J. Physiol. (1983), **340**, *pp.* 287–305
With 9 *text-figures*
Printed in Great Britain

THE CATION SELECTIVITY AND VOLTAGE DEPENDENCE OF THE LIGHT-ACTIVATED POTASSIUM CONDUCTANCE IN SCALLOP DISTAL PHOTORECEPTOR

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(*Received 3 June 1982*)

SUMMARY

1. Light-dependent voltage and current responses were measured from the distal hyperpolarizing photoreceptors of the scallop (*Pecten irradians*) retina.

2. In normal external solution, the hyperpolarizing receptor potential was caused by a light-dependent K^+ outward current. The magnitude of the hyperpolarizing receptor potential and the light-dependent outward current, measured at the resting potential, was graded with light intensity.

3. In normal external solution, during prolonged illumination the light-dependent K^+ outward current was characterized by an early peak and a subsequent plateau. Current responses to brief light flashes were reduced progressively during background illumination.

4. In the absence of external Na^+ ions, the reversal potential for the receptor potential changed 58 mV per 10-fold change in the extracellular K^+ concentration. The estimated internal K^+ concentration was 385 mM.

5. The hyperpolarizing receptor potential produced by prolonged bright illumination consists of an early peak which decays to a plateau. This decay was determined by a decrease in the light-dependent K^+ conductance during maintained illumination.

6. The light-dependent conductance pathway passed outward currents better than inward K^+ currents. The light-dependent K^+ conductance was estimated to increase e-fold per 23–34 mV depolarization at the peak and during the plateau of the light response.

7. The light-dependent conductance pathway was highly selective for K^+ ions. The selectivity sequence for monovalent cations was Tl^+, $K^+ > Rb^+ > NH_4 > Cs^+$, Li^+, Na^+.

8. External caesium and tetraethylammonium blocked inward but not outward K^+ currents through the light-dependent K^+ conductance pathway.

9. The data suggest that K^+ ions move through an aqueous pore which is controlled by light.

INTRODUCTION

The receptor potential of all of the photoreceptors studied so far is caused by a change in membrane conductance to cations (see Ebrey & Honig, 1975). The

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selectivity of the different light-dependent conductance pathways found in various vertebrate and invertebrate photoreceptors for inorganic and organic molecules could provide information about the structure of the conductance pathway, but has not been fully determined. For those invertebrate photoreceptors whose depolarizing receptor potential is caused primarily by an increase in membrane conductance to Na^+ ions, Na^+, Li^+, K^+ and perhaps Ca^{2+}, choline and Tris pass through the conductance pathway (Brown, Hagiwara, Koike & Meech, 1970; Brown & Mote, 1974). For the vertebrate photoreceptors, whose hyperpolarizing receptor potential is caused by a decrease in membrane conductance, there is evidence that the conductance pathway may be highly selective for Na^+ ions (Yau, McNaughton & Hodgkin, 1981, but see Woodruff & Fain, 1980). The hyperpolarizing receptor potential of the distal photoreceptors in the scallop retina is caused by an increase in membrane K^+ conductance (Gorman & McReynolds, 1978). To determine how selective this pathway is for K^+ ion movement a single-electrode voltage-clamp circuit was used so that current flow could be measured directly. In this paper we show that the light dependent pathway is highly selective for K^+ ions and the conductance of the pathway is dependent upon membrane potential as well as light intensity. The dependence of this K^+ conductance upon the wave-length of illumination is determined in the succeeding paper (Cornwall & Gorman, 1983).

METHODS

Preparation. All experiments were done on the distal photoreceptors in the isolated retina of the scallop *Pecten irradians*. Animals were obtained from the Marine Biological Laboratory at Woods Hole, MA. The cornea and lens were removed to expose the distal photoreceptors which are located directly beneath the retinal surface. The eye was held firmly against the bottom of a channel in a special chamber through which artificial sea water (ASW) flowed perfusing the exposed retina. Changes in the perfusion solution were made by manually switching a series of valves connected into a manifold located close to the retina. About 15 sec were required for a complete replacement of solution in the chamber at the normal flow rate. The time required for a complete change of solution surrounding the distal photoreceptors was about 30 sec (Cornwall & Gorman, 1979). To facilitate micro-electrode penetration through the fibrous septum which covers the retinal surface, the eye was treated with a mixture of 0·5 % pronase and 0·25 % collagenase for about 1·5 min.

Light stimuli. The optical stimulator contained a tungsten quartz-iodide lamp (FCS Westinghouse Quartzline, 24 V, 150 W) and was designed to provide either step increments or decrements in light intensity (see Cornwall & Gorman, 1979). Light was passed through a condenser, electrically controlled shutter and a box containing neutral density filters and subsequently through a field lens, a field stop and finally through a 6·0 × microscope objective lens which was used to focus the beam to an evenly illuminated spot that covered the entire retina. The intensity of the beam was varied with neutral density filters that were calibrated between 400 nm and 700 nm with a spectrophotometer. The light stimulator was controlled by an electronically operated shutter (Uniblitz model 225, Victor Assoc., Rochester, N.Y.) which could be driven either in the normally open or normally closed mode by a specially designed driver circuit (Cornwall & Thomas, 1979).

Light calibrations. The maximum white light intensity was calibrated in terms of the effective photons at the wave-length of maximum sensitivity of the distal cells under dark-adapted conditions (see Cornwall & Gorman, 1979). The spectral sensitivity curve for these cells has a maximum at about 500 nm (McReynolds & Gorman, 1970*b*). The equivalent quantum density of white light referenced to the distal photoreceptor sensitivity at 500 nm was calculated to be $5{\cdot}6 \times 10^{16}$ photons cm^{-2} sec^{-1}. The light, except where specifically noted, was attenuated by about 0·9 log units. At this light intensity the distal photoreceptor produces a maximum hyperpolarizing receptor potential (McReynolds & Gorman, 1970*a*).

Electrical measurements. Recordings were made with micro-electrodes pulled from 'Omega dot'

tubing (Glass Co. of America, N.J.) and filled with 4 M-potassium acetate. Micro-electrodes were selected with resistances between 50 and 75 MΩ when measured in ASW. In early experiments the micro-electrode was connected to a high impedance, negative capacity compensated electrometer (Mentor N-950, Mentor Corp., Minneapolis, MN, U.S.A.) which had provision for passing constant current across the cell membrane through an active bridge circuit. This facility was used routinely to set membrane potential at various levels and to assess the membrane resistance under different experimental conditions. The bridge was balanced prior to and after cell penetration. All recording were referenced to a grounded Ag–AgCl electrode connected through a 3 M-KCl bridge to the flowing ASW in the chamber downstream from the preparation.

In later experiments the recording micro-electrode was connected to a high impedance differential pre-amplifier (Thomas, 1977). The output of this amplifier was fed to a single-electrode clamp circuit that could be used in either the current or voltage-clamp mode. The circuit used to construct the clamp was based closely on a design published by Bader, MacLeish & Schwartz (1979). The theory of operation of a single electrode voltage clamp has been described previously by several groups (see Wilson & Goldner, 1975; Bader *et al.* 1979; Merickel, 1981). In this technique, the same electrode is used alternately to measure voltage and to pass current in order to control the membrane potential. Since the passage of current changes the potential across the micro-electrode as well as the potential across the membrane, ideally the time constant of the micro-electrode should be much smaller than the time constant of the membrane. The maximum possible switching rate (and, therefore, the frequency of signals that can be clamped) is determined by the time required for the potential measured by the intracellular micro-electrode to settle to the membrane potential at the end of a current pulse. The time constant (τ) for the rise or decay of potential across a micro-electrode connected to ground through a low resistance bathing medium is determined by its resistance (R) in combination with the capacitances (C) of the electrode and of the amplifier to which it is connected (i.e. $\tau = RC$). In practice, it is difficult to reduce the electrode resistance below a critical level (about 50 MΩ) and at the same time obtain stable intracellular recordings from the distal photoreceptors because of their small size (*ca.* 10 μm diameter). The capacitance, however, can be reduced appreciably. The capacitance associated with the input and a part of the capacitance associated with the micro-electrode were neutralized by a positive feed-back circuit in the high input impedance pre-amplifier (see Thomas, 1977) of the single-electrode voltage clamp. The capacitance of the portion of the micro-electrode that is immersed in the bathing media is separated from the amplifier input capacitance by part of the electrode resistance and can never be neutralized completely by the positive feed-back circuit where it is assumed that the total capacitance (both amplifier input and micro-electrode) behaves as a single component. This distributed capacitance makes a significant contribution to the *minimum effective capacitance* that can be achieved. The micro-electrode was coated with a wax of high dielectric constant to about 75 μm of its tip using a technique described by Cornwall & Thomas (1981). This reduced the capacitance of the distal portion of the electrode about 7-fold. The advantage of a coated electrode is that the distributed capacitance is reduced thereby decreasing the minimum effective value of the capacitance that can be achieved by neutralization (see Cornwall & Thomas, 1981).

Each micro-electrode that was used with the single-electrode voltage clamp was tested in the recording chamber at the level of the retina before and after impalement of photoreceptor cells with 300 μsec constant current pulses of different intensity. The amplitude of the change in voltage produced by a current pulse of any intensity was used to measure the electrode resistance and the decay of the voltage change was used to determine the time constant of the electrode. Electrodes of about 60 MΩ were usually chosen. When the high impedance amplifier was adjusted for optimum neutralization of the total input capacitance the decay of a change in voltage was exponential with a time constant of less than 50 μsec. The resistance and time constant of these electrodes were unchanged for repetitive current pulses up to about 3×10^{-9} A of either polarity, but both increased non-linearly when currents of greater intensity were used.

Following penetration of a photoreceptor cell, the membrane was usually stimulated with 300 μsec current pulses at a frequency of 1·67 kHz. In some cases, 500 μsec current pulses at a frequency of 1 kHz were used. A 50% duty cycle was used in both cases. The resulting changes in voltage were sampled 270 μsec after the end of each 300 μsec current pulse (or 470 μsec after a 500 μsec current pulse) and fed to the input of one side of the voltage-clamp amplifier. Because of the time delay between sampled voltage and injected current, the clamping circuit was not stable for sudden changes of command voltage that were fed into the other side of the voltage-clamp amplifier. When the voltage was stepped between two levels, the rate of change in command voltage

was limited to a constant dV/dt of 1·6 V sec^{-1}. Photoreceptor cells were usually clamped to their resting potential in the dark using a low gain setting. The gain and phase settings were then adjusted to achieve a rapid rise time. The maximum rate of rise of the hyperpolarizing light response of the distal photoreceptor was measured previously (Cornwall & Gorman, 1979) and is 1·0 V sec^{-1}.

The voltage response of the photoreceptor membrane in the dark to a step change in current decays exponentially with a minimum time constant of 15 msec. The resistance of the membrane, and therefore the time constant, is a function of light intensity, however. We estimate that at the peak of the response to maximal white light stimulus, the membrane time constant can be as small as 3 msec, and at the peak of the response to a just saturating light flash, the time constant is about 7·5 msec.

The voltage across the micro-electrode and across the membrane produced by current pulses is expected to decay according to exp $(-t/\tau)$ where t is time. For a micro-electrode time constant of 50 μsec and a membrane time constant of 7·5 msec, 270 μsec after the cessation of a current pulse the fraction of the original voltage across the electrode would be $< 0{\cdot}5\%$ whereas the membrane voltage would have only decayed by $< 4\%$. Thus, it is possible to inject current and to sample the membrane potential in alternate 300 μsec intervals during the peak of the response to a just saturating flash or step of light with only a minor error. This error becomes greater at higher light intensities, e.g. for a membrane time constant of 3 msec, the fraction of voltage across the membrane would have decayed by about 9% 270 μsec after the end of a current pulse. This error becomes proportionally greater when longer intervals are used (*ca.* 500 μsec).

Solutions. The ionic composition of the normal ASW and of various Na^+-free and Ca^{2+}-free ASW solutions in mM are given in Table 1. Na^+-free ASW solutions containing various concentrations of K^+ were made by mixing the appropriate amounts of Na^+- and K^+-free ASW (solution no. 5) with high K^+ ASW (solution no. 6). Similarly, Na^+- and Ca^{2+}-free ASW solutions containing various amounts of K^+ were made by mixing the appropriate amounts of Na^+-, Ca^{2+}-, and K^+-free ASW (solution no. 7) with high K^+ and Ca^{2+}-free ASW (solution no. 8). In experiments where various monovalent test ions were tested, some or all of the choline Cl in the Na^+- and K^+-free ASW (solution no. 5) was replaced with an equivalent amount of a test salt. The following salts were tested: LiCl, RbCl, CsCl, NaCl, Tl acetate, NH_4Cl, methylamine HCl and Hydrazine 2 HCl. In the Li- and Na^+-containing test solutions all the choline Cl (434 mM) was replaced by 868 mM of the test ion. In preliminary experiments we found that a temporary increase in the osmolarity of the bathing solution had no obvious effect on the light response. The Tl^+ ASW was made entirely from acetate salts and tested on photoreceptor cells in comparison with a K^+-containing Na^+-free ASW which was also made from acetate salts. This was done because Tl acetate, but not TlCl, is soluble at the concentration used (50 mM). Only four ions (K^+, Rb^+, NH_4^+ and Tl^+) were measureably permeable through the light-dependent K^+ channels. At the concentrations used in these experiments, the activity coefficients for these ions are assumed to be in the ratios 1·00:0·98:1·00:0·97 (Robinson & Stokes, 1965). Activity coefficient corrections were not used for the other test ions. Finally, solutions containing various concentrations of K^+ and of tetraethylammonium (TEA^+) were made by mixing the appropriate concentrations of Na^+- and K^+-free ASW (solution no. 5) and high K^+ASW (solution no. 6) with TEA^+ ASW. The pH of various solutions was adjusted to 7·8, except for the hydrazine ASW which was used at a pH of 6·5. All experiments were done within a temperature range of 21–22 °C.

Several experimental manipulations were used to improve the measurement of light-dependent currents. Voltage-dependent Ca^{2+} currents were eliminated in some experiments by the removal of external Ca^{2+} or by the addition of La^{3+} ions to the ASW to block Ca^{2+} influx. The block of Ca^{2+} currents had the additional advantage that this procedure also eliminated the Ca^{2+}-activated K^+ current. The inability of the clamp to hold membrane potential constant during large light-dependent outward currents was solved partially by making most measurements near the reversal potential for the light-dependent current where currents were appreciably smaller at all light intensities or by using less intense light to produce smaller currents. The single electrode voltage-clamp technique provides information about the electrical behaviour of small cells which is impossible to obtain by other means, but any interpretation of this data must be weighted carefully against the types of problems discussed above (see also Fig. 1*A*).

TABLE 1. Composition of external solutions (mM)

Solutions	Na	K	Ca	Mg	Choline	Tris	Cl
(1) ASW	425	9	9	49	0	15	565
(2) Na-free	0	9	9	49	425	15	565
(3) Ca-free	425	9	0	58	0	15	565
(4) Na-, Ca-free	0	9	0	58	425	15	565
(5) Na-, K-free	0	0	9	49	434	15	565
(6) High K	0	434	9	49	0	15	565
(7) Na-, K-, Ca-free	0	0	0	58	434	15	565
(8) High K, Ca-free	0	434	0	58	0	15	565

RESULTS

The light-dependent current and voltage responses

The single-electrode voltage clamp makes it possible to compare directly the light-dependent voltage and current responses from the same photoreceptor. Voltage and current responses produced by brief (100 msec) steps of white light at different intensities and recorded in normal ASW are shown in Fig. 1*A*. For dim lights the hyperpolarizing receptor potential reached a peak in about 270 msec and thereafter slowly declined (Fig. 1*A*, right). At higher intensities the time to peak was reduced to less than 140 msec, but after reaching the peak the response declined with a more rapid time course ending in a depolarizing overshoot of the membrane potential in the dark. The membrane potential was clamped to a potential which was slightly more negative than the resting membrane potential in the dark. The current responses, produced by 100 msec steps of light of identical intensity (Fig. 1*A*, left side), show that the hyperpolarizing phase of the receptor potential was caused by a flow of outward current which was graded by light intensity and that the depolarizing overshoot was associated with an inward current.

The single-electrode voltage clamp was capable of holding membrane potential constant during responses produced by dim and intermediate lights, but was only partially successful during responses produced by brighter lights (Fig. 1*A*). This inability to follow changes in membrane potential was manifested in a lack of constancy of the voltage record at the peak of the light-dependent current. The currents produced by bright lights in cells immersed in normal ASW and held at the normal resting potential of -45 to -55 mV were usually in excess of 1 nA and were seldom clamped perfectly. In addition, in normal ASW the repolarizing phase of the hyperpolarizing receptor potential is modified by voltage-dependent changes in membrane conductance to Ca^{2+} and to K^+ ions (Cornwall & Gorman, 1979). These changes are accentuated by prolonged bright illumination which drives membrane potential to a very negative value for an extended period of time, thereby removing inactivation of Ca^{2+}-channels, but can also be detected in responses associated with brief lights (*ca.* 100 msec). Activation of the Ca^{2+} channels sometimes produced a small overshoot of membrane potential (see Fig. 1*A*), but was more often manifested as a more rapid decay of the receptor potential. Fig. 1*A* also shows the very fast and small inward currents which sometimes occurred in the falling phase of the outward current responses. These inward currents represent the electrotonic invasion of action potentials from unclamped regions of the distal cell's axon. They were not present in most cells, but when present, they were blocked by the removal of external Na^+.

The light-dependent voltage and current responses from the same cell are plotted against the logarithm of light intensity in Fig. 1*B*. The measurements were made at the peak of the light response produced by 100 msec steps of light of different intensity. The voltage responses are fitted by a rectangular hyperbola or Michaelis equation $y/y_{max} = i/i(i+K)$ where y is the response amplitude produced by a flash of intensity i, y_{max} is the maximum response obtainable, and K is a constant equal to the intensity that produces a half-maximal response. In most cells the voltage responses reached saturation at light intensities where the current response was not fully saturated (Fig. 1*B*).

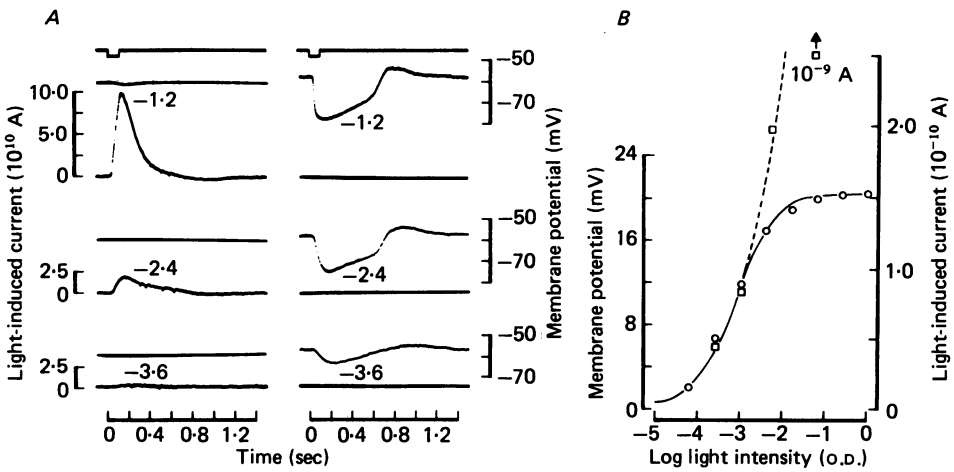

Fig. 1. Light-activated current and voltage responses from scallop distal photoreceptor cells. *A*, current (left) and voltage (right) responses activated by 100 msec steps of white light at the indicated intensities. At each intensity the current is shown below and the voltage above. Outward currents are plotted as an upward deflexion in this and subsequent Figures. *B*, plot of the peak light-activated current (□) and the peak of the hyperpolarizing receptor potential (○) *versus* the logarithm of the light intensity for the same cell shown in part *A*.

The outward membrane current during prolonged illumination

Fig. 2 shows the outward membrane current responses to a long (47 sec) step of white light of moderate intensity ($I = -2{\cdot}4$). The current rose initially to a peak, but decayed thereafter to a plateau. A small inward current occurred at the offset of light (see Cornwall & Gorman, 1983). When more intense steps of light were used the response was similar, but the difference between the peak and plateau was greater and the decay to a plateau was faster and occurred earlier (see Fig. 5). The decay of the outward current during a maintained illumination explains the characteristic peak and plateau of the hyperpolarizing receptor potential in these cells (see Fig. 3).

The amplitude and duration of the receptor potential are only slightly changed by a previous bright white light (McReynolds & Gorman, 1970*a*), which suggests that any after effects of illumination are short lived. Fig. 2 also shows changes in the membrane current response to flashes of light (100 msec, $I = -1{\cdot}8$) during maintained illumination. The magnitude of the outward current produced by the light flashes was progressively reduced during the step of white light. This reduction was greater

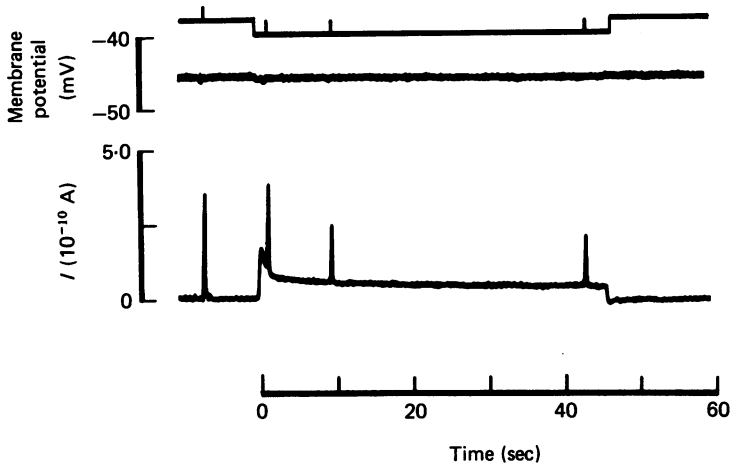

Fig. 2. The effects of prolonged illumination upon the light-dependent current. The current record shows the membrane response to a 47 sec step of light of moderate intensity ($I = -2{\cdot}4$) and to 100 msec flashes of light ($I = -1{\cdot}8$) given before and at various times during the light step. The membrane voltage record is shown above the current record. The light monitor (top) indicates the period when the light step was present.

and occurred earlier during illumination with more intense background lights. The results show that a form of light adaptation occurs in these cells during illumination. The recovery from light adaptation is analysed in the succeeding paper (Cornwall & Gorman, 1983*b*).

The light-dependent K^+ conductance

The distal photoceptors have a high permeability in the dark to Na^+ ions, and to a lesser extent, Ca^{2+} ions, (Gorman & McReynolds, 1978; Cornwall & Gorman, 1979). Fig. 3 shows the voltage responses of the cell to a prolonged (6 sec) step of light at different membrane potentials. The responses were recorded from a retina that was maintained in an ASW solution from which all the Na^+ and Ca^{2+} were removed and which contained about 1·6 times normal K^+ (15 mM). The records (Fig. 3*B*) show that the rising phase of the receptor potential and its subsequent decay to a less negative value (at potentials positive to the reversal potential) during maintained illumination occur in the absence of external Na^+ and Ca^{2+}. The decay of the receptor potential following the cessation of light was also present, but was slowed because the omission of external Ca^{2+} abolishes the voltage-dependent Ca^{2+} current activated by depolarization (see Cornwall & Gorman, 1979). The records also show that the receptor potential reverses polarity at negative membrane potentials and its magnitude depends on membrane potential, but the presence of a peak and plateau does not depend upon the direction of current flow.

Fig. 3*A* shows a plot of the voltage–current relation in the dark (filled circles), at the peak (open circles) and at the end of the plateau phase (open squares) of the receptor potential. Both the initial and the final phases of the receptor potential reverse polarity at the same potential. The data also show that whereas the voltage–current relation is linear in the dark and at the peak of the light response, the relation at the end of the response rectified strongly. The decrease in membrane

resistance at the peak of the light response *versus* the resistance in the dark (from $3 \times 10^9\ \Omega$ to $5 \times 10^8\ \Omega$) is much less apparent at the end of a prolonged light stimulus at all potentials. Moreover, the slow *increase* in membrane resistance that occurs during prolonged illumination and which was associated with the slow decay of the receptor potential depends upon membrane potential. The increase in membrane resistance was more pronounced at negative than at positive potentials.

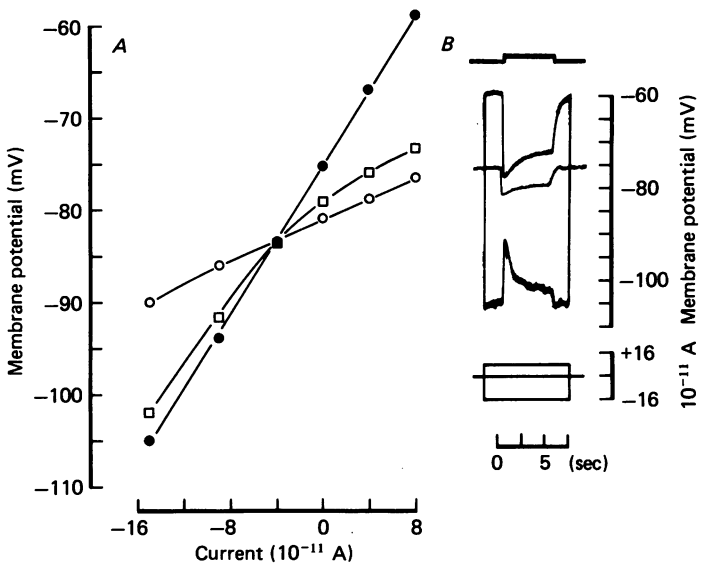

Fig. 3. Voltage–current relation of photoreceptor membrane in the dark and in light. *A*, plot of the membrane potential at the peak (○) and at the end (□) of the light response and in the dark (●) *versus* applied current. *B*, photoreceptor responses from the same cell at three different membrane potentials. The light stimulus is shown above and the current below the responses. The external solution contained 15 mM-K^+ and was Na^+- and Ca^{2+}-free.

In addition, substitution of Na^+-free (Tris substituted) or Ca^{2+}-free (replacement of Mg^{2+} for Ca^{2+} or addition of 2 mM-EGTA to Ca^{2+}-free ASW) sea water did not change the reversal potential of the receptor potential.

Similar reversal potential measurements were made in Na^+-free ASW containing different values of K^+ between 9 and 100 mM. Fig. 4 shows examples of one set of measurements made in 50 mM-K^+. The cell's response to a short (200 msec) step of light at various membrane potentials near the reversal potential is shown in Fig. 4*A*. The reversal potential for the response was −53 mV. Fig. 4*B* shows the same cell's response to a short (200 msec) step to complete darkness which interrupted a background of light of the same intensity used to produce the responses shown in part *A*. The reversal potential was also −53 mV. Reversal potential measurements from six cells for the onset and for the decay of the receptor potential are plotted *versus* the logarithm of the extracellular K^+ concentration in Fig. 4*C*. The reversal potentials for the onset and decay of the receptor potential were identical at all K^+ concentrations. The line drawn through the experimental points has a slope of 58 mV per ten-fold change in extracellular K^+, $[K]_o$, which is consistent with the predicted slope from the Nernst equation for K^+ ions. The reversal potential for the receptor

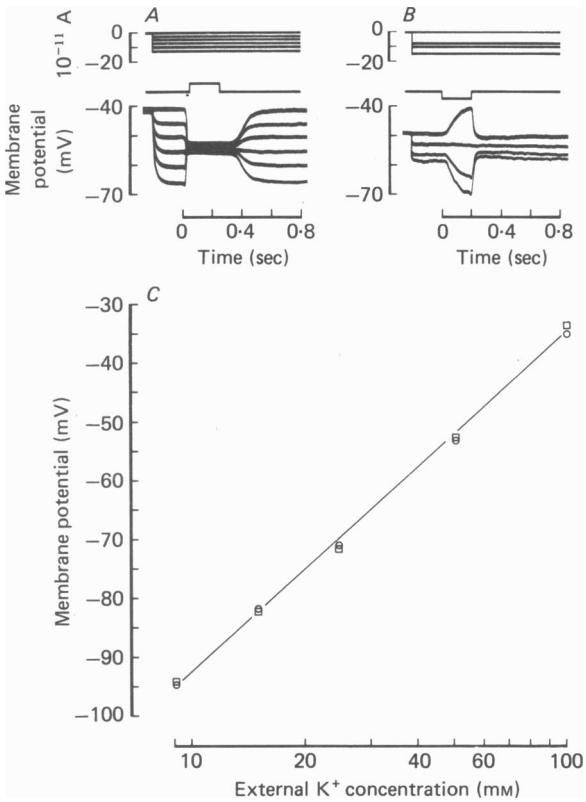

Fig. 4. Effects of the external K^+ concentration upon the reversal potential of the hyperpolarizing receptor potential. *A*, superimposed responses to a 200 msec step of white light at different membrane potentials. *B*, superimposed responses to a 200 msec step of darkness during continuous light at different membrane potentials. *C*, plot of the reversal potential for the receptor potential at the onset (○) and offset (□) of a light *versus* the logarithm of the external K^+ concentration. The external Na^+ was replaced by choline. External K^+ replaced choline on a one-for-one basis at different K^+ concentrations.

potential therefore, is equal to the K^+ equilibrium potential (E_K) for all values of $[K]_o$ where $E_K = 58$ mV $\log_{10}$ $[K]_o/[K]_i$. Because both $[K]_o$ and E_K are known, the intracellular K^+ concentration, $[K]_i$, can be estimated and for these cells was equal to $385 \pm 8{\cdot}02$ mM (S.E. of the mean).

Voltage dependence of the light-dependent K^+ conductance.

A more direct measure of the light-dependent K^+ conductance was obtained using the single-electrode voltage clamp. Fig. 5*A* shows the current produced by a prolonged (10 sec) step of white light measured at different membrane potentials. The currents were measured in Na^+- and Ca^{2+}-free ASW which contained 1 mM-La^{3+} and about 5 times the normal K^+ concentration (50 mM). The current was outward at positive membrane potentials and inward at negative membrane potentials. The current rose to an early peak, decayed to a lesser value during illumination and returned to the resting value following the cessation of light. This pattern was independent of the direction of current flow across the membrane. The voltage records

shown in the inset indicate that the current at positive membrane potentials was underestimated slightly.

The currents in the dark (filled circles), at the peak (open squares) and at the end of the 10 second light step (open triangles) are plotted *versus* membrane potential in Fig. 5*B*. Both the peak current and the current at the end of the plateau phase reverse polarity at the same value. The current–voltage relation in the dark was reasonably linear whereas the relation at the end of the light-step rectified. These

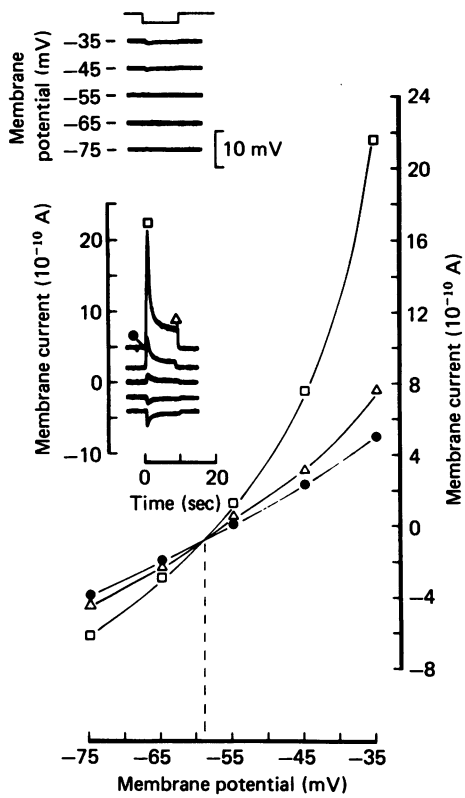

Fig. 5. Current–voltage relation of photoreceptor membrane in light and in darkness. The inset records show the current responses (bottom traces) activated by 10 sec steps of light (top) at the indicated membrane potentials (middle traces). The plot shows the current measured 1 sec before the onset of the white light (●), at the peak of the light response (□) and at the end of the white light (△) *versus* membrane holding potential. The external bathing solution was Na^+- and Ca^{2+}-free and contained 50 mM-K^+ and 2 mM-La^{3+}.

results are consistent with results obtained using current clamp (see Fig. 3*A*). The current–voltage relation measured at the peak of the response, however, was not linear. The rectification at the peak was greater than at the end of the prolonged light. Probably the change in the K^+ conductance at the peak of the receptor potential is underestimated at membrane potentials on either side of the reversal potential when the membrane is not voltage clamped (see Fig. 3*A*).

The data shown in Fig. 5 imply that the light-dependent K^+ conductance increases as the membrane potential is made more positive. They also indicate that the

light-dependent conductance decreases during continuous illumination. The data can be analysed further if we assume that current flow through the light-dependent K^+ channel (I_K) depends only on the K^+ conductance (g_K) and the driving force ($V_m - E_K$), or $I_K = g_K/(V_m - E_K)$ where g_K is the chord conductance. I_K is determined experimentally, V_M is known and E_K is determined from the reversal potential of the light-dependent current. Fig. 6*A* shows a plot of the light-induced current (I_K) measured at the peak and at the end of the 10 sec step of light, *versus* membrane potential. The data represent the difference between the current in the light and in the dark. Fig. 6*B* plots the logarithm of the chord conductance *versus* the

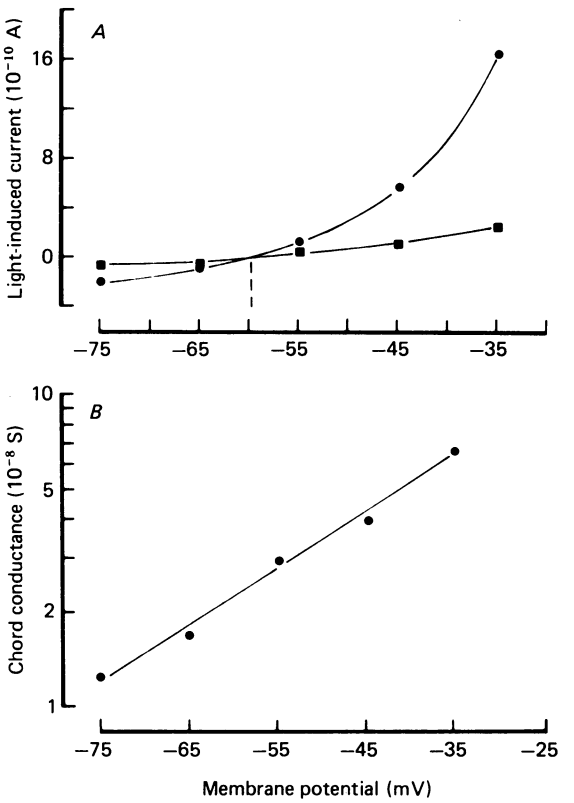

Fig. 6. The dependence of the light-activated K^+ conductance upon membrane potential. A, plot of the difference currents measured at the peak (●) and at the end of the plateau phase (■) of a response to a 10 sec step of white light *versus* membrane potential. The currents represent the difference between the current at the peak, or at the end of the stimulus, and the current in the dark at each membrane potential. *B*, plot of the chord conductance measured at the peak of the light response *versus* membrane potential.

membrane potential. The light-dependent K^+ conductance (measured at the peak of the current) increased e-fold per 23 mV change in potential. Although the current was appreciably smaller at all potentials at the end of the 10 sec light step (plateau region), the increase in conductance was of similar magnitude. In a second cell, studied under identical external ionic conditions and using maximum light intensities, the increase in conductance was e-fold per 34 mV change in potential, but in this cell

the membrane potential was less well clamped at potentials on either side of the reversal potential. In cells where weaker light intensities were used, the e-fold change in conductance occurred over a range of 40–60 mV change in potential (see Figs. 7 and 9*A*) suggesting that the voltage dependence of the light-dependent K^+ conductance may depend on light intensity, but we did not investigate this further.

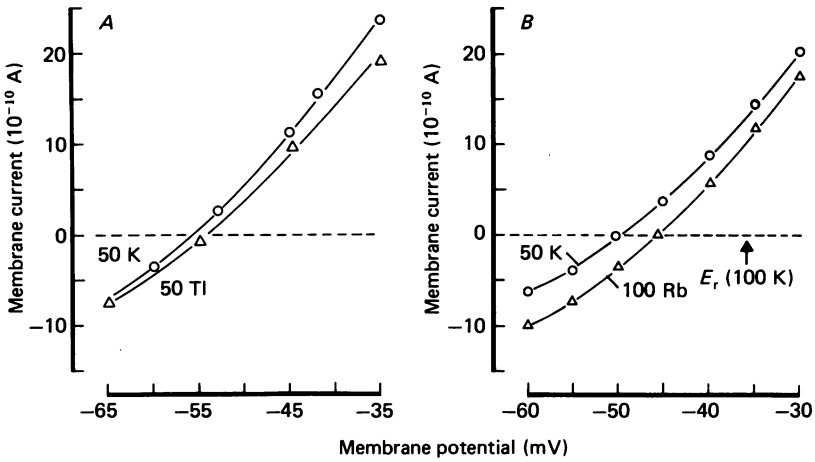

Fig. 7. Reversal potential of the light-activated current under different external ionic conditions. *A*, comparison of the current reversal potential when the external solution contained equal concentrations (50 mM) of K^+ or Tl^+ ions. *B*, comparison of the current reversal potential when the external solution contained 50 mM-K^+ or 100 mM-Rb^+. The estimated reversal potential in 100 mM-K^+ is indicated. The external solution was Na^+-free in *A* and *B*.

The selectivity of the light-dependent K^+ conductance

The selectivity of the light-dependent K^+ channel can be determined from measurements of the reversal potential of the light-dependent current. To determine the selectivity of the light dependent K^+ channel we used a method similar to the one used by Hille and his colleagues (Dwyer, Adams & Hille, 1980; Adams, Dwyer & Hille, 1980). The photoreceptor was immersed first in a Na^+-free ASW containing a known concentration of K^+ ions, and then in a Na^+-free ASW containing a known concentration of the test ion. The membrane potential was held at different potentials under voltage clamp, and the light-dependent current produced by a brief (200 msec) flash of moderate intensity white light was measured in the two solutions at each potential. Fig. 7*B* illustrates the experimental determination of the reversal potential in a cell immersed first in 50 mM-K^+ and then in 100 mM-Rb^+. We found the reversal potential for the light dependent current in each solution by plotting the difference current (see Fig. 6*A*) flowing about 60 msec after the end of the light flash. There was a positive shift of the reversal potential of +4·5 mV in the 100 mM-Rb^+ ASW. The reversal potential shifted −7 mV and −8 mV in two other cells tested in 50 mM-Rb^+ ASW. Fig. 7*A* shows a similar set of measurements from a cell immersed first in 50 mM-K^+ and thereafter in 50 mM-Tl^+. There was a positive shift of the reversal potential of +2 mV in the Tl^+ ASW.

In Table 2 we summarize the reversal potential measurements. Permeability ratios

TABLE 2. Reversal potentials and permeability ratios for monovalent cations

X	[X] (mM)	E_K (mV)	E_X (mV)	n	P_X/P_K
Lithium	868	−40·0	> −75·0	1	< 0·013
Sodium	868	−50·0	> −95·0	2	< 0·008
Potassium	50	−45·5	—	15	1·00
Thalium	50	−37·0	−35·0	2	1·07
Rubidium	50	−48·5	−56·0	2	0·730
	100	−51·0	−46·0	1	0·615
Ammonium	100	−44·5	−82·5	2	0·103
Hydrazine	100	−53·0	> −103·0	1	< 0·062
Caesium	434	−47·5	> −85·0	2	< 0·024
Methylamine	434	−42·0	> −77·5	2	< 0·026

X is the test cation. [X] is its concentration in mM. E_K is the potassium equilibrium potential measured when the external bathing solution contained only K^+ ions and choline. E_X is the reversal potential measured when the external solution contained only X ions. n is the number of experiments. P_X/P_K is the permeability ratio for ion X to K^+ ions.

(P_X/P_K) for the monovalent cations and organic molecules (X) were determined from reversal potential measurements ($V_{r,X}$ and $V_{r,K}$) made when the external solution contained only K^+ or X^+ as the permeable cations, using a modified form of the Goldman, Hodgkin, Katz equation (Hille, 1972).

$$\frac{P_X}{P_K} = \frac{[K]_o}{[X]_o} exp\left(\frac{F\{V_{r,X} - V_{r,K}\}}{RT}\right), \quad (1)$$

where $[X]_o$ and $[K]_o$ are the external concentration of X^+ and K^+ and R, T and F have their usual meanings. Clear reversal potentials were measured in external solutions containing K^+, Tl^+, Rb^+ and NH_4^+. These ions pass through the light-dependent K^+ channels and have ionic diameters between 2·66 and 3·00 Å. The rest of the ions and organic molecules were no more than 3% as permeable as K^+ ions. For these ions, the values given for P_X/P_K represent the upper limits determined from estimated reversal potential measurements. The selectivity sequence for the passage of alkali metal cations through the K^+ channel is $K^+ > Rb^+ > Cs^+ > Na^+$, Li^+. In addition to K^+ and Rb^+, Tl^+ and NH_4 were the only other cations that were measurably permeable.

The reversal potential measurements would be in error if substantial changes in concentration occurred transiently on either side of the membrane during the period when the K^+ channels are opened by light. We minimized this problem by making measurements using small changes in potential near the reversal potential to preclude large fluxes of K^+ or of the test ions. The reversal potential measurements might also be in error if any internal accumulation of the test ion, and the consequent decrease in the intracellular K^+ concentration, occurred during the period of exposure. This would have occurred if there was substantial inward movement of the test ion through leakage pathways. This problem was minimized by keeping the exposure of the cell to the test ion as brief as possible (about 3 min). The reversal potential in the presence of the test ion stayed reasonably constant during the period of exposure suggesting that any influx through leakage channels was probably small.

The reversal measurements can be related to the movement of ions through the light-dependent K^+ channel by making some assumptions about its molecular structure. A variety of evidence suggests that ions move discontinuously through

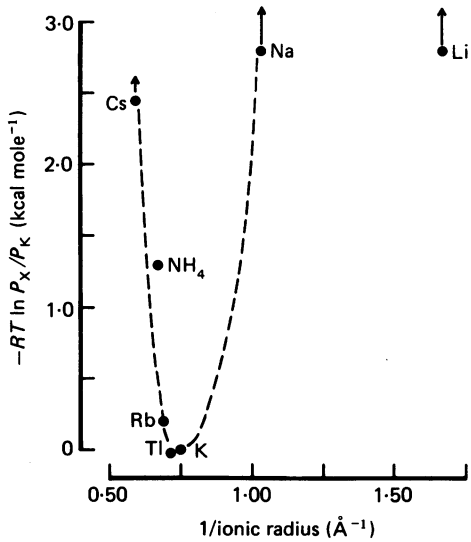

Fig. 8. Plot of the relative barrier height for the passage of an ion through the light-activated K^+ channel *versus* the reciprocal of the ionic radius. The values estimated for Cs^+, Na^+ and Li^+ represent lower limits (see text for further details).

membrane channels which are composed of multiple energy barriers (see Hille, 1975). We assume that there is one position within the channel which has the highest free energy, i.e. the barrier at this location is sufficiently higher than all others to be rate limiting, and that movement in this region must be single file (see Hille 1973; Reuter & Stevens, 1980). This region determines the selectivity of the channel. The change in barrier height (ΔB) that a monovalent cation experiences relative to the barrier height for a K^+ ion is related to the permeability coefficient by (Reuter & Stevens, 1980)

$$\Delta B = -RT \ln (P_X/P_K) \quad (2)$$

In Fig. 8, the barrier free energy (in k cal/mole) that a monovalent cation experiences relative to the K^+ ion barrier is plotted *versus* the reciprocal ionic radii of the ions. The values for ΔB were determined from the mean value of P_X/P_K) in Table 2 using eqn. 2 and the ionic radii were Pauling radii. The data agree with similar data published by Reuter & Stevens (1980) for the relative barrier height for monovalent cations in the delayed outward K^+ channel in *Helix* neurones. The values plotted for Cs^+, Na^+ and Li^+ are based on estimated reversal potential measurements and, therefore, must be viewed as lower limits for the barrier height.

Effects of external Cs^+ and TEA^+ on the light-dependent K^+ current

The results shown in Table 2 and in Fig. 8 suggest that the selectivity of the light-dependent K^+ channel is much the same as the selectivity of voltage-dependent K^+ channels (Hille, 1973; Gay & Stanfield, 1978; Reuter & Stevens, 1980). It is of interest to determine whether ions which block voltage-dependent K^+ channels. e.g. Cs^+ and tetraethylammonium (TEA), also block the light-dependent K^+ current.

The block of K^+ currents by Cs^+ ions depends upon the side of the membrane to which the Cs^+ is applied and upon the direction of ion movement. External Cs^+ blocks

inward, but not outward voltage-dependent K^+ currents (Adelman, 1971; Bezanilla & Armstrong, 1972). Fig. 9*A* shows the current–voltage relation from a cell immersed first in a Na^+-free ASW containing 50 mM-K^+ before and after the addition of 50 mM-Cs^+. The reversal potential was unchanged by Cs^+, but the inward K^+ current measured at the peak of the light-dependent current was reduced significantly. By contrast, the outward K^+ current was increased slightly by external Cs^+. The time course of inward and outward current was not changed appreciably by Cs^+.

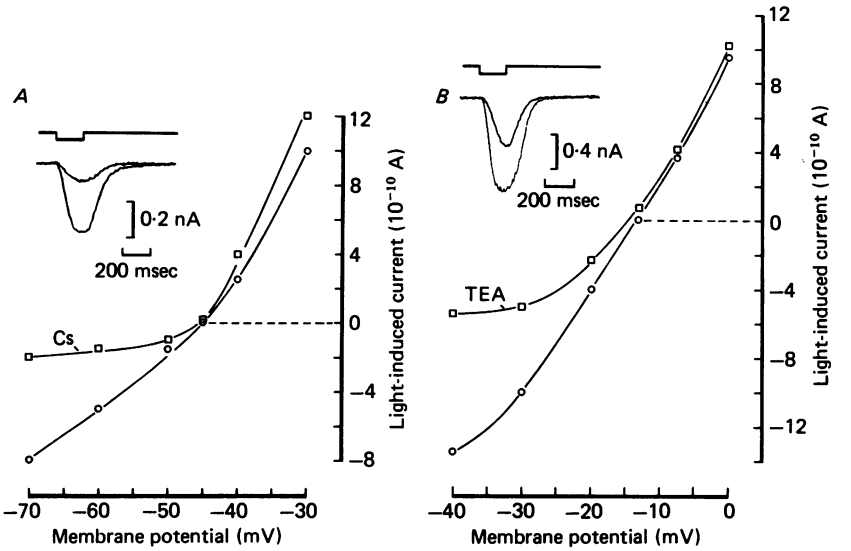

Fig. 9. Effects of external Cs^+ and TEA^+ on the light-activated K^+ current. *A*, plot of the light-activated current *versus* membrane holding potential in an external solution containing 50 mM-K^+ and a solution containing 50 mM-K^+ and 50 mM-Cs^+. *B*, plot of the light-activated current *versus* membrane holding potential in an external solution containing 200 mM-K^+ and a solution containing 200 mM-K^+ and 200 mM-TEA^+. The inset traces show light-induced inward currents measured at the same potential before and after the addition of Cs^+ (*A*) and before and after the addition of TEA (*B*). The external solution was Na^+- and Ca^{2+}-free in *A* and *B*.

External TEA blocks outward as well as inward voltage-dependent K^+ currents in myelinated axon (Hille 1967), presumably because there is an external receptor site which controls the movement of ions through the channel. The block produced by external TEA in myelinated axon occurs at very low concentrations (disassociation constant of 0·4 mM) and is complete at concentrations of about 50 mM. External TEA had very little effect on the light-dependent K^+ current at low concentration (< 10 mM). Fig. 9*B* illustrates the effect of a high concentration (200 mM) of TEA on the light-dependent K^+ current. The cell was immersed in Na^+-free ASW containing 200 mM-K^+. We used a high concentration of K^+ to move the membrane resting potential in a positive direction. This made inward K^+ currents substantially larger because of the voltage dependence of the light dependent K^+ conductance. The current–voltage relation shows that external TEA reduced inward K^+ current (measured at the peak of the light response) without any appreciable effect on outward K^+ currents. This effect is analagous to the action of *internal* TEA on

voltage-dependent K^+ currents in squid axon (Armstrong & Binstock, 1965) where *outward*, but not inward currents are blocked. The reversal potential and the time course of the light-dependent inward K^+ current were not changed significantly by external TEA.

DISCUSSION

The principal conclusions from these experiments are that the change in K conductance that underlies the hyperpolarizing response of the distal photoreceptor depends on membrane potential as well as on light, and that the conductance mechanism is highly selective for K^+ ions.

In excitable cells, studies of voltage-dependent conductances suggest that ions move through aqueous pores which are made up of a selectivity filter and a gating mechanism which allows an increase in conductance upon depolarization of the membrane (see Hille, 1978). Our evidence suggests that the movement of ions through the light-dependent channels is likely to also be through a pore, rather than by way of a carrier mechanism. First, channels are permeable to K^+ and to other monovalent cations of similar diameter (Tl^+, Rb^+, NH_4^+) but less than 3 % as permeable to larger (hydrazine, methylamine) or smaller diameter cations (Na^+, Li^+). Secondly, the selectivity sequence is Tl^+, $K^+ > Rb^+ > NH_4^+ > Cs^+ > Na^+$, Li^+. Lastly, extracellular Cs^+ blocks inward but not outward current. These observations are all consistent with similar data obtained for the voltage-dependent K^+ channels (delayed outward current) in several tissues (Bezanilla & Armstrong, 1972; Hille, 1973; Reuter & Stevens, 1980) where there is strong evidence that K^+ ions move through aqueous pores (see Armstrong, 1975).

The permeability ratios for Tl^+ and Rb^+ are smaller than the values reported for the delayed outward K^+ channels of vertebrate myelinated axon (Hille, 1973) and of striated muscle (Gay & Stanfield, 1978), but are very similar to the values determined for the delayed outward and the Ca^{2+}-activated K^+ channels of molluscan neurones (Reuter & Stevens, 1980; Gorman, Woolum & Cornwall, 1982). The monovalent cations which pass through the light dependent channel, as well as those which are excluded are identical to those which pass through and those which are excluded from other K^+ channels (Hagiwara, Eaton, Stuart & Rosenthal, 1972; Hille, 1973; Hagiwara & Takahashi, 1974; Gay & Stanfield, 1978; Reuter & Stevens, 1980; Gorman *et al.* 1982). Finally, the block by external Cs^+ of inward, but not outward K^+ movement through the light-dependent channel also occurs in all other K^+ channels so far studied (Adelman, 1971; Bezanilla & Armstrong, 1972; Hagiwara, Miyazaki & Rosenthal, 1976; Gay & Stanfield, 1977; Reuter & Stevens, 1980; Gorman *et al.* 1982).

In the distal photoreceptors, external TEA blocks inward, but not outward K^+ currents and is only effective at high concentrations. The effects of *external* TEA on the light dependent K^+ channels are analagous to the effects of *internal* TEA on voltage-dependent K^+ channels where outward, but not inward K^+ currents are blocked (Armstrong & Binstock, 1965). This occurs presumably because the TEA molecule (diameter 8 Å) can move into a wider portion of the K^+ channel, but does not pass through the selectivity filter (see Armstrong, 1975). The blocking effect of external TEA on the light-dependent K^+ channel could be explained by a pore which

contains an outer mouth of about 8 Å diameter which TEA could enter, thereby blocking the inward movement of K^+ ions. If TEA did not bind tightly in the mouth of the channel, then a high external concentration would be needed to depress the inward K^+ current and TEA could be easily flushed out of its blocking site by the outward movement of K^+ ions.

The light-dependent K^+ channel passes outward currents more easily than inward currents. The voltage sensitivity of this K^+ conductance is at least 4 times smaller than the conductance change which occurs in voltage-dependent K^+ channels in squid axon (Hodgkin & Huxley, 1952), but is close to the value determined for the Ca^{2+}-activated K^+ conductance of molluscan nerve cells (Gorman & Thomas, 1980). That light-activated K^+ conductance depends on membrane potential is not completely unexpected. The light-dependent conductance of barnacle photoreceptors (Brown *et al.* 1970) and of vertebrate rods (Bader *et al.* 1979) are also dependent upon membrane potential. If K^+ ions move through aqueous pores which are controlled by light, then it is possible that the number of open K^+ channels increases or that individual K^+ channels stay open longer at positive membrane potentials.

The light-dependent K^+ conductance is maximal at the beginning of a step of bright light, but falls thereafter to a lesser value during maintained illumination. This change in conductance accounts for the shape of the hyperpolarizing receptor potential and is analagous to the changes which occur in depolarizing invertebrate photoreceptors under similar conditions (Millecchia & Mauro, 1969; Brown *et al.* 1970; Lisman & Brown, 1975*a*; Bader *et al.* 1976). Our results show that the decline of the K^+ conductance occurs under conditions where other non-light dependent conductances are minimized or blocked. The decline of the light-activated Na^+ conductance of depolarizing photoreceptors is thought to be caused by an increase in the intracellular Ca^{2+} concentration (see Lisman & Brown, 1975*b*). If a similar mechanism occurs in the distal photoreceptors then the increase in intracellular Ca^{2+} has to be from internal stores since the complete removal of extracellular Ca^{2+} has no effect upon the decline of the K^+ conductance during maintained illumination.

We thank John Lisman for his criticisms of an earlier draft of this paper. This work supported by U.S.P.H.S. grant EY 01157.

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