1983, 1984) has shown that the mean open time of the light-activated channel is in the millisecond range at resting potential. Fig. 5 shows that the value of the mean open time increases when the patch voltage is strongly depolarized. Fig. 5A shows a series of current traces that were obtained at various patch potentials during steady illumination (−1.3). The mean open time of the channel is plotted vs. the patch potential in Fig. 5B. The mean open time was ~4 ms between −32 and +8 mV, but rose sharply between +8 and +40 mV. The experiment of Figs. 5 and 6 was done on a cell that had been injected with EGTA. This patch contained only a single channel, whereas all other patches in which we examined the voltage dependence of the open time contained multiple light-activated channels and/or voltage-dependent channels. In most patches, the maximum light-induced inward current was 2–10

times larger than the single channel current, which indicates that the patch contained many light-activated channels. In such cells, it was not possible to measure unambiguously the mean open time at positive voltages because the overlap of events was large, even during the steady state part of the response. It was nevertheless obvious from such records that the mean open time of the light-activated channel increased dramatically at positive voltages.
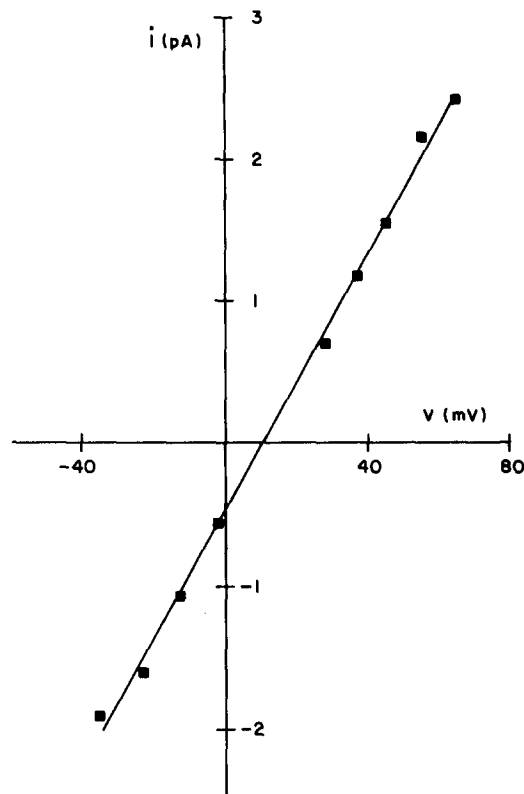

FIGURE 4. Current-voltage relationship for current through a single light-activated channel. The patch potential was varied by imposing a voltage in the patch pipette. The abscissa is the absolute patch potential. The straight line was fit by the least-squares method. The single channel conductance obtained from the slope of the curve was 45 pS. The reversal potential was +10 mV.

In the same patch as in Fig. 5, we measured the rate of channel opening during the maintained response to a steady light at a series of patch potentials. The data are plotted in Fig. 6. The plot shows that the rate of opening of the channel increased approximately fourfold between −32 and +40 mV, an effect that was observed in all patches in which the opening rate was examined ($n = 4$). In the cells used for this type of analysis, the rate of channel opening in the dark was insignificant compared with that in the light over a wide voltage range (Bacigalupo and Lisman, 1983), so no correction for dark events was necessary. In a

few cells that we studied, channel activity in the dark was significant, but we are not yet certain whether these events were due to some other channel with properties similar to the light-activated channel or whether they represent spontaneous openings of the light-activated channel.
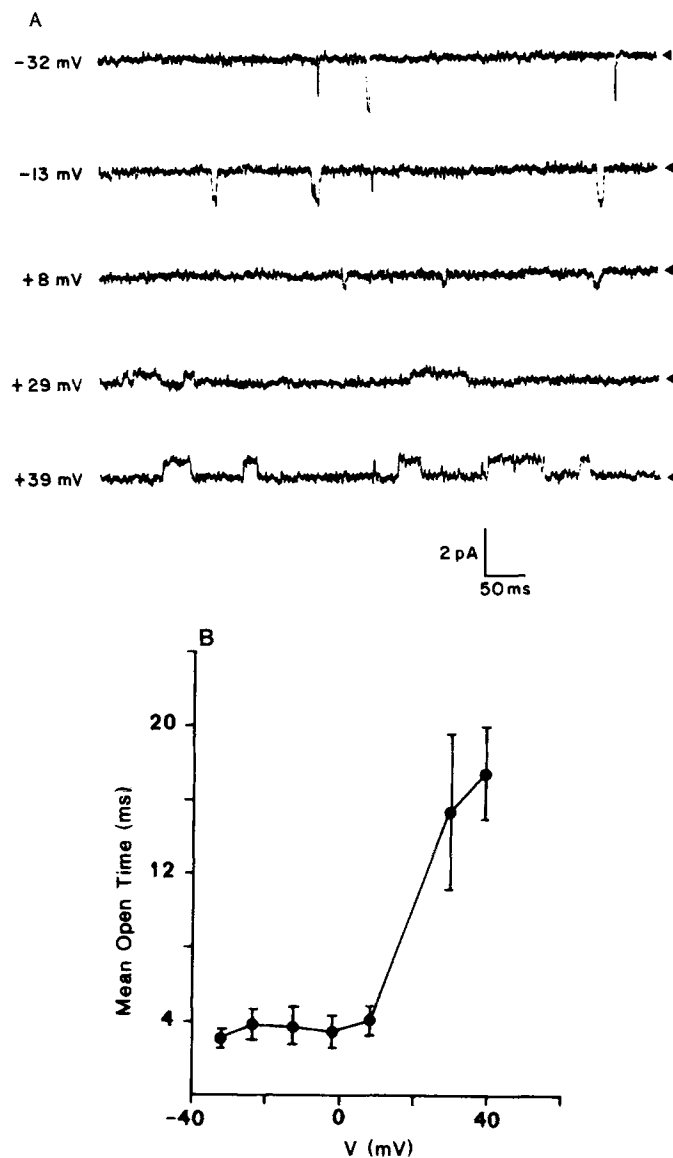
FIGURE 5.   Voltage dependence of mean open time of the light-activated channel. (A) Patch current recordings obtained at various patch potentials. The numbers at the left indicate absolute patch potentials. (B) Plot of the mean open time vs. voltage from the same patch as in A. The number of events used to compute the open time at each voltage (starting with the most negative) was 58, 8, 10, 12, 19, 24, and 40.

The steady state macroscopic conductance depends on the product of the number of channels in the cell times the single channel conductance times the probability of a channel being in the open state (P_o). Of these three terms, only P_o is voltage dependent. P_o, in turn, depends on the product of the rate of opening and the mean open time. This product, which is computed from the data in Figs. 5B and 6, is plotted as a function of voltage in Fig. 7 and will be compared with the voltage dependence of the macroscopic conductance in the next section.

Voltage Dependence of Macroscopic Currents

To measure the effects of voltage on the macroscopic light-induced currents, intact cells were voltage-clamped at a holding potential of -70 mV and were depolarized to a variety of different potentials. 200 ms after the onset of each
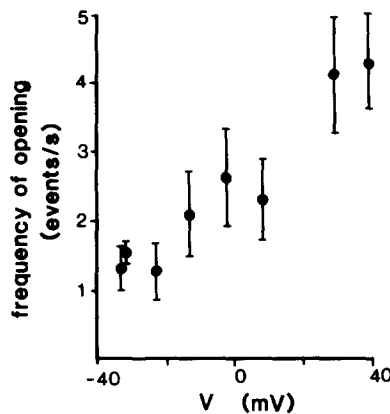

FIGURE 6. Opening frequency (number of events per second) as a function of voltage during steady light (-1.6). Data are from same patch as in Fig. 5.

voltage step, the cell was exposed to a 100-ms light flash. The reason for using flashes to quantitate the macroscopic conductance rather than steady light is that the latter could lead to a voltage-dependent rise in Ca^{2+}, a rise that by itself can reduce the light-activated conductance (O'Day et al., 1982). Fig. 8 shows the superimposed response to light obtained at potentials from -30 to $+60$ mV (in 10-mV increments). The reversal potential in this cell was $+10$ mV. The two traces marked by stars ($+50$ and -30 mV) had electrochemical driving forces of equal magnitude, but the outward response at $+50$ mV was much larger than the inward response at -30 mV, which indicates a marked outward rectification. In addition, the duration of the falling phase of the response was longer at very positive voltages and the time of the peak current occurred at a later time. Because of the voltage dependence of these kinetic parameters, we quantified the magnitude of the conductance by integrating the current during each response and dividing this value by the driving force. The resulting number, which we term the pseudoconductance, g', is equal to the single channel conductance times the number of channel openings per flash times the mean open
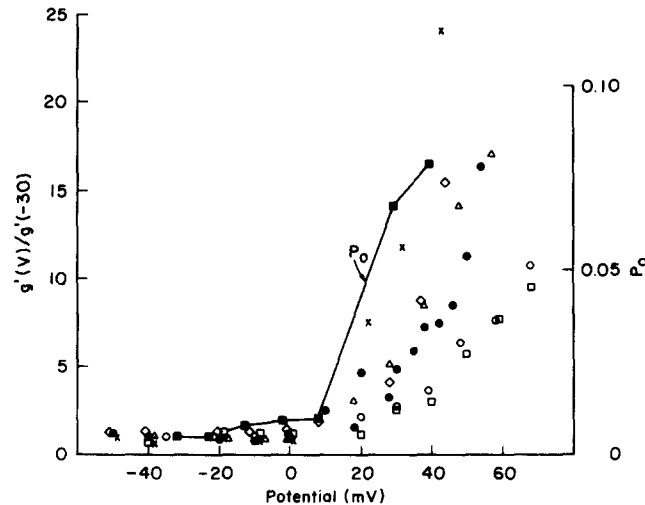
FIGURE 7. Comparison of the voltage dependence of the macroscopic light-activated conductance with the voltage dependence of a light-activated channel. The probability of a channel being open (P_o), computed from the data in Figs. 5 and 6, is designated by the solid line. The voltage dependence of the normalized macroscopic pseudoconductance is designated by the unconnected points for six cells. The pseudoconductances were calculated by integrating the current evoked by a brief flash (see Fig. 8) and dividing this number by the driving force. The pseudoconductances were then normalized to the value at −30 mV. The abscissa is the membrane potential: the absolute membrane potential of the voltage-clamped cells and the absolute patch potential in the patch-clamped cell.

time of the channel. Only these last two parameters are voltage dependent. Their product is proportional to the probability that a channel is in the open state (P_o) during a steady light. Thus, if the channels we have observed underlie the macroscopic response, g' should be directly proportional to P_o.
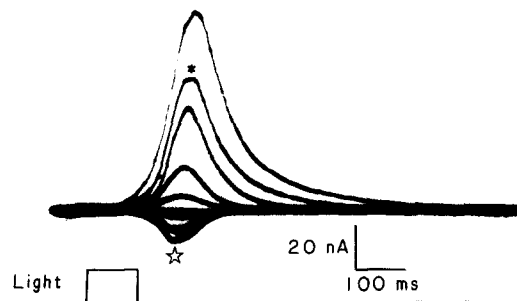

FIGURE 8. Effect of membrane potential on the kinetics and amplitude of the macroscopic light-activated current. The cell was voltage-clamped at a holding potential of −70 mV. Voltage steps of various values were applied from this holding potential; 200 ms after the onset of each voltage step, the cell was stimulated with a 100-ms light flash. The reversal potential was +10 mV. The rectifying nature of the light-induced current is illustrated by the two starred traces (*, +50 mV; ☆, −30 mV), which have equal but opposite driving forces.

g'-V curves computed from the macroscopic data from several cells are shown in Fig. 7. The curves have been normalized at 1 to -30 mV. The reversal potentials for different cells varied slightly, but all were in the range of 0 to $+15$ mV, as previously described (Millecchia and Mauro, 1969). In the voltage range of -60 to 0 mV, g' is nearly independent of voltage. In contrast, g' increases dramatically in the range of 0 to $+60$ mV. The g' at $+40$ mV is 5–25 times larger than at -30 mV. The reason for the large variation in this ratio is unclear. The variation is probably due in part to shifts in the curve along the voltage axis. Some of this shift may be artifactual because of the variation in electrode tip potential. However, the curves cannot be made to superpose exactly, even by arbitrarily shifting them along the voltage axis. This suggests that the slope and position of the rectification may depend on factors unique to each cell. Despite this variation, the macroscopic voltage dependence shows a close qualitative resemblance to the voltage dependence of the channel, which strongly suggests that the voltage dependence of channel gating properties can account for the voltage dependence of the macroscopic conductance. It should be pointed out that in making this comparison, we have assumed that the adaptational state of the cell and the level of internal transmitter are constant at all voltages at which channel properties were measured. This assumption seems reasonable since only the patch voltage was changed.

DISCUSSION

The similarities between the macroscopic light-activated current and single channel currents activated by light are as follows.

(a) Both the macroscopic current (Millecchia and Mauro, 1969; J. E. Brown and Mote, 1974) and the single channel currents (Bacigalupo and Lisman, 1983) have a reversal potential near $+10$ mV.

(b) Injecting the Ca^{2+} buffer EGTA increases the maintained macroscopic current (Lisman and Brown, 1975b) and increases the probability of opening of the light-activated channels during a maintained light (Fig. 3). Thus, neither the macroscopic current nor the single channel currents require the light-induced rise in Ca_i^{2+} (J. E. Brown and Blinks, 1974) for their activation.

(c) Both the macroscopic current and the single channel activity are graded with light intensity (Bacigalupo and Lisman, 1983) and affected by a preceding adapting light (Fig. 2); both have a latency after the onset of light that decreases when the intensity of the light is increased (Bacigalupo and Lisman, 1983). During a sustained light, the macroscopic current is initially high, but then declines to a smaller maintained value. A similar pattern of activity is seen in patches having a short latency of channel activation (Fig. 1), but not in patches having very long latencies (see below).

(d) The macroscopic light-activated conductance is nearly voltage independent at voltages more negative than zero but increases sharply as the voltage is made positive (Figs. 7 and 8). Similarly, the channels have a probability of being in the open state that is nearly voltage independent at negative voltages but sharply increases at positive voltages (Fig. 7).

(e) From the maximal macroscopic current ($\sim$0.5 μA; J. E. Brown and Coles, 1979), the current through a single channel (2 pA) at resting potential, and the

area of transducing membrane (2×10^{-3} cm^2; Calman and Chamberlain, 1982), the channel density is estimated to be $1.2/\mu$m^2. When light-dependent channels are observed in patch recordings, the maximum patch current is usually 2–10 times larger than the current through a single channel, which indicates that the average patch contains on the order of five channels. The area of the patched membrane can be estimated as 2–10 μm^2 (Sakmann and Neher, 1983); this indicates that the channel density is ~$1/\mu$m^2, which is in good agreement with the estimate from macroscopic data.

The largest discrepancy between the properties of the single channel and macroscopic currents relates to the kinetics of channel activation. The latency of the single channel currents was usually greater than that of the macroscopic response, sometimes by as much as several seconds. Two plausible hypotheses could explain these discrepancies. One possibility is that the channels we have studied may be abnormally affected by light because of a local deformation of the membrane within the suction pipette (Hamill et al., 1981). This deformation might, for example, increase the latency by separating the channels from their source of second messenger. A second possibility is that the channels we have sampled are a small, normal subset of the light-activated channels that open with a latency different from that of the typical light-activated channel. Preferential sampling might occur if the regions generating the bulk of the light-induced current were regions with typical microvillar morphology, and if such microvillar regions were unfavorable for the formation of gigohm seals. Thus, the successful formation of seals might occur with greater probability on atypical regions of the membrane. Whereas in most patches the discrepancy between channel and macroscopic latencies was large, there were patches in which it was negligible. This kind of variation would be hard to explain on the hypothesis that these channels are responding secondarily to changes in cell energy metabolism, as suggested by Auerbach and Sachs (1984).

Finally, it is of interest to compare our estimate of ~40 pS for the single channel conductance and 3 ms for the channel lifetime with the values of 18 pS and 19 ms, respectively, derived by noise analysis of the macroscopic current (Wong, 1978). Given the many assumptions associated with noise analysis in photoreceptors (Fain and Lisman, 1981) and the fact that high-frequency noise (>50 Hz) was not measured, these differences do not seem large or unexplainable.

Mechanism of Voltage Dependence

The voltage independence of the single channel conductance (Fig. 4) and the voltage dependence of the channel lifetime (Fig. 5) and opening probability (Fig. 6) clearly demonstrate that the light-activated conductance is voltage dependent because voltage affects channel gating. This conclusion is consistent with previous measurements of the voltage dependence of the macroscopic light-activated conductance in barnacle photoreceptors (H. M. Brown et al., 1970). These measurements showed that the instantaneous current-voltage curve was linear. However, with a time constant of 13–15 ms, the current-voltage curves became strongly outwardly rectifying. It is intriguing that *Limulus*, barnacle photoreceptors, and vertebrate rods (Bader et al., 1979) all have a light-dependent conductance that shows strong outward rectification.

Implications for Light Adaptation

Light adaptation in *Limulus* photoreceptors is due, in part, to a reduction of the conductance activated by a given light (Lisman and Brown, 1975a); this reduction is mediated by the light-induced rise in Ca_i^{2+} (Lisman and Brown, 1972; J. E. Brown and Blinks, 1974). Ca^{2+} might reduce the conductance by affecting the gain-producing reactions that create the transmitter that opens the channel. Alternatively, Ca^{2+} might reduce the macroscopic conductance by acting on the channel and reducing the mean open time, opening rate, or conductance. Our data indicate (Fig. 4) that light adaptation acts, at least in part, by reducing the probability of channel opening. Whether this is a direct or indirect effect on the channel is unclear. If light adaptation also affected the channel's conductance or open time, these channel properties ought to depend on the intensity of light, since intensity determines the level of light adaptation (Lisman and Brown, 1975a). In our experiments, however, we detected no obvious change in the single channel conductance or the mean open time at the various light intensities that we used to stimulate cells (−3.0–0). It therefore seems unlikely that changes in the sensitivity of the cell occur because of changes in the channel open time or single channel conductance. However, since we have not observed channel activation by very dim lights, our data do not exclude the possibility that channels have longer open times under dark-adapted conditions, a possibility suggested by Raggenbass (1983) and Dirnberger et al. (1985).

Comparison with Other Channels

The light-activated channels in *Limulus* are relatively nonselective for cations (J. E. Brown and Mote, 1974) and in this regard are similar to the acetylcholine-activated channel at the neuromuscular junction (Adams et al., 1980). Under conditions of temperature and ionic strength similar to those we have used, the acetylcholine-activated channel has a conductance of 48 pS and a mean open time in the millisecond range (Montal et al., 1984), values that are close to those of the light-activated channel in *Limulus*. Thus, in contrast to the situation in vertebrate rods, where ion permeation appears to occur by some unusual mechanism (Detwiler et al., 1982), the light-activated channels in *Limulus* appear to be classic channels similar to those underlying other neuronal events.

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