

Decline of Electrogenic Na^+/K^+ Pump Activity in Rod Photoreceptors During Maintained Illumination

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ABSTRACT Light-evoked changes in membrane voltage were recorded intracellularly from rod photoreceptors in the isolated retina preparation of the toad, *Bufo marinus*, during superfusion with a solution containing pharmacological agents that blocked voltage-dependent conductances. Under these conditions, the amplitude of the hyperpolarizing photoresponse became much greater than under control conditions. The results of several experiments support the conclusion that this increase in photoresponse amplitude was due primarily to a voltage that was produced when the electrogenic current from the rods' Na^+/K^+ pump flowed across an increased membrane resistance (Torre, V. 1982. *Journal of Physiology*. 333:315). At the onset of a period of continuous illumination, the rod membrane first hyperpolarized and then began to repolarize, and after 180 s of illumination, the membrane voltage had recovered by 60–72% of its initial hyperpolarization. There did not appear to be any significant decrease in rod membrane resistance associated with this repolarization. Both the enhanced hyperpolarization at light onset and the slow repolarization during maintained illumination were blocked by superfusion with 10.0 μM strophanthidin. These data support the hypothesis that the activity of the rods' Na^+/K^+ pump declines progressively during maintained illumination. It is likely that the decline in pump activity produces significant changes in $[\text{K}^+]_o$ in the subretinal space during maintained illumination.

INTRODUCTION

Upon illumination of the vertebrate retina, there is a decrease in the extracellular potassium ion concentration, $[\text{K}^+]_o$, which is of maximal amplitude at the depth of the rod inner segments (Oakley and Green, 1976; Tomita, 1976). Studies of this light-evoked decrease in $[\text{K}^+]_o$ have shown that it is produced when the active uptake of K^+ by the rods' Na^+/K^+ pump exceeds the passive flux of K^+ out of the rods (Matsuura et al., 1978; Oakley et al., 1979; Shimazaki and Oakley, 1984). After light onset, $[\text{K}^+]_o$ rapidly falls to a minimum value. However, after

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reaching this minimum value, $[K^+]_o$ begins to recover, and during maintained illumination, $[K^+]_o$ eventually recovers to a value that in many species is only ~ 0.1 mM less than its dark-adapted value (Steinberg et al., 1980; Oakley and Steinberg, 1982; Oakley, 1983; Shimazaki and Oakley, 1984). Much of the mechanism that determines this recovery process is likely to be intrinsic to the rods, since the recovery process is present in the isolated retina preparation, it survives after synaptic transmission to second-order neurons is blocked with aspartate, and it is not affected by substances that should block spatial buffering of $[K^+]_o$ by glial cells (Oakley, 1983; Shimazaki and Oakley, 1984).

It was hypothesized that the activity of the rods' Na^+/K^+ pump decreases progressively during maintained illumination, thereby reducing the active uptake of K^+ and allowing diffusion of K^+ to restore the level of $[K^+]_o$ (Steinberg et al., 1980; Oakley, 1983). In support of this hypothesis, we found that treatments that reduce the activity of the Na^+/K^+ pump before the onset of illumination, including ouabain, lowered $[K^+]_o$, lowered temperature, and severe hypoxia, all reversibly block the recovery process (Shimazaki and Oakley, 1984). However, our experiments did not provide any direct evidence that the activity of the rods' Na^+/K^+ pump actually decreases during maintained illumination. Therefore, in order to have a more complete understanding of the behavior of rods during maintained illumination, it became important to assess the activity of their Na^+/K^+ pump by a more direct method.

The rods' Na^+/K^+ pump is electrogenic (Torre, 1982), and a decrease in pump activity might be expected to produce membrane depolarization. However, the rod membrane normally has a very low resistance, primarily caused by voltage-dependent conductances in the rod inner segment (Fain et al., 1978, 1980; Bader et al., 1979, 1982; Fain and Quandt, 1980; MacLeish et al., 1984; Bader and Bertrand, 1984). Therefore, the electrogenic current from the Na^+/K^+ pump normally does not contribute appreciably to membrane voltage. However, when certain of the inner segment conductances are blocked pharmacologically, and when the light-dependent conductance is blocked by light, the rod membrane resistance increases significantly (e.g., Fain et al., 1978; Bader et al., 1979). Under these conditions, the electrogenic current produces a very large hyperpolarizing component of the voltage response to illumination (Torre, 1982; Marroni et al., 1983). In the present experiments, we have used this electrogenic component to assess the activity of the rods' Na^+/K^+ pump. Our results provide additional support for the hypothesis that the pump activity decreases progressively during maintained illumination.

METHODS

All experiments were performed on the isolated retina preparation of the toad, *Bufo marinus*, as described in detail recently (Oakley, 1983; Shimazaki and Oakley, 1984). Briefly, the dark-adapted retina was pinned receptor-side up in a small chamber (~ 0.3 ml in volume), and was superfused at 1.0–1.5 ml/min with an oxygenated Ringer's solution that had the following composition (in mM): 108 NaCl, 2.4 KCl, 0.9 CaCl₂, 1.3 MgCl₂, 3.0 HEPES, 5.6 glucose, 0.01 EDTA, and 0.003 phenol red. The pH of this solution was adjusted to 7.8 by the addition of NaOH. A test solution was prepared by substitution of 10.0 mM CsCl and 10.0 mM tetraethylammonium (TEA⁺) chloride for equimolar amounts

of NaCl (modified from Bader et al., 1982, and MacLeish et al., 1984). For certain experiments, this test solution was modified further, either by the addition of 2.0–10.0 μM strophanthidin (Torre, 1982), or by the lowering of CaCl_2 to 90 μM (1/10 of control; see Oakley, 1984).

Single-barreled micropipette electrodes were used to record intracellularly from rod photoreceptors. The impaled rods were probably the larger and more numerous red rods, based upon their response waveform and sensitivity to 500-nm light. A rod was considered acceptable only if its resting membrane voltage in the dark was more negative than -30 mV and the plateau amplitude of its saturated light response exceeded 10 mV. In all illustrations of rod membrane voltage in this paper, light- and drug-evoked responses are superimposed upon the resting membrane voltage, which typically was -35 mV under control conditions, and varied from this value under test conditions.

In some experiments, the input resistance of the impaled rod was measured using a preamplifier (Axoprobe-1, Axon Instruments, Inc., Burlingame, CA) that had the ability to pass current through the microelectrode, and thus into the rod. A bridge circuit was used to cancel the current-evoked voltage drop across the microelectrode; the bridge was balanced before each impalement, when the tip of the microelectrode was extracellular.

Double-barreled K^+ -selective microelectrodes were used to measure $[\text{K}^+]_o$. As described previously (Oakley and Green, 1976; Shimazaki and Oakley, 1984), one barrel was an ion-selective electrode that contained a K^+ -selective ligand (Fluka 60031, Fluka Chemical Corp., Hauppauge, NY) in its tip; the other barrel was a reference electrode. Each K^+ -selective microelectrode was calibrated after an experiment, in order to obtain the relationship between electrode voltage (V_{K^+}) and $[\text{K}^+]_o$. Because of interference from the pharmacological agents (primarily Cs^+ ; Oehme and Simon, 1976; Shimazaki and Oakley, 1984) that were added to the test solutions, it was necessary for the calibrations to be done in modified test solutions, which contained identical concentrations of the pharmacological agents used in the experiment. In the presence of the interfering ions, a given change in $[\text{K}^+]_o$ produced a much smaller change in V_{K^+} than it did under control conditions. In order to prevent the signal-to-noise ratio from becoming unacceptably low under these conditions, K^+ -selective microelectrodes having large, beveled tips (up to 25 μm along the beveled surface) were used, and the V_{K^+} waveforms were filtered using a low-pass filter that had an upper -3 -dB frequency of 2.0 Hz. Using the electrode calibration data and a computer, digitized records of V_{K^+} could be converted into waveforms representing $[\text{K}^+]_o$ (Shimazaki and Oakley, 1984).

The retina was stimulated (full-field) with flashes or steps of 500-nm light, whose duration could be varied. The irradiance of the unattenuated stimulus beam in the plane of the retina was measured with a calibrated photodiode (model 40X, United Detector Technology, Culver City, CA). For each stimulus, the number of quanta absorbed was calculated, based upon an effective collecting area for toad red rods of $\sim 30 \mu\text{m}^2$ (Fain, 1976).

RESULTS

Superfusion of the retina with certain pharmacological agents can block the voltage-dependent conductances that help to shape the waveform of the light-evoked change in membrane voltage (for reviews, see Fain and Lisman, 1981, and Bader et al., 1982). Specifically, Cs^+ blocks a conductance increase that normally is activated by membrane hyperpolarization (Fain et al., 1978) and TEA^+ blocks a voltage-dependent K^+ conductance (Fain and Quandt, 1980; Bader et al., 1982). When these agents are included in the superfusion solution, the rods' current-voltage relationship becomes more linear (Bader et al., 1979,

1982; Fain and Quandt, 1980), and the light-evoked change in membrane voltage (the "photoresponse") is altered (e.g., Fain et al., 1978). The combined effects of these pharmacological agents upon rod photoresponses are illustrated in Fig. 1. Under control conditions (Fig. 1, response 1), an intense, 100-ms flash evoked a photoresponse that had a hyperpolarizing transient, which decayed to a plateau. After the solution superfusing the retina was switched to the test solution, the photoresponse was altered significantly. Approximately 30 s after the test solution entered the chamber (Fig. 1, response 2), the photoresponse amplitude increased from 22 to 50 mV, and the initial (hyperpolarizing) transient was abolished. With increasing time in the test solution, the photoresponse amplitude decreased slightly. After ~150 s in the test solution, the photoresponse stabilized at 42 mV (Fig. 1, response 5).

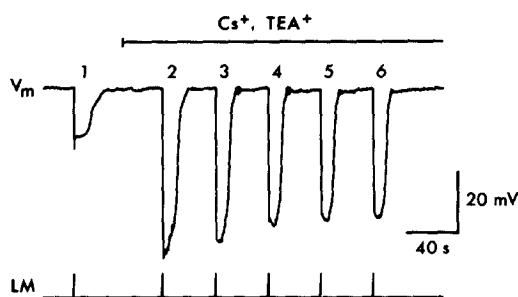


FIGURE 1. Effects of the test solution upon rod photoresponses. The changes in rod membrane voltage are shown in the waveform labeled V_m . The retina was stimulated repetitively with 100-ms flashes of light (3.0×10^4 quanta absorbed per rod-flash), as indicated by the waveform labeled LM (light monitor). The hyperpolarizing photoresponses in this series are numbered consecutively from 1 to 6. During the 244-s time period indicated by the horizontal bar above the V_m waveform, the solution entering the recording chamber was switched from the control solution to the test solution, which contained 10.0 mM Cs^+ and 10.0 mM TEA^+ . The effects of this test solution were reversible (data not illustrated). Similar results were observed in all rods superfused with this test solution ($n = 24$).

A component of the enhanced photoresponse observed during superfusion with the test solution is probably due to current from the rods' electrogenic Na^+/K^+ pump (Torre, 1982). Therefore, this component should be reduced in amplitude if the pump activity is decreased. This notion was tested in Fig. 2. At the start of the record illustrated in Fig. 2, the retina had been superfused with the test solution for ~13 min. An intense, 100-ms flash evoked a typical photoresponse of 41 mV (Fig. 2, response 1). The test solution superfusing the retina then was switched to one that contained 10.0 μ M strophanthidin, a cardiac glycoside that reversibly inhibits the Na^+/K^+ pump in rods (Torre, 1982). Immediately after this solution reached the preparation, the rod membrane began to depolarize and the photoresponse amplitude began to decline. After 210 s in strophanthidin solution, the photoresponse amplitude was only 17 mV (Fig. 2, response 7). At this point, the solution bathing the retina was switched

back to the test solution (without strophanthidin), and after a 260-s washout period, the photoresponse amplitude increased to 29 mV (Fig. 2, response 14). In earlier studies, similar effects of strophanthidin upon photoresponse amplitude were observed when the superfusate contained 10.0 mM Cs⁺ (Torre, 1982; Marroni et al., 1983). Overall, the data obtained using strophanthidin support the hypothesis that a significant fraction of the enhanced photoresponse observed in the test solution is due to current from the electrogenic Na⁺/K⁺ pump.

If our interpretation of the strophanthidin data is correct, then the photoresponse (measured during superfusion with the test solution) should become larger when the pump activity is increased. It is possible to increase pump activity

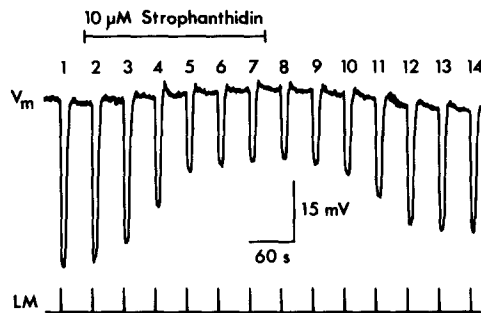


FIGURE 2. Effects of strophanthidin upon rod photoresponses. The changes in rod membrane voltage are shown in the waveform labeled V_m . At the start of the illustrated time period, the retina had been bathed in the standard test solution for ~ 13 min. Every 40 s, the retina was stimulated with a 100-ms flash of light (3.0×10^4 quanta absorbed per rod-flash), as indicated by the waveform labeled LM. The hyperpolarizing photoresponses in this series are numbered consecutively from 1 to 14. During the 230-s time period indicated by the horizontal bar above the V_m waveform, the solution entering the recording chamber was switched from the test solution to a similar solution that contained 10.0 μM strophanthidin. This figure represents the second exposure of this rod to the strophanthidin solution. The first exposure lasted for 200 s, and was followed by a 310-s washout period, before the start of the time period illustrated. Similar effects were observed in all rods superfused with 10.0 μM strophanthidin ($n = 6$). In other experiments, 2.0 μM strophanthidin had little effect ($n = 2$), whereas 4.0 μM strophanthidin produced effects similar to 10.0 μM strophanthidin ($n = 6$).

by lowering the extracellular calcium concentration, $[\text{Ca}^{2+}]_o$, which increases the Na⁺ conductance in the rod outer segment and leads to an increased influx of Na⁺ (Hagins, 1972); in response to the increased influx of Na⁺, the activity of the rods' Na⁺/K⁺ pump increases (Hodgkin et al., 1984; Oakley, 1984). Therefore, we observed the effects of increasing pump activity by lowering $[\text{Ca}^{2+}]_o$, as shown in Fig. 3. At the start of the record illustrated in Fig. 3, the retina had been superfused with the test solution for 6 min. An intense, 100-ms flash evoked a typical photoresponse of 44 mV (Fig. 3, response 1). The solution superfusing the retina then was switched to a similar test solution that contained 90 μM Ca²⁺

(1/10 of control). As this new solution reached the retina, the rod depolarized by 19 mV. After 200 s in the low- Ca^{2+} solution, the amplitude of the photoreponse increased to 91 mV (Fig. 3, response 7). At the peak of the photoreponse, the rod membrane was 27 mV more hyperpolarized than in the normal Ca^{2+} test solution. The enhanced amplitude of the photoreponse was maintained in the low- Ca^{2+} test solution. The additional hyperpolarization observed in low- Ca^{2+} solution was probably due to increased current from the electrogenic Na^+/K^+ pump. Similar effects of decreased $[\text{Ca}^{2+}]_o$ were obtained previously by others when the superfusate contained 10.0 mM Cs^+ (Torre, 1982; Marroni et al., 1983).

Overall, the data in Figs. 1–3 strongly support the interpretation that a major component of the enhanced receptor potential, observed during superfusion

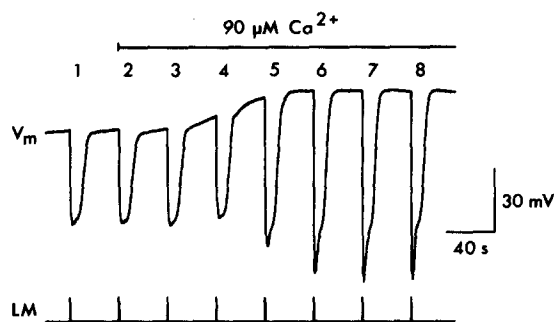


FIGURE 3. Effects of lowered $[\text{Ca}^{2+}]_o$ upon rod photoreponses. The changes in rod membrane voltage are shown in the waveform labeled V_m . At the start of the illustrated time period, the retina had been bathed in the test solution for ~6 min. Every 40 s, the retina was stimulated with a 100-ms flash of light (3.0×10^4 quanta absorbed per rod-flash), as indicated by the waveform labeled LM. The hyperpolarizing photoreponses in this series are numbered consecutively from 1 to 8. During the 277-s time period indicated by the horizontal bar above the V_m waveform, the solution entering the recording chamber was switched from the test solution to a similar solution that contained $90 \mu\text{M} \text{Ca}^{2+}$ (1/10 of that in the test solution). Similar effects were observed for all rods superfused with the low- Ca^{2+} test solution ($n = 4$). The effects of low- Ca^{2+} solution were reversible (data not illustrated).

with Cs^+ and TEA^+ , is due to electrogenic current from the rods' Na^+/K^+ pump. This current produces a significant component of membrane voltage only when the rod membrane resistance is increased (Torre, 1982), which in the present experiments was done by a combination of pharmacological agents and illumination.

It was hypothesized that the activity of the rods' Na^+/K^+ pump decreases progressively during maintained illumination (Steinberg et al., 1980; Oakley, 1983; Shimazaki and Oakley, 1984). This hypothesis was tested in Fig. 4, using the electrogenic component of the photoreponse to assess pump activity. At the start of the waveform shown in Fig. 4A, the retina had been superfused with the test solution for >8 min. The retina was stimulated by a 180-s step of illumination, which had an irradiance that was 100 times less than that of the 100-ms flashes

used in Fig. 1–3. At the onset of illumination, the rod hyperpolarized by 48 mV. After reaching this maximum hyperpolarization, the rod then began to depolarize (repolarize). At 90 s after light onset, the rod had depolarized by 24 mV, and by the end of the 180-s period of illumination, the rod had depolarized by 29 mV. At the offset of illumination, the rod depolarized rapidly and overshoot the dark-adapted baseline, before recovering to a stable value. Since a major component of the membrane hyperpolarization under these test conditions is due to electrogenic current from the rods' Na⁺/K⁺ pump, the decline in membrane voltage observed in Fig. 4A could have been caused by a decline in the electrogenic current from the pump. A similar decline in membrane voltage during maintained illumination was observed in all rods superfused with this test solution

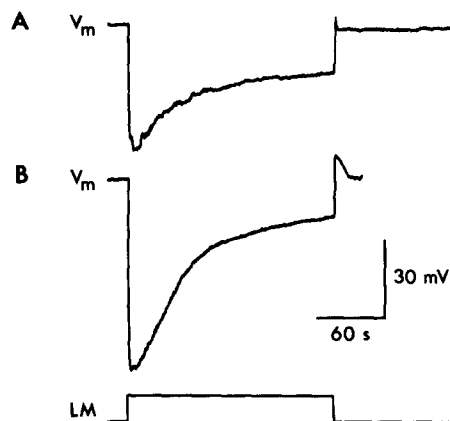


FIGURE 4. Effects of maintained illumination upon rod membrane voltage, V_m . Each V_m waveform was recorded from a different rod during a different experiment. For each response illustrated, the retina was stimulated by a 180-s period of maintained illumination, as indicated by the waveform labeled LM, which produced 3.0×10^9 quanta absorbed per rod per second. (A) Response recorded during superfusion with the standard test solution. The retina had been bathed in the test solution for more than 8 min before the onset of the stimulus. (B) Response recorded during superfusion with the low- $[Ca^{2+}]$ test solution. The retina was superfused with a modified test solution that contained $90 \mu M Ca^{2+}$ (1/10 of control) for 4.5 min before the onset of the stimulus.

($n = 3$). In these cells, the membrane voltage by the end of the 180-s period of illumination recovered by an amount ranging from 60 to 72% of the maximum hyperpolarization at light onset.

The activity of the rods' Na⁺/K⁺ pump is increased in the low- Ca^{2+} test solution, leading to an increase in the amplitude of the photoresponse (Fig. 3). If the decline in membrane voltage during maintained illumination observed in Fig. 4A was due to a decrease in the activity of the rods' Na⁺/K⁺ pump, then there should be an even larger decline in rod membrane voltage in the low- Ca^{2+} test solution. This idea was tested in Fig. 4B. At the start of the illustrated record, the retina had been superfused with the low- Ca^{2+} test solution for ~4.5 min. At the onset of illumination, the rod hyperpolarized by 72 mV. After

reaching this maximum hyperpolarization, the rod then began to depolarize. At 90 s after light onset, the rod had depolarized by 49 mV, and at the end of the 180-s period of illumination, the rod had depolarized by 58 mV. At the offset of illumination, the rod depolarized rapidly and overshoot the dark-adapted baseline, before recovering to a stable value. As expected, the decline in membrane voltage was much larger than that observed during superfusion with the normal- Ca^{2+} test solution (Fig. 4A). A similar decline in membrane voltage during maintained illumination was observed in all rods studied in low- Ca^{2+} test solution ($n = 3$). In these cells, the membrane voltage by the end of the 180-s period of illumination had recovered by an amount ranging from 77 to 87% of the maximum hyperpolarization at light onset.

When the inner segment conductances are blocked pharmacologically, strophanthidin abolishes the major component of the photoresponse elicited by brief flashes, probably by reducing the electrogenic current from the rods' Na^+/K^+ pump (Fig. 2). The effects of strophanthidin during maintained illumination



FIGURE 5. Effects of strophanthidin upon the rod response to maintained illumination. The changes in rod membrane voltage, V_m , were produced when the retina was stimulated by a 180-s period of maintained illumination. The light stimulus, which is indicated by the waveform labeled LM, produced 3.0×10^3 quanta absorbed per rod per second. During the illustrated time period, the retina was superfused with a modified test solution, which contained $10.0 \mu\text{M}$ strophanthidin. Before the onset of the stimulus, the retina first was superfused with the test solution for 12.5 min, and then superfused with the strophanthidin solution for another 6.5 min. Similar results were obtained in all rods superfused with $10.0 \mu\text{M}$ strophanthidin during maintained illumination ($n = 4$).

were examined next, as shown in Fig. 5. At the start of the record illustrated in Fig. 5, the retina had been bathed in a test solution containing $10.0 \mu\text{M}$ strophanthidin for ~ 6.5 min. In response to a 180-s light stimulus, which had the same irradiance as the light stimuli used in Fig. 4, the rod membrane response consisted of a 9.4-mV hyperpolarization at light onset, which declined slightly throughout the period of illumination, and a rapid repolarization (depolarization) at light offset. When the responses in Figs. 4 and 5 are compared, it can be seen that strophanthidin eliminated the major component of the photoresponse, which consisted of a large hyperpolarization at light onset, followed by a slow repolarization during maintained illumination.

The strophanthidin data in Fig. 5 are consistent with the notion that the repolarization of membrane voltage during maintained illumination is due to a decline in electrogenic current flowing across the rod membranes. However, a similar change in membrane voltage might have been produced if the electrogenic current remained constant and the membrane resistance decreased. An

experiment was performed to search for any decrease in membrane resistance during maintained illumination. In this experiment, pulses of current were injected repetitively into the impaled rod to measure its input resistance, R_{in} . Because of electrical coupling between rods, R_{in} is in large part determined by

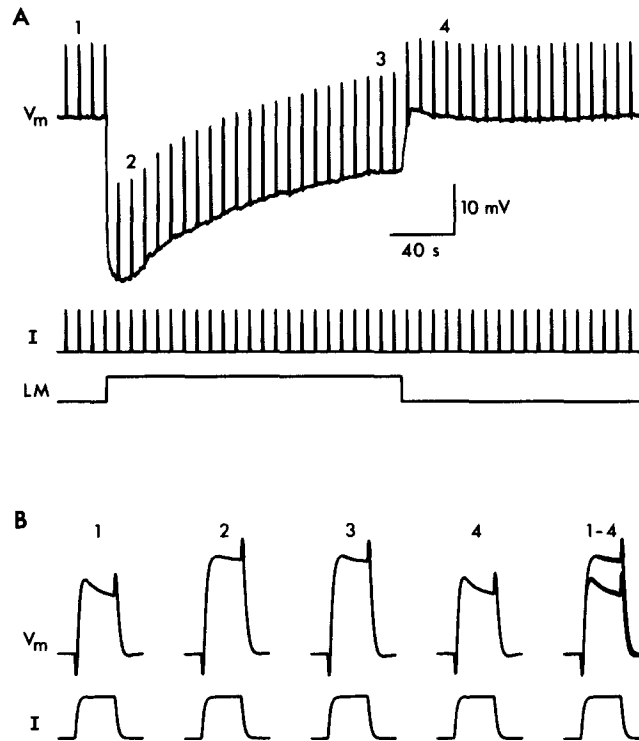


FIGURE 6. Changes in input resistance evoked by maintained illumination. (A) The retina was bathed in the test solution for 13 min before (and during) the illustrated time period. Every 8.0 s, a current pulse (60 pA, 0.5 s) was injected into the rod, as indicated by the waveform labeled I. The current pulses produced the upward (depolarizing) deflections of membrane voltage, V_m . The retina was stimulated by a 180-s period of maintained illumination (LM), which produced 1.2×10^4 quanta absorbed per rod per second. In digitizing the overall V_m waveform, the protocol was adjusted to avoid sampling the transient (capacitive) artifacts that occurred at the onset and offset of the current pulses (see part B). (B) Current-evoked changes in V_m from part A are shown on an expanded time scale (each waveform is 1.0 s in duration; same vertical scale as in A). Each V_m waveform in part B (labeled 1–4) is the average of three consecutive responses (the second of the three responses is labeled with the corresponding number in part A). Responses 1–4 are superimposed in the lower right of the figure.

the coupling resistance (Lamb and Simon, 1976; Torre and Owen, 1983). However, when voltage-dependent conductances are blocked pharmacologically, light-evoked increases in membrane resistance can be detected by measuring changes in R_{in} (Fain et al., 1978). At the start of the waveforms illustrated in Fig. 6, the retina had been bathed in the test solution for 13 min. A current pulse

(60 pA, 0.5 s) evoked a maximum change in membrane potential of 14.2 mV (response 1), corresponding to an R_{in} of 237 M Ω . The current-evoked potential declined to 11.4 mV in 0.5 s, corresponding to an R_{in} of 190 M Ω . A similar relaxation of this type of current-evoked potential was observed earlier by Torre and Owen (1983). At light onset, the membrane was hyperpolarized by 32.1 mV, and the current-evoked potential increased in amplitude to 19.0 mV (response 2), corresponding to an R_{in} of 317 M Ω . During illumination, there was much less relaxation of the current-evoked potential during the 0.5-s current pulse. As the membrane voltage repolarized during the 180-s period of maintained illumination, the current-evoked potentials remained of essentially constant amplitude (e.g., response 3). At light offset, both the membrane voltage and the current-evoked potential (response 4) recovered promptly to their dark-adapted values. In all rods examined in this manner ($n = 7$), R_{in} increased at light onset, and then remained essentially constant during maintained illumination, within the limits of detectability of $\sim 10\%$ of the increase in R_{in} at light onset. Thus, there do not seem to be significant decreases in membrane resistance associated with the repolarization of the rod membrane during maintained illumination.¹ This result supports the conclusion that the membrane repolarization is due to a decrease in the electrogenic current from the Na⁺/K⁺ pump.

The decline in rod membrane voltage during maintained illumination (Fig. 4) occurs over the same time period as the recovery of [K⁺]_o in the subretinal space that was observed in previous experiments under control conditions (e.g., Oakley, 1983). However, in the present experiments, the addition of pharmacological agents to block inner segment conductances (including K⁺ conductances) might have affected the time course of the recovery of [K⁺]_o during maintained illumination. Therefore, we decided to repeat the measurements of the light-evoked decrease in [K⁺]_o in the subretinal space, under conditions identical to those used when recording rod responses intracellularly. In the experiment illustrated in Fig. 7, the tip of the K⁺-selective microelectrode first was placed extracellularly in the subretinal space, at the level of the rod inner segments, and the retina was superfused with control solution. In response to a 180-s light stimulus, which had the same irradiance as the stimuli used in Figs. 4 and 5, the K⁺ electrode voltage, V_{K^+} , decreased initially and then slowly recovered toward its dark-adapted baseline, as shown in Fig. 7A. At light offset, V_{K^+} overshoot its dark-adapted baseline, and then slowly recovered to a stable value. This waveform was typical of the changes in V_{K^+} during maintained illumination recorded previously (e.g., Oakley, 1983).

¹ Because of electrical coupling between rods, a measured value of R_{in} is a complex function of both membrane resistance and coupling resistance (Lamb and Simon, 1976; Torre and Owen, 1983). Therefore, the light-evoked increase in R_{in} measured in these experiments is not a linear measure of the increase in membrane resistance that actually occurred. Since we failed to detect any significant decrease in R_{in} during maintained illumination, we conclude that any decrease in membrane resistance would be too small to produce the observed 60–72% decline in membrane voltage during maintained illumination (e.g., Fig. 4A), if the electrogenic current from the rods' Na⁺/K⁺ pump remained constant. However, in order to provide a better measure of changes in membrane resistance during illumination, isolated (solitary) rods must be studied, and we anxiously await the results of similar experiments on these cells.

When the solution superfusing the retina was changed to the test solution, the value of V_{K^+} increased by 33 mV, as shown in Fig. 7C. Such an increase in V_{K^+} was expected, because of the response of the K⁺-selective microelectrode to the interfering ions (primarily Cs⁺) in the test solution. During superfusion with the test solution, another 180-s light stimulus was presented. As shown in Fig. 7B,

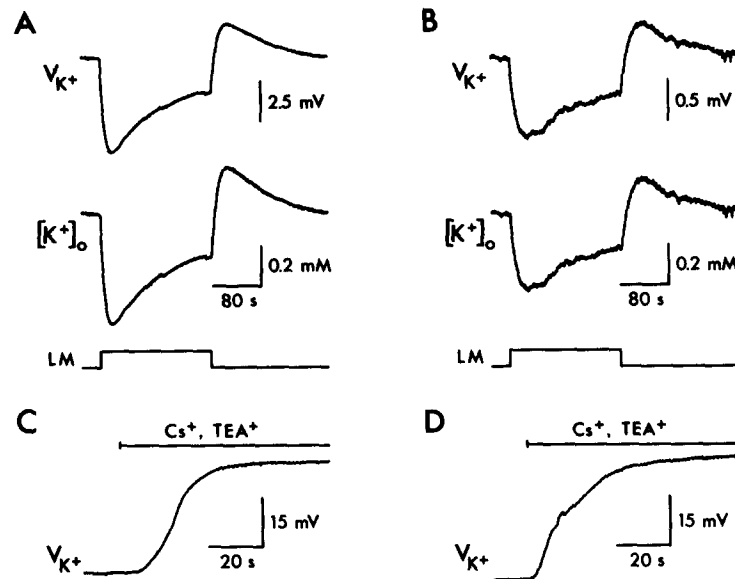


FIGURE 7. Effects of maintained illumination upon $[K^+]_o$. (A and B) The tip of the K⁺-selective microelectrode was placed in the subretinal space, at the depth of the rod inner segments. The upper waveforms, labeled V_{K^+} , were logarithmic measures of $[K^+]_o$. The middle waveforms, labeled $[K^+]_o$, were linearized from the corresponding V_{K^+} waveforms using the electrode calibration data (see Methods). Each V_{K^+} waveform, and thus each $[K^+]_o$ waveform, was evoked by a 180-s step of light (waveforms labeled LM; 3.0×10^5 quanta absorbed per rod per second). The retina was superfused with control solution for the responses in part A. The retina was superfused with the test solution for 4.5 min before, and during, the time period illustrated in part B. Note that although the V_{K^+} waveform in part B is plotted with a vertical gain five times larger than used in part A, both $[K^+]_o$ waveforms are plotted with identical gains. (C and D) For part C, the tip of the K⁺-selective microelectrode was positioned at the same retinal depth as in parts A and B, while for part D, the electrode tip was withdrawn from the retina into the superfusion solution. During the time period indicated by the horizontal bar above each V_{K^+} waveform, the solution superfusing the retina was switched from the control solution to the test solution. Note that the V_{K^+} waveforms in parts C and D are plotted with a vertical gain five times smaller than that used in part A.

this stimulus evoked a change in V_{K^+} that was similar in time course to the one observed under control conditions, although it was much smaller in amplitude (note that the V_{K^+} waveform in Fig. 7B is shown with a five-times-greater vertical gain than the V_{K^+} waveform in Fig. 7A). Similar changes in V_{K^+} were always observed during superfusion with the test solution (nine retinas). The effects of

the test solution upon the light-evoked changes in V_{K^+} were reversible (data not illustrated).

After the V_{K^+} waveforms illustrated in Fig. 7, A–C were recorded, the retina was again superfused with control solution and the tip of the K^+ -selective microelectrode was withdrawn into the bathing solution. Upon switching from the control solution to the test solution, the value of V_{K^+} increased by 36 mV, as shown in Fig. 7D. Since the concentration of K^+ remained constant at 2.4 mM during this solution change, this increase in V_{K^+} was due solely to the response of the K^+ -selective microelectrode to the interfering ions in the test solution. When the electrode tip was in the bathing solution, the increase in V_{K^+} (Fig. 7D) was only 3 mV larger in amplitude than it was when the electrode tip was in the retina (Fig. 7C), where the dark-adapted level of $[K^+]_o$ initially was 2.6 mM. Therefore, most of the increase in V_{K^+} in Fig. 7C was due to the interfering ions present in the test solution. Based upon the electrode calibration curve, however, it seems that there was a slight decline in the dark-adapted level of $[K^+]_o$ during superfusion with the test solution (from 2.6 to 2.5 mM).

The light-evoked changes in V_{K^+} in Fig. 7, A and B, were converted into changes in $[K^+]_o$ (see Methods), and the $[K^+]_o$ waveforms are shown just below the respective V_{K^+} waveforms. During superfusion with the test solution, the amplitude of the light-evoked decrease in $[K^+]_o$ was ~72% as large as it was under control conditions, and the recovery of $[K^+]_o$ during maintained illumination was slightly slower in time course than it was under control conditions.

The decline in rod membrane voltage evoked by a 180-s period of maintained illumination (Fig. 4B) now can be compared with the recovery in $[K^+]_o$ observed under identical conditions (Fig. 7B). Based upon this comparison, it seems that the decline in rod membrane voltage, and thus the decline in the activity of the rods' Na^+/K^+ pump, is slightly faster than the recovery of $[K^+]_o$, although both processes occur over a similar time period.

DISCUSSION

The data presented in this paper provide additional evidence in support of the hypothesis that the activity of the rods' Na^+/K^+ pump decreases progressively during maintained illumination. Moreover, the decrease in pump activity occurs slightly faster than the recovery of $[K^+]_o$ in the subretinal space, which supports the hypothesis that a decline in pump activity determines the recovery of $[K^+]_o$ during maintained illumination. There is no reason to doubt that a similar decline in pump activity during illumination occurs under control conditions, even though it is not readily detected (e.g., Fig. 7 in Oakley, 1983). The rod membrane resistance normally is quite low, and thus the electrogenic current from the Na^+/K^+ pump normally does not produce a significant component of membrane voltage.

When the retina was superfused with the test solution, maintained illumination produced a hyperpolarization of the rod membrane that recovered to a steady state level that was ~10–15 mV more hyperpolarized than the dark-adapted level (Fig. 4 and 6). This level of membrane voltage was approximately the same as the level reached at light onset during superfusion with 10.0 μ M strophanthidin

(Fig. 5). Thus, it appears that illumination is a potent regulator of the activity of the rods' Na⁺/K⁺ pump, since it eventually can produce the same decrease in pump activity as strophanthidin, as assessed by the pump-mediated (electrogenic) component of membrane voltage. This result could have important implications for rod function, since rods typically are exposed daily to maintained illumination during daylight hours. Assuming that the pump activity remains low during periods of maintained illumination, ATP will be spared for other cellular functions. Kimble et al. (1980) found that frog rods decreased their consumption of oxygen during maintained illumination, as would be expected if the activity of their Na⁺/K⁺ pump decreased.

In our earlier study (Shimazaki and Oakley, 1984), we suggested that a decline in intracellular sodium concentration, [Na⁺]_i, was responsible for the decrease in the activity of the rods' Na⁺/K⁺ pump during maintained illumination. At the onset of illumination, the decrease in sodium conductance in the rod outer segment will lead to reduced entry of Na⁺ into the rods. Coupled with an initially unchanged efflux of Na⁺ (caused by the Na⁺/K⁺ pump), the reduced entry of Na⁺ will lead to a decline in [Na⁺]_i. There is ample support (summarized in Shimazaki and Oakley, 1984) for the conclusion that this putative decline in [Na⁺]_i could decrease the activity of the rods' Na⁺/K⁺ pump (see also Torre, 1982). Future experiments must use Na⁺-selective microelectrodes to measure [Na⁺]_i during maintained illumination.

We found in the present experiments that the electrogenic component of rod membrane voltage decreases in amplitude during maintained illumination. We have interpreted this result to mean that the activity of the Na⁺/K⁺ pump decreases, thus reducing the overall transport of Na⁺ and K⁺. Similar results might have been obtained if rod membrane resistance decreased during maintained illumination. However, there does not appear to be a significant decrease in rod membrane resistance associated with the decline in membrane voltage during maintained illumination. Similar results also might have been obtained if the pump activity remained constant and the ratio coupling the Na⁺ and K⁺ fluxes changed, causing the pump to become more electroneutral during maintained illumination. We cannot rule out such changes in this ratio, but in light of the observation that the recovery of [K⁺]_o occurs over the same time period as the decline in membrane voltage, it seems likely that the pump activity itself decreases during maintained illumination.

In an earlier study, Fain et al. (1978) concluded that the increase in the amplitude of the photoresponse, observed upon superfusion with Cs⁺, was due to an effect of Cs⁺ upon a voltage-dependent conductance in the rod inner segment. Assuming that Cs⁺ blocked a voltage-dependent increase in conductance for an ion with a more positive reversal potential, Fain et al. (1978) argued that the photoresponse became larger when the rods were allowed to hyperpolarize toward the K⁺ equilibrium potential. The present study, as well as that of Torre (1982), would argue that much of this enhanced amplitude is due to the electrogenic current flowing across an increased membrane resistance. Moreover, earlier studies found that the hyperpolarizing transient observed under control conditions is abolished promptly when the retina is superfused with ouabain or

strophanthidin (Oakley et al., 1979; Torre, 1982). Thus, it seems that the hyperpolarizing transient is due, at least in part, to current from the electrogenic pump. At light onset, closing of the light-dependent channels increases the membrane resistance, so that a component of the initial hyperpolarization is due to electrogenic current flowing across the increased membrane resistance. Subsequently, the increase in the Cs^+ -sensitive conductance upon hyperpolarization decreases the membrane resistance, thereby decreasing the amplitude of the electrogenic component of membrane voltage.

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