Effects of the Rod Receptor Potential upon Retinal Extracellular Potassium Concentration

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ABSTRACT It has been hypothesized that the light-evoked rod hyperpolarization (the receptor potential) initiates the light-evoked decrease in extracellular potassium ion concentration, [K⁺]₀, in the distal retina. The hypothesis was tested using the isolated, superfused retina of the toad, Bufo marinus; the receptor potential was recorded intracellularly from red rods, and [K⁺]₀ was measured in the photoreceptor layer with K⁺-specific microelectrodes. In support of the hypothesis, variations in stimulus irradiance or duration, or in retinal temperature, produced qualitatively similar effects on both the receptor potential and the decrease in [K⁺]₀. A mechanism for the relationship between the receptor potential and the decrease in [K⁺]₀ was suggested by Matsuura et al. (1978. Vision Res. 18:767-775). In the dark, the passive efflux of K⁺ out of the rod is balanced by an equal influx of K⁺ from the Na⁺/K⁺ pump. The light-evoked rod hyperpolarization is assumed to reduce the passive efflux, with little effect on the pump. Thus, the influx will exceed the efflux, and [K⁺]₀ will decrease. Consistent with this mechanism, the largest and most rapid decrease in [K⁺]₀ was measured adjacent to the rod inner segments, where the Na⁺/K⁺ pump is most likely located; in addition, inhibition of the pump with ouabain abolished the decrease in [K⁺]₀ more rapidly than the rod hyperpolarization. Based upon this mechanism, Matsuura et al. (1978) developed a mathematical model: over a wide range of stimulus irradiance, this model successfully predicts the time-course of the decrease in [K⁺]₀, given only the time-course of the rod hyperpolarization.

INTRODUCTION

When a vertebrate retina is stimulated with light, there is a decrease in the extracellular potassium ion concentration, [K⁺]₀, in the space surrounding the photoreceptors (Oakley and Green, 1976; Tomita, 1976; Oakley, 1977; Matsuura et al., 1978). In rod-dominated retinas, the light-evoked decrease in [K⁺]₀ seems to be generated solely by the rods (Oakley and Green, 1976; Matsuura et al., 1978). It is important to understand the mechanism by which...
rod excitation generates the light-evoked decrease in \([K^+]_o\), since this ionic change (a) hyperpolarizes the retinal pigment epithelium (RPE), producing the c-wave of the electroretinogram (Oakley, 1977), (b) alters the active transport across the RPE of taurine, an amino acid that is concentrated in photoreceptors (Miller and Steinberg, 1979), and (c) diffuses throughout the entire retina during light adaptation, possibly affecting the sensitivity of proximal retinal neurons (Steinberg et al., 1979).

Matsuura et al. (1978) suggested that the decrease in \([K^+]_o\) is generated as a direct consequence of the light-evoked rod hyperpolarization. Specifically, they proposed that the rod hyperpolarization reduces the passive efflux of \(K^+\) from the rod, with little effect on the active influx of \(K^+\) (from the Na\(^+/K^+\) pump), thus causing \([K^+]_o\) to decrease. In order to test this hypothesis, light-evoked changes in both the rod membrane potential and \([K^+]_o\) have been measured under a variety of stimulus conditions. The results of these experiments support the hypothesis of Matsuura et al. (1978). Thus, the light-evoked decrease in \([K^+]_o\) seems to be a consequence of the rod membrane response to light.

**METHODS**

**Preparation**

These experiments were performed on the isolated retina of the toad, *Bufo marinus*. Most aspects of the preparation, and the techniques for intracellular recording, have been described previously (Brown and Flaming, 1977, 1978). Before an experiment, a toad was dark-adapted for 3-4 h. Under dim red illumination, the toad was decapitated and immediately pithed. An eye was enucleated and sectioned into anterior and posterior portions. The posterior portion was submerged in a modified Ringer's solution (see below) and cut into several pieces. The retina was isolated from a piece of the eyecup that was lacking the optic nerve. The isolated retina was placed receptor-side up on a glass contact lens, which had its convex surface facing upwards. This contact lens was part of a perfusion chamber that was mounted on the stage of a compound microscope. Infrared light (>800 nm) illuminated the retina from below through the microscope condenser lens. The receptor surface was imaged by a Zeiss ×40 water-immersion lens (Carl Zeiss, Inc., New York) upon a silicon diode image tube in a television camera (model 4415, Cohu Inc., Electronics Div., San Diego, Calif.). The output signal from this camera was displayed on a television monitor (model SNA9/C, Conrac Corp., Stamford, Conn.). This system provided high-resolution conversion from infrared to visible light.

Microelectrodes were advanced towards the receptor surface using a high-speed hydraulic advancer (Brown and Flaming, 1977). The electrode made a 23° angle with the retinal surface. Contact of the microelectrode tip with the preparation was visualized by a small movement of the tip of a single outer segment. The position of the microelectrode along the electrode track was then measured in one micron steps, so that the depth of the electrode tip below the receptor surface could be calculated accurately. By restricting intracellular recording to the first 50 \(\mu\)m below the receptor surface, all recordings were made in rod outer segments (Brown and Flaming, 1977). Outer segments were impaled, either by using a rapid, 3-\(\mu\)m advance, or by briefly increasing the “negative capacitance,” causing the potential of the micropipette to oscillate. The cells studied were red rods, as identified by their spectral sensitivity, which peaks at \(~500\) nm (Hárosi, 1975; Fain, 1976).
Perfusion

The retina was superfused with a modified Ringer's solution, which had the following composition (in millimolar): 94.0 NaCl, 2.0 KCl, 1.0 MgCl₂, 1.8 CaCl₂, 15.0 NaHCO₃, and 10.0 glucose. This solution was continuously bubbled with 98.5% O₂-1.5% CO₂ and had a pH of 7.7-7.8. In one set of experiments, 10⁻⁴ M ouabain (Sigma Chemical Co., St. Louis, Mo.) was added to the control solution.

The superfusate flowed into the chamber via a gravity feed system at 5.0 ml/min. The chamber volume was ~ 1.5 ml. Before entering the chamber, the solution flowed through a stainless-steel loop, which was in contact with the surface of a Peltier device (Cambridge Thermionic Corp., Cambridge, Mass.). By varying the current through this device (bidirectionally), the temperature of the solution in the perfusion chamber could be varied from 10 to 30°C. The temperature of the preparation was measured with a miniature thermistor probe (No. 524, Yellow Springs Instrument Co., Yellow Springs, Ohio) located in the chamber. This temperature was held constant at 22°C, except for one set of experiments in which temperature was deliberately varied.

Light Stimuli

Light stimuli were imaged upon the retina from below through the microscope condenser lens. The stimulus spot was centered on the electrode tip and focused upon the outer segments, using direct viewing with infrared light in the stimulus beam. The stimulus spot was 1.0 mm in diameter at the outer segments, which more than covered the rod's receptive field (Leeper et al., 1978). An electromagnetic shutter (Uniblitz, Vincent Associates, Rochester, N.Y.) was placed in the stimulus beam and electronically controlled, to provide stimulus durations from 10 ms to 10 s. An interference filter (Balzers Corp., Nashua, N.H.) with peak transmission at 500 nm was also placed in the stimulus beam. The stimulus irradiance was attenuated with calibrated neutral density filters and a calibrated neutral density circular wedge (Optical Industries, Inc., Melles Griot, Irvine, Calif.). At 500 nm, the irradiance of the unattenuated light beam in the plane of the retina was 2.1 × 10¹³ quanta s⁻¹ cm⁻², as measured by a calibrated photodiode (Optometer model 40A, United Detector Technology Inc., Santa Monica, Calif.). When referring to the stimulus irradiance, the negative logarithm of the attenuation will be used (e.g., with a 3.0 log unit neutral density filter in the beam, the stimulus irradiance would be -3.0 log units).

The effective collecting area of the red rod was calculated using 6.0 μm for the average diameter of the outer segment (Brown and Flaming, 1978), 0.89 as the fractional light absorbance by the photopigment of the rod (Fain, 1975), and 1.00 for the assumed retinal transmittance of light to the rod outer segment (Fain, 1975). Using these values, a 100-ms, unattenuated light stimulus would yield 5.3 × 10⁶ quanta absorbed rod⁻¹ flash⁻¹.

Electrodes

Micropipette electrodes for intracellular recording were made from glass capillary tubing (1.0 mm o.d., 0.5 mm i.d., "Omega-Dot", Glass Co. of America, Bargaintown, N.J.) using an "air-blast" microelectrode puller (Brown and Flaming, 1977). The electrodes were filled by injection with 0.15 M KCl and stored in a humid chamber. The resistance of the electrodes ranged from 400 to 500 MΩ.

Double-barrel, potassium-specific microelectrodes were used to measure [K⁺]₀. These electrodes were constructed as described by Oakley (1977). One barrel was an ion-specific electrode containing a K⁺-specific liquid ion-exchanger (Walker, 1971), and the other barrel was a reference electrode containing 5.0 M LiCl (Oakley et al.,
The K+-specific microelectrodes were bevelled on a surface embedded with 1.0 μm diamond dust (Brown and Flaming, 1975, 1979), with the ion-specific barrel making initial contact with the abrasive surface. After beveling, the resistance of the reference barrel was 20-40 MΩ. The resistance of the K+-specific barrel was estimated to be 2,000 times larger than that of the reference barrel (Zeuthen et al., 1974).

Each K+-specific microelectrode was calibrated immediately after an experiment. The solutions used in the calibration procedure were identical to the modified Ringer’s solution, except that their [K+] was varied from 0.1 to 50.0 mM. Using a least-squared error regression analysis, the electrode calibration data was fit by an empirical equation of the form:

\[ V_{K+} = \frac{2.303 \, nRT}{F} \log_{10} \left( \frac{[K^+] + [Na^+]}{S} \right) + V_o, \]  

where \( V_{K+} \) is the differential voltage between the two barrels, \( n \) is a factor (<1.0) which accounts for the nonideal slope of the electrode, \( R \) is the gas constant, \( T \) is the absolute temperature, \( F \) is the Faraday number, \( S \) is the selectivity coefficient for K+ over Na+, and \( V_o \) is a constant (Walker, 1971; Oakley and Green, 1976). The term \( \left( \frac{2.303 \, nRT}{F} \right) \) is called the electrode slope, and had a value ranging from 53-58 mV/decade at 22°C. The value of \( S \) ranged from 50 to 70 at 22°C.

Recording

A unity-gain, capacity-compensated preamplifier having an input impedance of 10^14 Ω was used to measure the potential of the intracellular microelectrode with respect to a grounded Ag/AgCl disc in the perfusion chamber. This signal was amplified ×100 and low-pass filtered (DC-30 Hz). In rod outer segments, the membrane potential (\( V_m \)) in the dark was typically −30 mV (range: −25 to −40 mV) with respect to the perfusate.

The potential difference between the two barrels of the K+-specific microelectrode (\( V_{K+} \)) was measured as follows. The potential of each barrel was measured with respect to ground by separate unity-gain preamplifiers, each having an input impedance of 10^14 Ω. The resulting voltages were amplified differentially (K+-specific barrel positive) by a low-noise amplifier (PAR-113, Princeton Applied Research, Corp., Princeton, N.J.), having a gain of 10 and a DC − 10 Hz bandpass.

The amplified signals (\( V_m \) or \( V_{K+} \)) were displayed on both an oscilloscope (model D-11, Tektronix, Inc., Beaverton, Ore.) and a digital millivoltmeter (model 160, Keithley Instruments Inc., Cleveland, Ohio). These signals were recorded on both a chart recorder (Brush 220, Gould Instruments, Inc., Cleveland, Ohio) and an FM tape recorder (Store 4D, Lockheed Electronics Co., Inc., Los Angeles, Calif.). The FM recordings could be digitized by a computer (Nova 2, Data General Corp., Westboro, Mass.), and then plotted (Zeta plotter, model 100, Zeta Research, Inc., Div. of Nicolet Instrument Corp., Lafayette, Calif.).

**RESULTS**

The light-evoked hyperpolarization of the rod (the receptor potential), recorded by an intracellular micropipette as the change in rod membrane voltage (\( V_m \)), will be termed the \( V_m \) response. Similarly, the light-evoked decrease in [K+]o, recorded as the change in voltage (\( V_{K+} \)) of an extracellular, K+-specific microelectrode, will be termed the \( V_{K+} \) response.

In testing the hypothesis that the light-evoked decrease in [K+]o in the distal retina is generated as a direct consequence of the receptor potential, it should
be emphasized that these two responses need not have the same time-course in order for this hypothesis to be valid. For example, if the change in \([K^+]_o\) were in some manner related to the integral of the receptor potential, then the ionic change could be much slower in time-course than the receptor potential and still be a direct consequence of it.

**Effects of Stimulus Irradiance and Duration**

The hypothesis was tested initially by varying the stimulus irradiance and recording both the \(V_m\) and \(V_{K^+}\) responses. A rod outer segment was impaled with a micropipette, and the \(V_m\) responses shown in the upper half of Fig. 1 were elicited by stimuli of increasing irradiance. The micropipette was then withdrawn, and a \(K^+\)-specific microelectrode was advanced into the same area of the retina. With the tip of the electrode 40 μm below the receptor surface, near the retinal depth where the light-evoked decrease in \([K^+]_o\) was maximal (see below), the \(V_{K^+}\) responses shown in the lower half of Fig. 1 were recorded sequentially in the same preparation. For each type of response, the stimulus irradiance was increased in 1.0 log unit steps, from \(-5.0\) to \(0.0\) log units. The stimulus (indicated by the arrow) was 100 ms in duration and was presented repetitively with an interstimulus interval of either 50 s (\(-5.0\) to \(-3.0\) log units) or 150 s (\(-3.0\) to \(0.0\) log units). The \(V_m\) responses were recorded first and were superimposed upon a resting membrane potential of \(-31\) mV. Following the 0.0 log unit flash, \(~10\) min were required to recover maximum sensitivity. The \(V_{K^+}\) responses were then recorded at a retinal depth 40 μm below the tips of the rod outer segments. The logarithmic scale at the right of the \(V_{K^+}\) responses represents \([K^+]_o\) and was obtained from the electrode calibration data.
recorded in response to the same stimuli. The $V_{K^+}$ response was a measure of the light-evoked decrease in the logarithm of $[K^+]_o$ (Eq. 1).

In the case of the $V_m$ response, both the response amplitude and the initial rate of hyperpolarization increased as the stimulus irradiance was increased from $-5.0$ to $-3.0$ log units. The $-3.0$ log unit stimulus evoked a response having an initial, hyperpolarizing transient, which decayed to a sustained plateau. Further increases in the stimulus irradiance (from $-3.0$ to $0.0$ log units) increased the duration of this plateau.

For the $V_{K^+}$ responses, the initial slope, the maximum amplitude, and the time-to-peak all increased as the stimulus irradiance was increased from $-5.0$ to $-3.0$ log units. Further increases in the stimulus irradiance (from $-3.0$ to $0.0$ log units) did not further increase the initial slope, but did increase both the peak amplitude and the time-to-peak of the response. The saturation of the initial slope of the $V_{K^+}$ response was not due to the response time of the $K^+$-specific microelectrode, since this type of electrode responds to step changes in $[K^+]_o$ with a time constant of less than 100 ms (Lux, 1974; Oakley, 1975). Similar effects of stimulus irradiance upon the $V_m$ and $V_{K^+}$ responses were recorded in three other retinas.

The temporal relationship between the $V_m$ and $V_{K^+}$ responses is shown in greater detail in Fig. 2, in which two pairs of responses are replotted from Fig. 1. The upper pair of responses was evoked by the $-4.0$ log unit stimulus; the lower pair by the $0.0$ log unit stimulus. A dashed line has been drawn through the peak of each $V_{K^+}$ response. For the $-4.0$ log unit stimulus, the $V_m$ response had decayed nearly to its baseline when the $V_{K^+}$ response peaked. For the $0.0$ log unit stimulus, however, the $V_m$ response barely had begun to decay from

![Figure 2](https://example.com/figure2.png)

**Figure 2.** $V_m$ and $V_{K^+}$ responses from Fig. 1 have been superimposed for two different stimuli. The stimulus irradiance was $-4.0$ log units for the upper pair of responses and $0.0$ log units for the lower pair. The stimulus (indicated by the arrow) was 100 ms in duration. In each set of responses, a dashed line has been drawn through the time at which the $V_{K^+}$ response was maximum.
its plateau when the $V_{K^+}$ response peaked. Thus, the temporal relationship between the two responses varied as a function of stimulus irradiance. It will be shown that the large time lag between the peaks of the two responses, as seen with the $-4.0 \log$ unit stimulus, can be explained by the hypothesis (see Discussion).

The relationship between the $V_m$ and $V_{K^+}$ responses was characterized further by varying the stimulus duration. A rod outer segment was impaled and stimuli of $-3.0 \log$ unit irradiance, ranging in duration from 10 ms to 10 s, were presented. These stimuli evoked the responses shown in the upper half of Fig. 3. The receptor potential elicited by the 10-ms stimulus lacked a distinct plateau. When the stimulus duration was increased to 100 ms, however, the receptor potential had both an initial transient and a plateau. Further increases in the stimulus duration did not alter the response amplitude, but did alter the duration of the plateau phase.

In another retina, a $K^+$-specific microelectrode was advanced to a depth 40 $\mu$m below the receptor surface. The identical stimuli were then presented that had been used to elicit the $V_m$ responses. These stimuli elicited the $V_{K^+}$ responses shown in the lower half of Fig. 3. Increasing the stimulus duration increased both the peak amplitude and the time-to-peak of the $V_{K^+}$ response. As the stimulus duration was increased from 10 to 100 ms, the magnitude of the initial slope of the response increased. Additional increases in stimulus duration, however, did not further alter this slope.
Variations in the stimulus duration produced qualitatively similar effects on the waveforms of the two responses. Increasing the stimulus duration increased both the duration of the rod plateau and the time-to-peak of the decrease in $[K^+]_o$. For the briefest stimulus, the peak of the change in $[K^+]_o$ occurred when $V_m$ had decayed to $\approx 10\%$ of the plateau level, while for the longest stimulus, this peak occurred when $V_m$ had decayed to $\approx 45\%$ of its plateau level. Thus, the temporal relationship between the rod receptor potential and the decrease in $[K^+]_o$ also varied as a function of the stimulus duration.

Since the $V_m$ and $V_{K^+}$ responses had different time-courses, it was desired to test the hypothesis under conditions where the difference in time-course would not impair the comparison between the two responses. If the hypothesis is valid, then stimuli which have been equated to produce identical receptor potentials should also produce identical changes in $[K^+]_o$. This prediction was tested as follows.

A rod outer segment was impaled with a micropipette, and an unattenuated stimulus of 10 ms duration was presented. The stimulus duration ($d$) was then increased and its irradiance ($I$) decreased, so that the total number of quanta in each flash ($I \times d$) was held constant. The $V_m$ responses evoked by these stimuli were nearly identical, as shown by the superimposed responses in the upper half of Fig. 4. When stimulus durations longer than 7 s were used, no stimulus irradiance could be found that would produce a $V_m$ response identical to the other responses. For durations greater than 7 s, when ($I \times d$) was held constant, the $V_m$ responses had plateaus of smaller amplitude and longer duration than the other responses.

In another retina, a $K^+$-specific microelectrode was advanced so that its tip was 40 $\mu$m below the receptor surface. The same stimuli were then presented that had produced the $V_m$ responses, for which ($I \times d$) was held constant. As shown in the lower half of Fig. 4, these stimuli also produced nearly identical $V_{K^+}$ responses. Similar to the $V_m$ responses, when stimulus durations longer than 7 s were used, no stimulus irradiance could be found that would produce a $V_{K^+}$ response identical to the other responses. For durations greater than 7 s, when ($I \times d$) was held constant, the $V_{K^+}$ responses had smaller initial slopes and greater times-to-peak than the other responses.

The time over which there is perfect reciprocity between stimulus irradiance and duration is termed the integrating time. In Fig. 4, the integrating time of each response was $\approx 7$ s. In two other experiments (not shown), when ($I \times d$) was increased by a factor of 10, the rod plateau was prolonged and the $V_{K^+}$ response peaked at a later time. Under these conditions, the integrating time of each response increased to greater than 10 s. Similarly, when ($I \times d$) was decreased by a factor of 10, both the duration and the integrating time of each response decreased.

**Effects of Retinal Temperature**

If the rod receptor potential itself initiates the decrease in $[K^+]_o$, any variation in the waveform of the rod receptor potential should cause a corresponding
variation in the waveform of the light-evoked decrease in \([K^+]_o\). In order to vary the waveform of the rod receptor potential under constant stimulus conditions, and thus provide another test of the hypothesis, the retinal temperature was varied.

A rod outer segment was impaled, and a stimulus of \(-3.0\) log unit irradiance and 100 ms duration was presented once every 50 s. The retinal temperature was first lowered to 13.5°C, and the responses on the left side of Fig. 5 were recorded as the temperature was subsequently increased. Increasing temperature caused the rod receptor potential to become faster; the initial transient peaked at an earlier time, and the duration of the plateau decreased.

In another retina, a \(K^+\)-specific microelectrode was advanced to a retinal depth 40 \(\mu\)m below the receptor surface. The identical stimulus protocol was employed. The temperature of the retina was first decreased to 14.5°C, and the responses shown on the right side of Fig. 5 were recorded as the temperature was subsequently increased.\(^1\) Increasing temperature also caused the \(V_{K^+}\)

\(^1\) In Fig. 5, the scale relating \(V_{K^+}\) to \([K^+]_o\) has been omitted for the following reason. As the temperature was increased, the \(K^+\)-specific electrode slope increased and its selectivity for \(K\)
FIGURE 5. The effect of temperature upon the $V_m$ and $V_{K^+}$ responses. Each type of response was recorded from a different preparation under identical stimulus conditions. The stimulus was a 100-ms flash (arrows) of -3.0 log unit irradiance, presented repetitively at a rate of one flash every 50 s. The temperature of the retina was measured with a thermistor probe in the perfusion chamber (see Methods), and is indicated to the left of each response (in °C). For each set of responses, the retina was first cooled, and then data were recorded as the temperature was slowly increased. The $V_{K^+}$ responses were recorded 40 μm below the receptor surface.

Over Na$^+$ decreased. Consequently, the $V_{K^+}$ response at each temperature would have to have its own calibration curve in order to be converted into a change in $[K^+]_o$. The response time of the electrode, which was <100 ms (Lux, 1974; Oakley, 1975), was not significantly altered by these temperature changes. Thus, the time-to-peak of the $V_{K^+}$ response (range: 4–10 s) was not affected by the changes in the electrode properties, and was therefore a direct measure of the time-to-peak of the decrease in $[K^+]_o$. The initial rate of change of the $V_{K^+}$ response, however, was directly affected by the changes in the electrode slope and selectivity (see Eq. 1). The observed decrease in the magnitude of the initial $dV_{K^+}/dt$ at lower temperatures (Fig. 5), therefore, was due to changes in both $d[K^+]_o/dt$ and in the electrode response to $[K^+]_o$. Since decreasing the temperature increased the selectivity coefficient much more than it decreased the electrode slope, a given $d[K^+]_o/dt$ would produce a $dV_{K^+}/dt$ that would be greater in magnitude at a low temperature than at a high temperature (see Eq. 1). Thus, at low temperatures, the decrease in the magnitude of $dV_{K^+}/dt$ was indicative of an even greater decrease in $d[K^+]_o/dt$. 
response to become faster; its initial slope increased in magnitude, and its time-to-peak decreased.

Since temperature decreased both the duration of the rod plateau and the time-to-peak of the decrease in [K+]o, these effects were quantified for direct comparison. In Fig. 6, the time-to-peak of the V_K+ response is plotted (○) as a function of temperature. In previous experiments, with a retinal temperature of 22°C and the identical stimulus parameters used in this experiment, the peak of the V_K+ response occurred when the rod receptor potential had decayed to ~30% of its plateau level (Figs. 1 and 3). Thus, the time for the receptor potential to decay to 30% of its plateau level was used as a measure of the duration of the response. In Fig. 6, this time is also plotted (■) as a function of temperature. For each type of response, a single exponential of the form \( y = ae^{bt} \), where \( a \) and \( b \) are constants and \( T \) is the temperature in °C, was fit to the data using a least-squares regression analysis. The fractional change in the response parameter for a 10°C change in temperature is termed the \( Q_{10} \), and is equal in magnitude to \( e^{10b} \). In Fig. 6, the \( Q_{10} \) for \( V_m \) was 2.1 and the \( Q_{10} \) for \( V_K+ \) was 2.0. In another pair of experiments, the \( Q_{10} \) for \( V_m \) was 2.3 and the \( Q_{10} \) for \( V_K+ \) was 2.1. Thus, temperature affected both response

![Figure 6](https://gfp.rupress.org/doi/10.1083/jgp.723.17a)

**Figure 6.** The effect of temperature upon the \( V_m \) and \( V_K+ \) responses. These data were obtained from the waveforms in Fig. 5. (○) Time-to-peak of the \( V_K+ \) response; (■) Time for the \( V_m \) response to recover to 30% of its plateau level (see text). The solid lines are of the form \( y = ae^{bt} \), where \( a \) and \( b \) are constants and \( T \) is the temperature in °C. The parameters were obtained from a least-squares regression analysis. (Although a linear relationship might seem appropriate over this temperature range, the exponential curve provided a better fit, as indicated by the coefficient of determination, \( r^2 \).)
parameters in a quantitatively similar manner. The effects of temperature were reversible.

**Role of the Na\(^+\)/K\(^+\) Pump**

It has been suggested that \([K^+]_o\) will decrease when the amount of K\(^+\) that is pumped into the rod by the Na\(^+\)/K\(^+\) pump exceeds the passive efflux of K\(^+\) from the rod (Zuckerman, 1973; Matsuura et al., 1978). The Na\(^+\)/K\(^+\) pump is thought to be located in the rod inner segment (Zuckerman, 1973; Hagins and Yoshikami, 1975; Stirling, 1977). If this pump current is involved in decreasing \([K^+]_o\), then K\(^+\) ions should be removed primarily from the extracellular space surrounding the rod inner segments.

In Fig. 7, the depth profile of the light-evoked change in \(V_{K^+}\) is shown. In this experiment, the K\(^+\)-specific microelectrode was first advanced into the

![Figure 7](image-url)

**Figure 7.** The depth profile of the \(V_{K^+}\) response. These data were recorded as the K\(^+\)-specific microelectrode was withdrawn from the retina. The depth of the electrode tip below the receptor surface is indicated (in micrometers) to the left of each response. This depth was calculated by multiplying the distance travelled along the electrode track by the sine of 23°, the angle the electrode made with the receptor surface. The stimulus was a 100-ms flash (arrow) of -3.0 log unit irradiance, presented repetitively at a rate of one flash every 50 s. The logarithmic scale at the right of the figure represents \([K^+]_o\), and has been shifted to be valid for the response recorded at a depth of 66 \(\mu\)m. Since the resting level of \([K^+]_o\) remained constant with retinal depth, the scale of \([K^+]_o\) may be applied (when appropriately shifted) to any of the \(V_{K^+}\) responses.
retina, and the illustrated responses were then recorded as the electrode was withdrawn. At proximal retinal depths (e.g., 66-98 μm), there was initially a rapid, light-evoked increase in [K+]o, followed in time by a much slower decrease in [K+]o. The initial increase in [K+]o was probably the “distal potassium increase,” described recently by Dick and Miller (1978) and Kline et al. (1978). At more distal retinal depths, only the slow decrease in [K+]o was present.

The light-evoked decrease in [K+]o attained its maximum amplitude at a retinal depth ≈66 μm below the receptor surface. At this depth, its initial slope was greatest and its time-to-peak was shortest. The rod outer segments in *Bufo marinus* are ~60 μm in length (Hárosi, 1975; Fain, 1976). Thus, the largest and most rapid decrease in [K+]o seems to occur in the extracellular space surrounding the rod inner segments. At more proximal and more distal retinal depths, the light-evoked decrease was smaller in amplitude, with a smaller initial slope and a longer time-to-peak. These results would be expected if the decrease in [K+]o was generated primarily at the inner segments and was propagated radially by diffusion (Oakley, 1975).

If the Na+/K+ pump in the rod inner segment provides the mechanism for the uptake of K+ from the extracellular space, then the light-evoked decrease in [K+]o should be abolished rapidly when the pump is inhibited. This prediction was tested by perfusing the retina with ouabain, an inhibitor of the Na+/K+ pump (Skou, 1965; Frank and Goldsmith, 1968; Glynn and Karlish, 1975).

A rod outer segment was impaled, and a −3.0 log unit, 100 ms stimulus was presented at a rate of one flash every 30 s. After a 5-min control period in order to insure steady-state responses, the solution perfusing the retina was switched to a ouabain (10⁻⁴ M) Ringer’s solution. Selected receptor potentials recorded during this experiment are shown at the left side of Fig. 8; the time of switching is indicated as t = 0. The first effect of ouabain was to abolish the initial transient of the receptor potential. With continued application, the receptor potential decreased in amplitude and became more prolonged. After 17.5 min, the plateau had declined to ≈46% of its control value. This amplitude continued to decline until it was ≈35% of its control value when the cell was lost at t = 29 min. Similar effects of ouabain upon the receptor potential were observed in two additional cells. In seven other cells, the addition of ouabain caused the cell to be lost within several minutes, presumably due to changes in cell volume following inhibition of the Na+/K+ pump.

In another experiment, a K+-specific microelectrode was advanced to a depth 40 μm below the receptor surface. The identical stimuli were then presented that had been used to observe the effects of ouabain on the receptor potential. Certain of the light-evoked changes in VKP are shown at the right of Fig. 8. Again, at t = 0, the ouabain Ringer’s solution began to flow into the chamber. Ouabain had a rapid effect on the waveform of the VKP response; the initial slope decreased in magnitude and the time-to-peak increased. Within 5.5 min, the VKP response amplitude had decreased to ≈26% of its control value, and after 17.5 min, the VKP response was totally abolished.
Thus, ouabain affected the $V_K^+$ response much more rapidly than the $V_m$ response. Similar effects of ouabain on the $V_K^+$ response were seen in two other retinas. The effects of ouabain on both types of response were irreversible.

**DISCUSSION**

The light-evoked changes in $[K^+]_o$, measured in the present experiments, were similar to those reported previously (Oakley and Green, 1976; Tomita, 1976; Oakley, 1977; Matsuura et al., 1978), with one major exception: the maximum amplitude of the decrease in $[K^+]_o$ was much smaller in the present experiments. The largest $V_K^+$ responses in the isolated retina were 5 mV, corresponding to a reduction in $[K^+]_o$ from 2.0 to 1.4 mM (Fig. 1). In the frog retina—pigment epithelium—choroid preparation, for example, $V_K^+$ responses of 13 mV have been observed, corresponding to a reduction in $[K^+]_o$ from 3.1 to 1.0 mM (Oakley, 1977).
One possible reason for the smaller changes in \([K^+]_o\) in the isolated retina is that the retina was separated from its pigment epithelium. The apical processes of the pigment epithelium interdigitate with the rod outer segments and extend all the way down to the inner segments (Nilsson, 1964; Fain, 1976). By removing the pigment epithelium, the volume of the extracellular space surrounding the rods was undoubtedly increased. A fixed loss of \(K^+\) ions from the extracellular space would therefore cause a smaller decrease in \([K^+]_o\).

Another possible reason for the smaller decrease in \([K^+]_o\) is that the receptor surface was continuously perfused with a modified Ringer's solution containing only 2.0 mM \([K^+]\). This perfusion lowered \([K^+]_o\) in the extracellular space surrounding the rods to 2.0 mM, compared with 3.1-5.5 mM in preparations in which the receptor surface was not perfused (Oakley and Green, 1976; Oakley, 1977). By reducing \([K^+]_o\), the operating range over which \([K^+]_o\) could be modulated by light was also reduced.

Although it is likely that the continuous perfusion reduced the overall amplitude of the \(V_{K^+}\) response, it is unlikely that individual \(V_{K^+}\) responses were altered in time-course or relative amplitude. In this type of perfusion system, the bulk solution does not contact the tissue directly. Instead, an unstirred layer (as thick as 200 \(\mu\)m) surrounds the tissue, in which transport is limited by diffusion (Dainty and House, 1966; Miller et al., 1978). Evidence for such an unstirred layer is seen in Fig. 7, where there was a measurable \(V_{K^+}\) response 39 \(\mu\)m away from the receptor surface. It is unlikely, therefore, that any changes in \([K^+]_o\) generated near the receptor surface would be "washed away" by convective flow. Since the \(V_{K^+}\) response at the receptor surface had a longer time-to-peak, as well as a smaller amplitude, than the \(V_{K^+}\) response near the inner segments, the results are consistent with diffusion from a localized source.

**Relationship between \(V_m\) and \([K^+]_o\)**

There are many similarities between the light-evoked changes in the rod membrane potential and \([K^+]_o\). All variations in the stimulus parameters had qualitatively similar effects on both the receptor potential and \([K^+]_o\). Dim stimuli, which did not saturate the receptor potential, produced graded changes in the magnitude of the initial slope of the decrease in \([K^+]_o\). More intense stimuli, which saturated the receptor potential, did not alter the initial slope, but did prolong the duration, of the decrease in \([K^+]_o\). As the rod plateau became more prolonged, the decrease in \([K^+]_o\) continued to grow in amplitude. Thus, the decrease in \([K^+]_o\) can be thought of as an integral of the rod receptor potential (see Fig. 1).

A similar conclusion was reached indirectly by Schmidt and Steinberg (1971), who measured the rod-dependent pigment epithelial hyperpolarization in the cat retina. This response is caused directly by the light-evoked decrease in \([K^+]_o\). Schmidt and Steinberg (1971) observed that the rod "aftereffect" (the plateau response) was integrated by the pigment epithelial cell hyperpo-

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larization, and they concluded that the "pigment epithelial response probably is generated therefore as a consequence of the same excitatory events which lead to the receptor potential of the rod." The present data are consistent with their conclusion.

Several characteristics of the light-evoked decrease in $[K^+]_o$ were directly related to properties of the rod receptor potential. The integrating times of the $V_m$ and $V_{K^+}$ responses were virtually identical, and were at least 7 s in duration, as shown in Fig. 4. When stimuli were used that contained 10 times more quanta than those in Fig. 4, both integrating times increased to over 10 s. As long as the stimuli produced identical receptor potentials, the changes in $[K^+]_o$ were also identical. The measured integrating time for the $V_{K^+}$ response is consistent with previously reported data. Oakley and Green (1976) found in the frog eyecup preparation that the integrating time for the light-evoked decrease in $[K^+]_o$ was at least 5 s. Lurie (1976) found in the intact frog eye that the integrating time for the $c$-wave of the electroretinogram (which is caused directly by the decrease in $[K^+]_o$; Oakley, 1977) was as long as 18.5 s when intense stimuli were used.

Decreasing temperature of the retina slowed the $V_m$ and $V_{K^+}$ responses. The $Q_{10}$ for the time-to-peak of the $V_{K^+}$ response was 2.0, compared with a $Q_{10}$ of 2.1 for the time required for the $V_m$ response to recover to 30% of its plateau level. Thus, temperature affected both of these response parameters in the same manner, as expected if the decrease in $[K^+]_o$ was produced in response to the receptor potential. Since the generation of a complex waveform such as the receptor potential is likely to involve many sequential steps, the $Q_{10}$ of 2.1 reflects the $Q_{10}$ of the step that changes least as a function of temperature. This $Q_{10}$ is greater than the $Q_{10}$ of a purely diffusional process (Hodgkin and Keynes, 1955), so the duration of the rod plateau does not seem to be limited by passive diffusion.

**Role of the Na$^+$/K$^+$ Pump**

An intense light stimulus caused $[K^+]_o$ in the distal retina to decrease from 2.0 mM (the concentration in the perfusate) to 1.4 mM (Fig. 1). In order for the extracellular concentration to decrease below the level in the surrounding space, some type of uptake mechanism must be involved. The present evidence suggests that this mechanism involves the Na$^+$/K$^+$ pump in the rod plasma membrane.

The maximum amplitude of the light-evoked decrease in $[K^+]_o$ was recorded $\approx$66 $\mu$m below the tips of the rod outer segments (Fig. 7). At this depth, the initial slope of the response was also maximal. This depth measurement was made with an accuracy of $\sim\pm8\,\mu$m, since the K$^+$-specific microelectrode was moved at a 23° angle to the rods in 20-40-$\mu$m steps (8-16-$\mu$m steps in the radial direction).

There is good reason to believe that the 66-$\mu$m depth was near the level of the rod inner segments. Microscopic measurements of the rods of *Bufo marinus*, either in fresh or fixed material, have shown that the rod outer segments are 50-68 $\mu$m in length (Hárosi, 1975; Fain, 1976; footnote 3). In addition,

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intracellular staining with Procion yellow, in the same preparation used in the present study, has shown that a micropipette would not penetrate an inner segment until its tip was at least 60 μm below the receptor surface (Brown and Flaming, 1977; footnote 3). It seems, therefore, that the light-evoked decrease in [K+]o is generated at the level of the rod inner segments. This same conclusion was reached by Oakley and Green (1976), who found in the frog eyecup that the light-evoked decrease in [K+]o was of maximal amplitude and minimal time-to-peak at the level of the rod inner segments.

The Na+/K + pump is likely to be located in the plasma membrane of the rod inner segments. [3H]Ouabain binding experiments have shown a high density of binding to inner segments, with almost no binding to outer segments (Stirling, 1977). Measurements of the dark current along the rods, which appears to consist mainly of the Na+ pump current (Zuckerman, 1973), have also localized the pump to the rod inner segment (Hagins et al., 1970; Zuckerman, 1973; Hagins and Yoshikami, 1975). Thus, the present data are consistent with the notion that the Na+/K + pump clears K+ from the extracellular space surrounding the inner segments. When light stimulation decreases the passive efflux of K+ from the cell, [K+]o changes first outside the inner segments. As the concentration change is propagated to other retinal depths by diffusion, its initial slope decreases in magnitude and its time-to-peak increases (Oakley, 1975).

Ouabain had a much more rapid effect on the light-evoked decrease in [K+]o than on the receptor potential, as shown in Fig. 8. If the effect of ouabain was only to inhibit the Na+/K + pump, then ouabain would eliminate the decrease in [K+]o by eliminating the mechanism by which K+ ions are transported into the cell from the extracellular space. After the pump was inhibited, the Na+ and K+ gradients across the rod plasma membrane would begin to decline. These gradients are necessary for the generation of the receptor potential, since this response involves a decrease in the sodium conductance, polarizing the cell towards the K+ equilibrium potential. When the pump was inhibited by ouabain, these gradients were not abolished immediately, since a receptor potential could still be recorded more than 20 min after the application of ouabain.

Ouabain could also reduce the light-evoked decrease in [K+]o if it decreased the K+ conductance (gK) of the rod. This would decrease the steady efflux of K+ out of the rod, and thus decrease the magnitude of the light-evoked change in the efflux. Ouabain affected both types of response within the 1st min after its application (Fig. 8). It is unlikely that these rapid effects were due to ouabain-induced changes in gK, since in other systems, gK is unaltered by ouabain within the 1st h after its application (Baker et al., 1969; Gorman and Marmor, 1974; Miller and Steinberg, 1977). Thus, it is concluded that the major effect of ouabain upon the VK+ response was due to an inhibition of the Na+/K + pump.

Model of the Light-Evoked Decrease in [K+]o
The measurements of the rod receptor potential and the light-evoked decrease in [K+]o, made in the same retina, can be used to test the model of the rod-
mediated potassium fluxes into and out of the extracellular space that was suggested by Matsuura et al. (1978). Since this model will be shown to predict the form of the actual light-evoked decreases in $[K^+]_o$, the model will now be presented in detail.

The flux of $K^+$ into and out of the extracellular space is shown diagrammatically in Fig. 9 A. The net flux of $K^+$ into the extracellular space, $i_K$, is equal to the difference between the passive efflux of $K^+$ along its electrochemical gradient, $I_K$, and the active influx of $K^+$ due to the $Na^+/K^+$ pump, $I_K^\ast$ (Zuckerman, 1973). This relationship can be written explicitly as

$$i_K = I_K - I_K^\ast$$

or

$$I_K = i_K + I_K^\ast. \hspace{1cm} (2)$$

The passive efflux of $K^+$ is given by the relationship

$$I_K = g_K(V_m - E_K), \hspace{1cm} (3)$$

where $E_K$ denotes the potassium equilibrium potential.

In the steady state, the efflux of $K^+$ out of the rod must be balanced by an equal influx from the pump. When light stimulates a rod, it produces a decrease in $g_{Na}$ in the rod outer segment, and thus a membrane hyperpolarization (Toyoda et al., 1969; Korenbrot and Cone, 1972; Brown and Pinto, 1974; Hagins and Yoshikami, 1975). This hyperpolarization will reduce the driving force on $K^+$ (given by Eq. 3), and assuming that $g_K$ remains constant (see below), the efflux of $K^+$ into the extracellular space will decrease (Zuck-

![Figure 9](https://example.com/figure9.png)

**Figure 9.** A model for the mechanism by which the light-evoked decrease in $[K^+]_o$ is generated. This figure is patterned after Fig. 10 of Matsuura et al. (1978). (A) The passive efflux of $K^+$ ($i_K$) from the rod divides into two components. Some of the $K^+$ ions ($i_K^\ast$) are pumped back into the rod by the $Na^+/K^+$ pump, and the remainder ($i_K$) flow into the extracellular space, altering $[K^+]_o$. (B) An electrical circuit that is analogous to the suggested model of the $K^+$ fluxes. The linear differential equation governing this circuit (Eq. 8) is identical in form to the one governing the hypothesized fluxes of $K^+$ into and out of the rod. See text for additional details.
eran, 1973). During the initial part of the light-evoked hyperpolarization, therefore, the pump influx will exceed the reduced efflux, and the net flux of $K^+$ into the extracellular space, $i_K$, will be negative. It is this flux of $K^+$ that is assumed to produce the observed decrease in $[K^+]_o$.

In other systems, the $Na^+/K^+$ pump is sensitive to $[K^+]_o$ (Glynn, 1962; Glynn and Karlish, 1975). If this is the case in the rod, the reduction in $[K^+]_o$ will decrease the rate of the pump. For this model, it will be assumed that the pump rate varies linearly with $[K^+]_o$. Moreover, it will be assumed that the pump rate is not altered by the change in membrane potential.

It is possible to transform the model of Fig. 9A into the equivalent circuit shown in Fig. 9B, due to direct analogies between the differential equations governing chemical and electrical systems. In this analogy, voltage ($v$) is equated to concentration, capacitance ($C$) to volume, and current to ionic flux. Thus, the voltage $v$ represents $[K^+]_o$. The current, $i_K$, is given by

$$i_K = C \frac{dv}{dt} \quad (4)$$

The pump influx, $I_K$, was assumed to vary linearly with $[K^+]_o$ (and thus $v$), so that

$$I_K = \frac{v}{R} \quad (5)$$

In this context, the scalar $1/R$ is the constant of proportionality relating $[K^+]_o$ to pump current. Eq. 2 can now be rewritten as

$$g_K(V_m - E_K) = C \frac{dv}{dt} + \frac{v}{R} \quad (6)$$

This equation is a linear differential equation of the form

$$\frac{dV}{dt} + \frac{V}{\beta} = \frac{1}{\beta} g_K(V_m - E_K), \quad (7)$$

where $\beta$ equals $RC$ (the time constant of the network in Fig. 9B) and $V$ equals $v/R$. The variable $V$ is thus also a measure of $[K^+]_o$, since it is related to $v$ (or $[K^+]_o$) by the scalar $1/R$.

In the present experiments, only the light-evoked changes in $V_m$ and $[K^+]_o$ were considered to be important. By defining new variables ($V'_m$ and $V'$), where the prime indicates the light-evoked change in the variable, Eq. 7 can be rewritten in the form

$$\frac{dV'}{dt} + \frac{V'}{\beta} = \frac{1}{\beta} (g_K \cdot V'_m), \quad (8)$$

where the forcing function has been equated (as a first approximation—see below) to $g_K \cdot V'_m$. Eq. 8 can be solved for various forcing functions using actual light-evoked changes in $V'_m$. The resulting solutions to the differential equation ($V'$) must have time-courses similar to the corresponding light-evoked decreases in $[K^+]_o$ in order for the model to be valid.
The responses to dim stimuli were examined first. The receptor potential elicited by a -4.0 log unit, 100-ms flash ($V_m$ in Fig. 1) was sampled at 25-ms intervals. The digitized waveform was used as the forcing function, $V_m$, in Eq. 8 ($g_K$ was assumed constant). The integration was performed using a fourth-order Runge-Kutta method (Carnahan et al., 1969). The value of $\beta$ was varied to give the "best fit" of the modelled response, $V'$, to the actual change in $[K^+]_o$ recorded in the same retina in response to the same stimulus ($V_{K^+}$ in Fig. 1). Although $V_{K^+}$ was actually a measure of the logarithm of $[K^+]_o$, the deviation from linearity was small in the region of interest (note the logarithmic scale in Fig. 1), so the time-course of the $V_{K^+}$ response was very nearly the same as that of the light-evoked decrease in $[K^+]_o$. A value of $\beta$ equal to 5.5 s was judged to give the best fit of the model to the data. The solution to the equation, $V'$, is shown in Fig. 10 B, along with the actual $V_{K^+}$ response (replotted from Fig. 1). The $V'$ waveform was scaled to have the same peak amplitude as the $V_{K^+}$ response. In support of the model, these two responses were similar in time-course.

Since the value of $\beta$ is the only parameter that is variable in Eq. 8, the model became fixed once a value of $\beta$ was chosen. If the model is valid, then it should successfully predict the changes in $[K^+]_o$ in response to any change in $V_m$. To test this model in this manner, the change in $V_m$ in response to an intense, 0.0 log unit, 100-ms flash ($V_m$ in Fig. 1) was used as the forcing function in Eq. 8. The receptor potential was sampled at 100-ms intervals, and the same fourth-order Runge-Kutta method was used to integrate the differential equation. The solution, $V'$, is shown in Fig. 10 B, along with the actual change in $V_{K^+}$ elicited by the same stimulus in the same retina ($V_{K^+}$ in Fig. 1). The scale factor used to plot the $V'$ waveform in Fig. 10 B was identical to the one used in Fig. 10 A. Again, the modelled response and the actual response were very similar in waveform. Thus, it was possible to predict the waveform of the light-evoked decrease in $[K^+]_o$ solely by knowing the waveform of the receptor potential. This result strongly supports both the original hypothesis and the detailed model.

The reason for the delay between the peak of the rod receptor potential and the peak of the decrease in $[K^+]_o$, seen with dim stimuli, is easily explained by the model. The efflux of $K^+$ from the rod can be thought of as a current flowing into the $RC$ circuit of Fig. 9 B. For rapid changes in this current, there will be a phase lag between the peak current change and the peak voltage change. This phase lag was most noticeable with the dimmest flashes (Fig. 1). With intense or prolonged stimuli, the change in the efflux of $K^+$, due to the prolonged rod plateau, is kept constant for several "$RC$ time constants." At the end of the plateau, $[K^+]_o$ is almost stabilized at a new level (Fig. 1, log I = 0.0). At this point, the new efflux is equal in magnitude to the new pump influx, and there is no additional change in $[K^+]_o$; this is equivalent to charging the capacitor in Fig. 9 B to a new voltage. As soon as the rod begins to repolarize, the efflux of $K^+$ will become greater than the pump influx, causing $[K^+]_o$ to increase. Thus, the phase lag between the receptor potential and the decrease in $[K^+]_o$ becomes much less with stimuli that prolong the rod plateau (Figs. 1 and 3).
The model can also account for the finding that the light-evoked decrease in \([K^+]_o\) is rate-limited. Over a 3 log unit range, increases in the stimulus irradiance (from -3.0 to 0.0 log units) did not increase the magnitude of the initial slope of the decrease in \([K^+]_o\) (Fig. 1). For these stimuli, the rod receptor potentials all reached a saturated plateau level. Thus, it was the driving force behind the decrease in \([K^+]_o\) that was saturated. This, in turn, caused the initial slope of the decrease in \([K^+]_o\) (which is directly related to the driving force itself; see Eq. 8) to be saturated, also.

**Figure 10.** Responses of the equivalent circuit, compared with actual changes in \([K^+]_o\). (A) The forcing function had the identical time-course as the receptor potential elicited by a dim, -4.0 log unit flash \((V_m\) in Fig. 1). (B) The forcing function had the identical time-course as the receptor potential elicited by an intense, 0.0 log unit flash \((V_m\) in Fig. 1). In both parts of this figure, the response of the circuit, \(V'\), was obtained by integrating Eq. 8, using a numerical method. The actual changes in \([K^+]_o\) elicited by the same stimuli (labelled \(V_{K^+}\)) have been replotted from Figs. 1 and 2.

**Limitations of the Model**

For all rods impaled in a given retina, the receptor potentials elicited by stimuli of fixed irradiance were very similar in time-course, even though these responses differed somewhat in amplitude. Such results are consistent with a network model of interreceptor coupling (Fain, 1975; Fain et al., 1976; Gold, 1979). Since the time-course, and not the absolute amplitude, of the \(V_m\) response was used in testing the model, the results were unaffected by differences in response amplitude between rods.

A value of \(\beta\) (the RC time constant of the circuit in Fig. 9 B) equal to 5.5 s gave the "best fit" of the model to the actual data. In this model, \(R\) was the coefficient relating the Na\(^+\)/K\(^+\) pump current to \([K^+]_o\), and \(C\) represented the volume of the extracellular space. Removing the pigment epithelium...
would increase the volume of the extracellular space, and thus the value of C in the model. It is likely, therefore, that a smaller value of β would be more appropriate in the intact retina. In addition, the slowing of the initial slope of the light-evoked decrease in [K+]o, seen at low temperature (Fig. 5), could have been due in part to a slowing of the Na+/K+ pump. Thus, it is likely that the value of β would also vary as a function of temperature.

For this model it was assumed that the rod membrane hyperpolarization decreased the passive efflux of K+ solely by reducing the driving force on K+ (Eq. 3). Additionally, the Na+/K+ pump was assumed to vary its rate linearly with [K+]o. It was not necessary to consider other complexities, such as potential-dependent changes in either gK or the rate of the Na+/K+ pump. While it is likely that gK does vary with Vm (Werblin, 1975; Fain et al., 1977), the changes in gK are likely to be related monotonically to the changes in Vm. If that is the case, then the change in the passive efflux of K+ would still have a time-course similar to the receptor potential. If gK increases as the rod hyperpolarizes (Werblin, 1975), this factor would oppose the decrease in (Vm - EK) and tend to reduce the magnitude of the light-evoked decrease in the passive efflux of K+.

It is possible that the methods used to test the model masked the effects of a change in gK. For most stimuli, the receptor potential reached a saturated plateau level, which varied in duration as a function of stimulus irradiance and duration. Thus, the passive efflux of K+ (given by Eq. 3) was changed for varying durations, but by a constant amount. If the magnitude of the change in Vm was constant, any voltage-dependent changes in gK would have produced a constant effect. It would have been necessary to vary the amplitude of the plateau response in order to see these effects. In an attempt to vary this amplitude, 2 mM Cs++ was added to the perfusate (Fain et al., 1978). Although this treatment more than tripled the magnitude of the plateau response, the effects on the light-evoked decrease in [K+]o could not be assessed, since the K+-specific microelectrodes were ≈ 40 times more sensitive to Cs++ than to K+ (Wise et al., 1970).

A voltage-dependent change in gK also might have had a significant effect on the Vr responses following the application of ouabain. A ouabain-induced depolarization of the rod membrane could have decreased gK and reduced the passive efflux of K+. This factor could have contributed to the rapid loss of the light-evoked decrease in [K+]o following ouabain. In sum, the assumption of constant gK, and the resulting simplifications in the model, might be applicable only when the light-evoked changes in Vm are of nearly constant magnitude.

One other aspect of the model must be considered. In deriving Eq. 8, the light-evoked change in EK, denoted EK, was neglected. EK will become more negative as [K+]o decreases, which will increase the passive efflux of K+ (Eq. 3) and reduce the size of the decrease in [K+]o. Thus, the actual driving force behind the decrease in [K+]o would have to include a term gK·EK, although the present results suggest that this term has only a minor contribution.

Finally, it must be mentioned that although the present data are consistent
with the hypothesis of Matsuura et al. (1978), they do not prove the hypothesis. The possibility remains that other mechanisms also contribute to the generation of the decrease in \([K^+]_o\).

**Effects of the Light-Evoked Decrease in \([K^+]_o\)**

The light-evoked decrease in \([K^+]_o\) seems likely to be a direct consequence of the rod receptor potential. This change in ionic concentration alters the potential difference across the pigment epithelial cell membranes, producing the c-wave of the electroretinogram (Oakley, 1977). Thus, the c-wave, which can be recorded across the entire retina with macroelectrodes, is also likely a direct consequence of the rod receptor potential.

Through its effect on the pigment epithelium, the decrease in \([K^+]_o\) is a potent modulator of transport into and out of the retina. For example, the transepithelial flux of taurine, an amino acid that is concentrated in photoreceptors (Cohen et al., 1973), is altered by changes in retinal \([K^+]_o\) within the physiological range (Miller and Steinberg, 1979). By \(K^+\)-mediated interactions with the pigment epithelium, therefore, the rods can signal the absorption of light, and directly affect their extracellular environment. It is not unreasonable to speculate that similar ionic interactions could affect the transport of ions and molecules involved in the regeneration of visual pigment, or in the synthesis of disc protein.

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