Electrical and Adaptive Properties of Rod Photoreceptors in *Bufo marinus*

I. Effects of Altered Extracellular Ca^{2+} Levels

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ABSTRACT The effects of altering extracellular Ca^{2+} levels on the electrical and adaptive properties of toad rods have been examined. The retina was continually superfused in control (1.6 mM Ca^{2+}) or test Ringer's solutions, and rod electrical activity was recorded intracellularly. Low-calcium Ringer's (10^{-9} M Ca^{2+}) superfused for up to 6 min caused a substantial depolarization of the resting membrane potential, an increase in light-evoked response amplitudes, and a change in the waveform of the light-evoked responses. High Ca^{2+} Ringer's (3.2 mM) hyperpolarized the cell membrane and decreased response amplitudes. However, under conditions of either low or high Ca^{2+} superfusion for up to 6 min, in both dark-adapted and partially light-adapted states, receptor *sensitivity* was virtually unaffected; i.e., the V-log *I* curve for the receptor potential was always located on the intensity scale at a position predicted by the prevailing light level, not by Ca^{2+} concentration. Thus, we speculate that cytosol Ca^{2+} concentration is capable of regulating membrane potential levels and light-evoked response amplitudes, but *not* the major component of rod sensitivity. Low Ca^{2+} Ringer's also shortened the period of receptor response saturation after a bright but nonbleaching light flash, hence accelerating the onset of both membrane potential and sensitivity recovery during dark adaptation.

Exposure of the retina to low Ca^{2+} (10^{-9} M) Ringer's for long periods (7–15 min) caused dark-adapted rods to lose responsiveness. Response amplitudes gradually decreased, and the rods became desensitized. These severe conditions of low Ca^{2+} caused changes in the dark-adapted rod that mimic those observed in rods during light adaptation. We suggest that loss of receptor sensitivity during prolonged exposure to low Ca^{2+} Ringer's results from a decrease of intracellular (intradisk) stores of Ca^{2+}; i.e., less Ca^{2+} is thereby released per quantum catch.

INTRODUCTION

In 1971, Yoshikami and Hagins postulated that Ca^{2+} serves as an intracellular messenger in the outer segment of the vertebrate visual cell. According to their

hypothesis, Ca^{2+} is released into the cytosol of the outer segment upon excitation of rhodopsin by light. The increase in intracellular Ca^{2+} decreases the Na^+ permeability of the outer segment plasma membrane, resulting in a hyperpolarization of the photoreceptor cell. A number of studies have provided evidence compatible with the Ca^{2+} hypothesis (Hagins, 1972; Korenbrot and Cone, 1972; Yoshikami and Hagins, 1973; Hagins and Yoshikami, 1974; Hendriks et al., 1974), but unequivocal evidence is still lacking and thus far experiments have failed to demonstrate calcium release with the stoichiometry and time course required for the internal messenger. However, even if the Ca^{2+} hypothesis is not correct, the ion appears to be important in photoreceptor activity because it is present in substantial amounts in rod outer segments (Liebman, 1974; Hendriks et al., 1974; Hess, 1975; Weller et al., 1975).

Some information has been provided on the effects of altering extracellular levels of Ca^{2+} on the electrical and other properties of vertebrate photoreceptor cells. Yoshikami and Hagins (1973), Winkler (1974), and Snyder (1974) showed that changing extracellular Ca^{2+} levels significantly affected the amplitude of the extracellularly recorded receptor response of the isolated rat retina. These effects could be enhanced by the addition of ionophores to the bathing media (Hagins and Yoshikami, 1974). Lowered (10^{-5} to $<10^{-7}$ M) Ca^{2+} increased response amplitudes; increased (20 mM) Ca^{2+} decreased response amplitudes. Yoshikami and Hagins (1973) further reported that with time in low Ca^{2+} the receptor potential was desensitized; i.e., more light was required to produce a response of half-maximum amplitude. Brown and Pinto (1974) tested the effects of low and high exogenous Ca^{2+} levels on rod intracellular activity in the toad retina, and they observed changes in both membrane potential and response amplitudes. Low (0.6 mM) extracellular Ca^{2+} levels depolarized the cell and increased the amplitudes of light-evoked responses; high (12.5 mM) extracellular Ca^{2+} hyperpolarized the cell and decreased response amplitudes.

In the experiments described here we have further examined the effects of altering Ca^{2+} on the intracellular electrical activity recorded from single toad rods. We were particularly concerned wth the effects of altered Ca^{2+} levels on the adaptive properties of the photoreceptor cells. For example, a major portion of visual adaptation occurs too quickly in the photoreceptors to be explained by the slower changes in visual pigment concentration (for references see Grabowski and Pak, 1975). Thus several workers have speculated that the fast changes in photoreceptor sensitivity during adaptation are coupled to membrane potential (Boynton and Whitten, 1970; Grabowski et al., 1972; Norman and Werblin, 1974; Baylor and Hodgkin, 1974; Baylor et al., 1974*a*, *b*) or to the cytosol calcium ion concentration (Yoshikami and Hagins, 1973; Hagins and Yoshikami, 1974). Our results suggest, however, that the major change in receptor sensitivity during adaptation is not closely related either to the membrane potential or to the cytosol calcium concentration. On the other hand, receptor sensitivity is significantly altered by conditions that presumably decrease intradisk stores of Ca^{2+}.

MATERIALS AND METHODS

Preparation

Marine toads *Bufo marinus* were dark adapted for 24 h. After pithing and excision of an

eye, a segment of the globe was sliced off with a razor blade and placed in a Ringer's-filled chamber. The retina was gently peeled away from the pigment epithelium and laid receptor side-up in the perfusion chamber. This dissection was performed quickly (~2 min) in dim red light. The isolated retina was viewed subsequently under IR illumination by image conversion.

Recording

Glass microelectrodes were pulled from Pyrex capillary tubing (OD 1.0 mm, type 7740, Corning Glass Works, Corning, N. Y.) on a Livingston puller modified with a timed solenoid release. Fine micropipettes, filled with 4 M KAc and measuring 400–1,000 MΩ in resistance in Ringer's, were used to penetrate photoreceptors.

The retina was viewed from above at × 400 through an IR image converter. Under direct observation a micropipette was lowered toward the rods with a micromanipulator (JENA, New York). The reference electrode, containing 3 M KCl, had a diameter of 1 mm, was placed downstream from the retinal perfusion, and displayed no significant change in tip potential in any of the bathing media.

Intracellular signals were amplified by a high input impedance, negative capacitance electrometer (model M-4AR, WP Instruments, Hamden, Conn.), and high-frequency noise was attenuated by passive low pass (3 ms time constant) and active 60 cycle notch filters (Frequency Devices, Haverhill, Mass.). Photoreceptor response amplitudes and waveforms were negligibly affected by these filters. Potentials were displayed on an oscilloscope and permanently recorded on a pen recorder. Most responses were also recorded after frequency modulation on a four-channel stereotape recorder.

Superfusion

The isolated retina was superfused with control Ringer's or test solutions throughout the experiment. Simultaneous superfusion and intracellular recordings from receptors were performed in an apparatus originally designed by Brown and Pinto (1974). All solutions were oxygenated, allowed to equilibrate at 24°C, entered by gravity feed, and recovered by suction. Test solutions of known concentration were added to the superfusate for varying time periods. By turning a valve the test medium entered the experimental chamber and mixed rapidly. The concentration of the test solution in the chamber thus increased with time at an exponential rate (first order linear differential equation) determined by the flow rate (15 μl/s) and chamber volume (0.4 ml). At the end of the test period, the test solution was washed out of the chamber at a similar rate. Turnover of the solution in the chamber took approximately 1 min. Hence, at any particular time after introduction of a test agent, its concentration in the bath could be calculated by the differential equation. Varying the length of time of addition provided a way of increasing test solution concentration in a graded fashion. The absolute intracellular concentration of agent in the receptors could not, of course, be estimated accurately. This method is perhaps less desirable than addition of a square pulse of drug, but we found mechanical perturbations were avoided, and receptor responses could be continuously monitored as drug concentrations increased (Ames and Pollen, 1969).

The composition of the control Ringer's (Brown and Pinto, 1974) was 108 mM NaCl, 0.6 mM Na_2SO_4, 2.5 mM KCl, 1.2 mM $MgSO_4$, 1.6 mM $CaCl_2$, 0.13 mM $NaHCO_3$, 3.0 mM *N*-2 hydroxyethylpiperazine *N'*-2-ethanesulfonic acid (HEPES), and 5.6 mM glucose adjusted to pH 7.4 with 1 N NaOH. Much less than 1 mM Na^+ was added during the pH adjustment. Low calcium Ringer's contained approximately 10^{-9} M Ca^{2+}. It was prepared by adding 1 mM ethylenebis[oxyethylenenitrilo]tetraacetate (EGTA) to "Ca^{2+}-free" Ringer's containing Mg^{2+} and adjusting the pH to 7.4 with NaOH. Approximately 1.7 mM Na^+ was added during this pH adjustment. In a number of experiments the

divalent cation ionophore A23187 was used in conjunction with 10^{-9} M Ca^{2+} Ringer's to lower intracellular Ca^{2+} levels, or in the presence of normal Ca^{2+} (1.6 mM) to raise intracellular Ca^{2+} concentration (Reed and Lardy, 1972). The ionophore (donated by Robert L. Hamill, Eli Lilly and Co.) was first dissolved in ethanol which was later diluted to 0.03% in Ringer's. Control superfusion with 0.03% ethanol in Ringer's had no effect on the electrical responses of toad rods. All solutions were made with glass double-distilled water.

Photic Stimulation

A dual beam photostimulator (described in Kleinschmidt, 1974) illuminated the retina. Both beams originated from the same tungsten iodide quartz lamp. Light passed up through the retina before impinging axially upon the receptor layer. Electromagnetic shutters (Uniblitz, model 225, Vincent Associates, Rochester, N. Y.) interrupted, and neutral density filters (Bausch & Lomb or Eastman Kodak Wratten) attenuated, the stimulus beams.

Beam 1 provided diffuse, short (200 ms) test flashes of light; beam 2, diffuse background or adapting illumination. Interference filters with peak transmittance at 500 nm (10-nm bandwidth at half-peak transmittance, Ditric Optics, Marlboro, Mass.) and KG-1 and KG-3 IR blocking filters (Schott-Jena, Duryea, Pa.) were placed in the path of both beams. The absolute intensity of each beam was calibrated with a photodiode (pin 5, United Detector Technology, Santa Monica, Calif.) as previously described (Fain and Dowling, 1973; Fain, 1975*a*, *b*). The unattenuated retinal illuminance of beam 1 was 3.06 $\mu W/cm^2$ and of beam 2, 0.54 $\mu W/cm^2$. These energies correspond to 1.4×10^7 and 2.5×10^6 photons absorbed per receptor-second. The mean number of quanta absorbed per rod at each stimulus intensity was computed as in Fain (1975*a*).

When beam 2 was used to deliver a bright step of light for dark adaptation experiments, a "yellow" (Corning 3484) cutoff filter and IR blocking filters were interposed in the light path whose unattenuated intensity was about 1.1 mW/cm^2 with light between 550 and 700 nm. Calculations show that the two intensities of 12-s duration yellow light routinely used in the dark adaptation experiments yielded a negligible (~0.03%) bleach in one case and ≤3% bleach in the other.

Protocol

After a receptor had been impaled, control responses were obtained during superfusion in control Ringer's. While recordings were being taken from the same cell, test solutions were introduced into the experiment chamber, and electrical responses were studied under identical stimulus conditions. Finally, the test solution was washed out, and the photic stimulation repeated.

Unless otherwise stated, each reported effect was observed in at least 8 rods and often in as many as 30 or 40. The test of statistical significance of the effects of test agents on responses to brief flashes and light adaptation involved a least-squares fit linear regression analysis on Lineweaver-Burk or double reciprocal plots (1/V vs. $1/I$, where V is response amplitude and I is intensity of the light flash). Plotting the response-intensity data in this matter yielded a straight line whose slope was the reciprocal of V_{max}/σ (Fain, 1975*a*). Coefficients of correlation (r^2) for the straight line fit in Ringer's-perfused rods ranged from 0.972 to 1.000 (mean, 0.995); for drug-treated rods r^2 was 0.985–0.999 (mean, 0.994). A two-tailed t-test for each rod, comparing slopes of the straight line in control Ringer's and in drug, was used as the test of significance. t-tests comparing V_{max} or σ for control vs. test agent could also be computed for each rod. The test of statistical significance of the effects of test agents on dark adaptation consisted of a two-tailed t-test comparing the time of onset of membrane recovery in control Ringer's and test

medium. *t*-tests comparing the time of complete membrane potential recovery (to the dark-adapted value) or the return of sensitivity (determined by criterion response threshold) for control vs. test agent were also determined. Level of significance was $P < 0.01$.

Identity of Cell Penetrated

Intracellular recordings from toad rods may be identified by characteristic waveform responses to brief flashes of light (Brown and Pinto, 1974; Fain, 1975*a*, *b*). In our experiments, when the electrode was lowered into the tissue, receptor potentials were immediately encountered, suggesting that most recordings were from outer segments (Werblin, 1975). In other retinas staining has verified the penetration of outer segments when the retina is approached from the receptor side (Toyoda et al., 1969, 1970; Murakami and Pak, 1970; Grabowski et al., 1972). In several experiments, we recorded receptor potentials while perfusing the retina with 2 mM Na asparate or $CoCl_2$. This provided additional evidence that our recordings were from receptors. These treatments abolish all responses from second order cells but not from photoreceptors (Dowling and Ripps, 1972; Cervetto and MacNichol, 1972; Cervetto and Piccolino, 1974; Coles and Yamane, 1975).

RESULTS

The data in this and the following paper were derived from 790 intracellular recordings in control Ringer's, and 183 recordings which lasted sufficiently long ($\geq$10 min) and gave responses stable and large enough (maximum amplitude $\geq$10 mV) to monitor the effects of control Ringers *and* one or more test solutions. Several cells were recorded from for nearly 4 h.

Responses of Dark-Adapted Rods to Flashes

Fig. 1 shows the response of a dark-adapted toad rod to 200-ms flashes of 500-nm light during superfusion in control Ringer's. With increasing photons absorbed per rod-flash, the hyperpolarizing response increased in amplitude until it saturated at ~30,000 photons per rod. With the higher stimulus intensities, the response became more complex in waveform and consisted of an initial transient followed by a prolonged plateau. Rods in these experiments were capable of responding consistently to flashes of less than one photon absorbed per rod-flash, indicating that the receptors were pooling their responses at dim intensities (Fain, 1975*a*; Fain et al., 1976).

In the experiments of Fig. 2, low (10^{-9} M) Ca^{2+} Ringer's was infused into the perfusion chamber, and responses at one intensity were monitored over time. As extracellular Ca^{2+} was lowered, the membrane potential of the cell depolarized and the hyperpolarizing response amplitude grew larger. Note that after 2 min of continuous addition of 10^{-9} M Ca^{2+} the absolute magnitude of the increase of the hyperpolarizing response amplitude exactly equalled the depolarization of membrane (Fig. 2 A). After a 4-min infusion, the depolarization was of even greater magnitude, the response amplitude had further increased, and the initial transient of the photoreceptor response had disappeared. Within about 6–7 min of continuous infusion, however, the rod began to lose responsiveness. By 10–15 min the cell had depolarized by nearly 25 mV, and no longer could any responses be elicited from the cell even with the brightest flashes

available. The addition of micromolar concentrations of A23187 ionophore to the 10^{-9} M Ca^{2+} Ringer's resulted in effects identical to those seen with low Ca^{2+} Ringer's alone, but the events occurred sooner, e.g., the maximum increase in amplitude and the disappearance of the transient were apparent within 2–3 min of superfusion with these test agents, and the cell began to lose responsiveness sooner.

All effects of lowered (10^{-9} M) Ca^{2+} were readily reversible with superfusions up to about 5 min. For instance, Fig. 2 B shows an experiment in which Ringer's containing 10^{-9} M Ca^{2+} was infused for 3.5 min. The change in

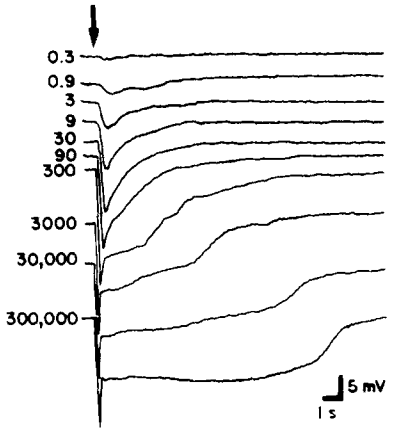

FIGURE 1. Intracellular responses of a dark-adapted toad rod superfused in control Ringer's. A typical intensity-response series for 200-ms flashes of diffuse 500-nm light is shown. Arrow indicates onset of 200-ms stimulus. The number to the left of each response is the average number of photons absorbed per rod per flash. Flashes were presented every 15 s for intensities up to 300 photons absorbed per rod-flash; at higher intensities flashes occurred at 1-min intervals. Responses to intensities greater than 300 photons absorbed per rod-flash have been slightly displaced for clarity. Resting membrane potentials in rods were typically about −25 mV. It is interesting to note that for extremely intense flashes (e.g., 300,000 photons absorbed per rod-flash) the plateau potential of the response slowly drifted more negative, toward the peak response of the transient.

response amplitude recorded at 2 min was almost identical to that observed in the experiment shown in Fig. 2 A. However, the response recorded at 4 min was already somewhat smaller than the response recorded at this time from the continuously superfused rod shown in Fig. 2 A. By 10–15 min the response shown in Fig. 2 B was virtually identical to the control record; i.e., the rod had completely recovered from the effects of the low Ca^{2+} Ringer's. With Ringer's that contained 0.8 mM Ca^{2+}, effects were entirely reversible for superfusions lasting up to 10 min (the longest tried). With superfusions of 10^{-9} M Ca^{2+} Ringer's of more than 6–7 min, on the other hand, the effects were not reversible and the behavior of the rods differed from that observed earlier. Thus, we will discuss separately the effects on rods of lowered (10^{-9} M) Ca^{2+}

superfused for up to 5–6 min and the effects of lowered (10^{-9} M) Ca^{2+} infused for longer than 6–7 min.

In Fig. 3, peak voltage amplitude as a function of flash intensity is plotted for a dark-adapted rod superfused first in control Ringer's and then in Ringer's containing 10^{-9} M Ca^{2+}. The latter voltage-intensity curve was determined between 4 and 6 min of low Ca^{2+} Ringer's infusion. Both V-log I curves follow

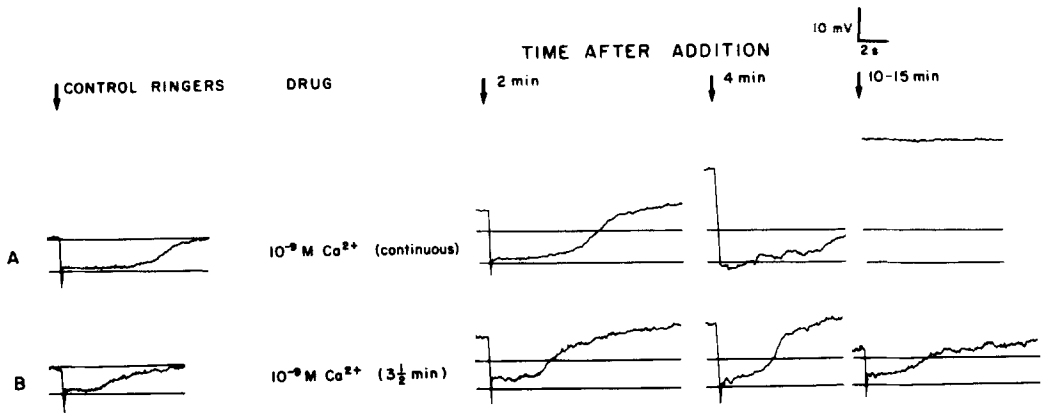

FIGURE 2. Effects of lowering Ca^{2+} upon intracellular responses of single dark-adapted toad rods to diffuse 200-ms flashes of 500-nm light. Each horizontal row presents responses from the same cell. The horizontal lines superimposed on each response indicate the original resting potential and plateau potential. Responses to one intensity only (30,000 and 3,000 photons absorbed per rod-flash for Fig. 2 A and 2 B, respectively) are shown. Concentration of agent in reservior is indicated in the test media column along with the length of addition. In both A and B, qualitatively similar effects were observed at 2 min. That is, both cells had depolarized and their response amplitudes had increased. At 4 min the effects of a 3.5-min superfusion in low Ca^{2+} (Fig. 2 A) were greater but qualitatively similar to the 2 min effects. However, continuous low Ca^{2+} (B) led to changes in waveform; e.g., the initial transient disappeared. By 10–15 min the effects of the short-term low Ca^{2+} infusion had reversed (B), while continuous low Ca^{2+} superfusion resulted in a loss of all light-evoked activity (A). For Fig. 2 A similar results were obtained in 15 of 15 intracellular recordings from rods, with a mean increase in V_{max} of 2.4-fold (range 1.3–7.5). For Fig. 2 B similar results were found in 12 of 13 rods with a mean increase in V_{max} of 1.6-fold (range 1.2–2.3). The change in V_{max} was significant with $P < 0.01$ in each case.

the relation $V = V_{max} I/(I + \sigma)$ (Naka and Rushton, 1966), where V is the peak incremental voltage amplitude of the response to flash intensity I, (i.e. peak voltage of response minus resting membrane potential), V_{max} is the peak amplitude at response saturation, and σ is the flash intensity which elicits a response of $1/2$ V_{max}. V_{max} may vary about threefold (10–30 mV) among intracellular recordings of dark-adapted rods in control Ringer's, but it is constant for a given rod while in control Ringer's. For a dark-adapted rod in control Ringer's, σ is about 30 photons absorbed per rod-flash at 500 nm. The

same value for σ has been observed for rod responses in a number of species (Penn and Hagins, 1972; Dowling and Ripps, 1972; Fain, 1975*a*).

At dim intensities ($I \ll \sigma$) the above expression states that the response amplitude (V) is linearly proportional to the flash intensity (I) and a constant, V_{max}/σ. This constant expresses the sensitivity or gain of the rod since it relates response amplitude to stimulus intensity and has the units millivolts per photon absorbed. In terms of the voltage-intensity curve in Fig. 3, σ is a determinant of the position of the curve on the horizontal intensity axis, while ΔV_{max} is a

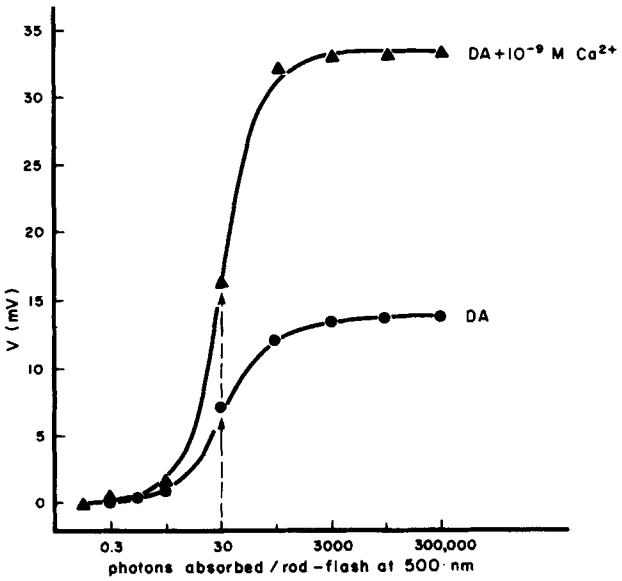

FIGURE 3. Plot of response amplitude vs. flash intensity for a single dark-adapted toad rod. The duration of each flash was 200 ms, and its intensity is expressed in photons absorbed per rod-flash. The lower curve was compiled during superfusion in control Ringer's, and the upper curve was between 4 and 6 min of superfusion in 10^{-9} M Ca^{2+}. Note that low Ca^{2+} affected the amplitude of responses compared to control but did not shift the curve along the abscissa, as reflected by little or no change in σ (indicated by arrows). Mean and range changes in V_{max} in low Ca^{2+} compared to control Ringer's are presented in the legend to Fig. 2*A*.

measure of response compression or amplification of the vertical axis (Kleinschmidt and Dowling, 1975). For example, Fig. 3 shows that the responses of a rod to brief flashes were simply vertically amplified (V_{max} increased) after superfusion of up to 6 min in Ringer's containing 10^{-9} M Ca^{2+}. That is, the position of the V-log I curve on the intensity scale (σ) remained virtually the same, as did the range of intensities over which the receptor responded. Thus, lowering extracellular Ca^{2+} for this period of time appeared to multiply responses by a constant factor. Hence, although V_{max} increased with 10^{-9} M Ca^{2+} superfusion for $\leq$6 min, σ displayed virtually no change, and the sensitivity (V_{max}/σ) increased no more than three to fourfold (Cf. Yoshikami and Hagins, 1973).

Several experiments were performed with Ringer's containing increased levels of Ca^{2+} (3.2 mM). Fig. 4 A shows the effects of a 3-min superfusion of 3.2 mM Ca^{2+} on the response of a rod. As extracellular Ca^{2+} was raised, the membrane potential of the cell hyperpolarized, and the amplitude of the light-evoked response decreased. After 2 min of continuous addition of 3.2 mM Ca^{2+}, the absolute magnitude of the decrease in the response amplitude equalled the increase in the hyperpolarization of the membrane. At 4 min there was observed a further hyperpolarization of the membrane potential and an equivalent decrease of response amplitude, but thereafter the rod began to recover. By 10–15 min of Ringer's washout, the effects of the increased Ca^{2+} were nearly entirely reversed. In Fig. 4 B records are displayed from a rod

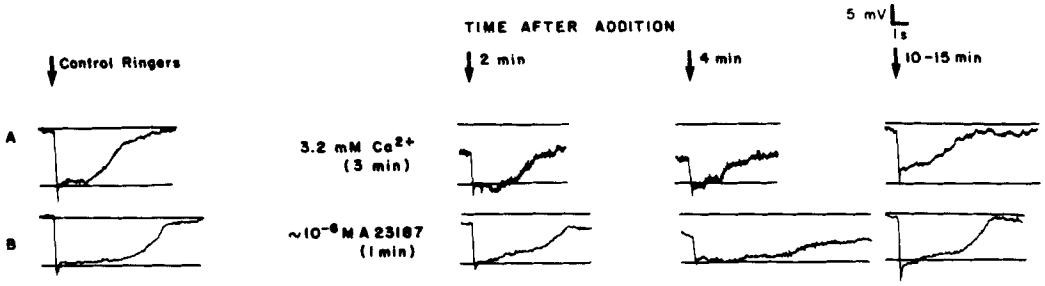

FIGURE 4. Effects of high (3.2 mM) Ca^{2+} and A23187 on responses of single dark-adapted toad rods to diffuse 200-ms flashes of 500-nm light. To produce an increase in intracellular Ca^{2+} levels, the ionophore A23187 was added in normal Ca^{2+} Ringer's (1.6 mM Ca^{2+}). The addition of high Ca^{2+} or A23187 resulted in similar effects. After 2 min of superfusion, the membrane potential had significantly hyperpolarized, and the light-evoked response was smaller than control. These effects were increased at 4 min but reversed by 10–15 min after the start of superfusion. Qualitatively similar results were obtained in all nine such experiments in high Ca^{2+} with a mean decrease in V_{max} to 0.6 of control (range 0.5–0.8). Similar effects were obtained in seven of eight intracellular recordings with A23187 with a mean decrease in V_{max} to 0.6 of control (range 0.5–0.7). All changes in V_{max} were significant with $P < 0.01$.

during a short exposure to Ringer's containing normal 1.6 mM Ca^{2+} plus the ionophore A23187. The effects observed were very similar to those seen with superfusion in high Ca^{2+} and were reversible as shown. In Fig. 5 the V-log I curves for a dark-adapted rod in control Ringer's and in Ringer's containing 3.2 mM Ca^{2+} are compared. In high Ca^{2+} V_{max} decreased, but σ remained virtually the same. Thus, varying Ca^{2+} levels from 10^{-9} M to 3.2 mM for superfusion periods of up to 6 min produced significant changes in membrane potential levels and response amplitudes but no significant shift of the V-log I curves on the intensity axis; i.e. rod sensitivity was very little affected.

Light Adaptation—Responses to Flashes Superimposed on Background Fields

Fig. 6 illustrates V-log I curves for a rod superfused in control Ringer's in the dark and in the presence of background fields of different intensity. Two

principal effects of background illumination were observed: (*a*) there was a decrease in V_{max}; and (*b*) there was a shift of the V-log *I* curve to the right on the intensity axis (i.e., σ increased). Thus, in light adaptation there was a large decrease in the sensitivity or gain (V_{max}/σ) of the rod, the extent of which was intensity dependent. Here, as in other receptors, the sensitivity loss was due almost entirely to the lateral shift of σ and *not* to the compression of response amplitude (V_{max}) (Dowling and Ripps, 1972; Kleinschmidt and Dowling, 1975). Hence, $1/\sigma$ and sensitivity (or gain, V_{max}/σ) are nearly equal in absolute

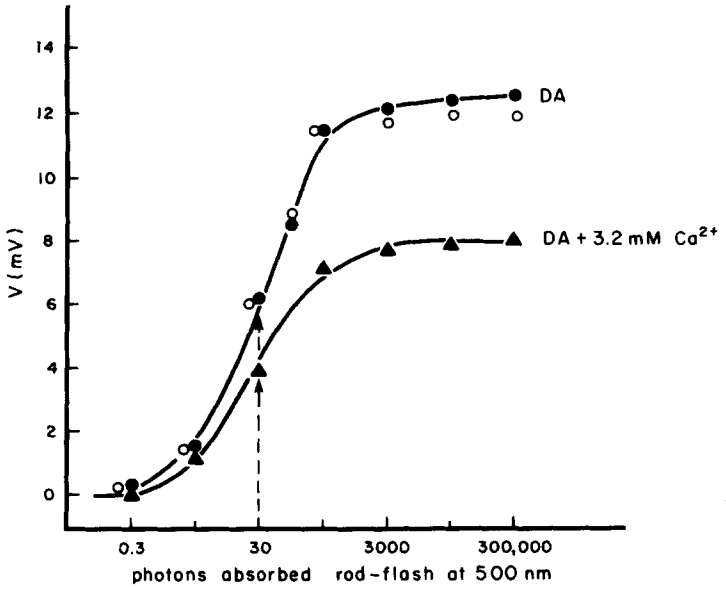

FIGURE 5. Plot of response amplitude vs. flash intensity for a single dark-adapted toad rod bathed in control Ringer's (closed circles) and for 3 min in high (3.2 mM) Ca^{2+} (triangles). High Ca^{2+} affected the amplitude of response but did not shift the V-log *I* curve on the intensity axis. The effects were reversible in 10–15 min (open circles). Similar results were observed in all nine intracellular recordings. Mean and range changes in V_{max} in high Ca^{2+} compared to control Ringer's are presented under Fig. 4.

magnitude. For example, when dark adapted, V_{max} for this rod was about 23 mV; with a background light (I_B) of such intensity that 850 photons were absorbed per rod-second at 500 nm, V_{max} was 15 mV. Therefore, with this background light, V_{max} decreased by a factor of about 3. On the other hand, this background light shifted the curve laterally from a σ value of 30 to almost 3,000 photons absorbed per rod-flash at 500 nm, a factor of almost 100. The entire response range of the light-adapted rod was shifted to higher flash intensities.

The effects of low Ca^{2+} superfusion for 4–6 min on V-log *I* curves determined in the dark and in background light (2,500 photons absorbed per rod-second at 500 nm) are shown in Fig. 7 and compared with V-log *I* curves obtained from the same rod in control Ringer's. In both solutions the V-log *I* curves were

shifted laterally in the light-adapted vs. the dark-adapted state by exactly the same amount. While dark adapted, σ was about 30 photons absorbed per rod-flash whether the retina was in control Ringer's or in Ringer's containing 10^{-9} M Ca^{2+}; when light adapted, σ was about 600 photons absorbed per rod-flash in both of the superfusing solutions. Note that V_{max} of the light-adapted rod in

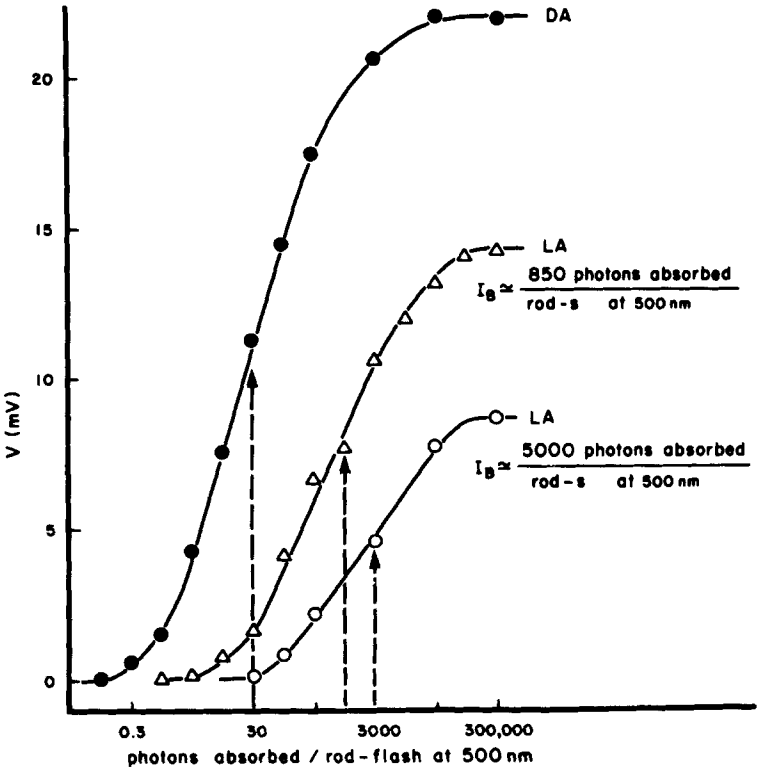

FIGURE 6. Plots of response amplitude vs. flash intensity for a single toad rod under dark-adapted (DA) and light-adapted (LA) conditions. All responses were obtained during superfusion of the retina in control Ringer's. The duration of each flash was 200 ms, and its intensity is expressed on the abscissa as photons absorbed per rod-flash. When background illumination (I_B) was superimposed on the brief flashes, the curves shifted to the right (increased σ) and were somewhat compressed (decreased V_{max}). See text for further explanation. Qualitatively similar effects were observed in 34 recordings.

low Ca^{2+} Ringer's was over twice the amplitude of the rod when dark adapted and in control Ringer's, yet the sensitivity (V_{max}/σ) of the light-adapted rod was about 10 times less. Similar experiments were performed with backgrounds varying from 140 to 14,000 photons absorbed per rod-second, and with low Ca^{2+} superfusions lasting as long as 6 min, with results similar to that shown in Fig. 7.

Fig. 8 shows the effects of background light on the intracellular electrical activity of the rod whose V-log I curves are shown in Fig. 7, during superfusion

in control Ringer's and in Ringer's containing 10^{-9} M Ca^{2+}. In control Ringer's (Fig. 8 A), a hyperpolarizing potential was maintained throughout background illumination. A single response to a superimposed flash is also illustrated at the end of the record and is typical of incremental responses recorded under control conditions. After a 3-min superfusion in 10^{-9} M Ca^{2+} (Fig. 8 B), a small

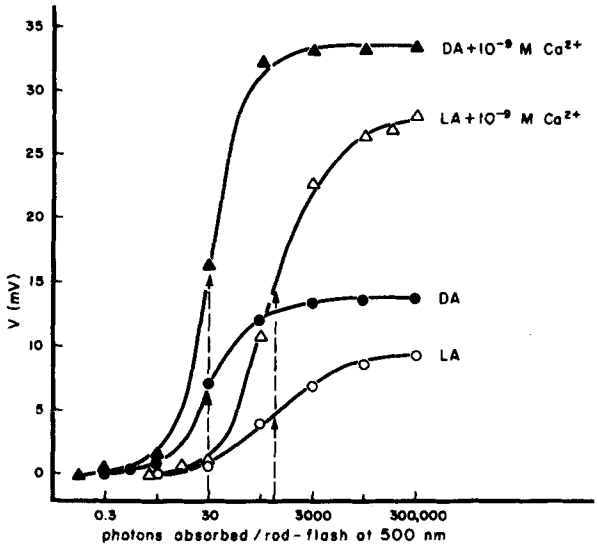

FIGURE 7. Plots of response amplitude vs. flash intensity for a single rod under dark-adapted (DA) and light-adapted (LA) conditions during superfusion in control Ringer's and short-term (≤6 min) 10^{-9} M Ca^{2+}. Responses were to 200-ms flashes of 500-nm light in the dark-adapted state and 200-ms flashes superimposed on background illumination (I_B = 2,500 photons absorbed per rod-second at 500 nm). Under both DA and LA conditions, superfusion of 10^{-9} M Ca^{2+} compared to control Ringer's increased V_{max} but had no effect on half-maximum amplitude, σ (dotted line and arrow). Data shown in figure were collected during 4–6 min of continuous superfusion in 10^{-9} M Ca^{2+}. During this interval the response amplitude to a given intensity was approximately constant. Similar experiments were repeated in 11 of 11 recordings from rods. Mean and range changes in V_{max} in control Ringer's and low Ca^{2+} during DA are presented in the legend to Fig. 3. During LA V_{max} increased an average of 3.1-fold (range 1.8–8.1) in 10^{-9} M Ca^{2+} compared to control Ringer's. In each case the increase was significant with $P < 0.01$.

increase in the initial response to the background light was observed, along with an increase in the amplitude of the incremental response. The maintained hyperpolarization induced by the background illumination was slightly greater in amplitude than that observed when the rod was superfused with control Ringer's, and it remained constant for the duration of the background illumination. After a 5-min superfusion with Ringer's containing 10^{-9} M Ca^{2+} (Fig. 8 C), the response to the background light was greatly increased in amplitude relative to the control response, as was the incremental response. The plateau potential to background illumination was *not* maintained, however; under these

conditions the plasma membrane recovered (depolarized) all the way back to its original dark-adapted resting potential within 1 min of the onset of the background light (compare before and after interruption of the record). It is interesting that the response to the background light under these conditions shows no transient, but the response to the incremental flash did. This was

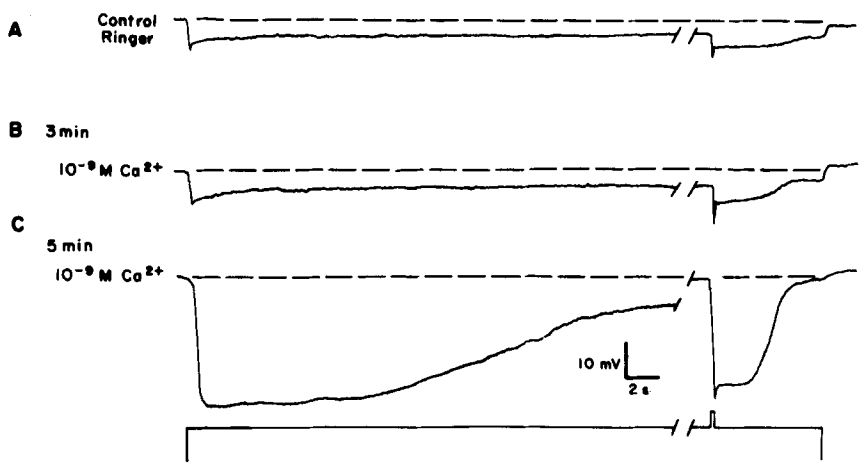

FIGURE 8. Effects of low Ca^{2+} upon intracellular responses of a single toad rod to background illumination and superimposed flashes. Lowest trace represents light stimulus: long step is background (I_B = 2,500 photons absorbed per rod-second at 500 nm), and shorter pulse is superimposed 200-ms flash (I = 3,000 photons absorbed per rod-flash). Hatch marks represent a break in the record of 34 s. A, Electrical response during superfusion in control Ringer's (1.6 mM Ca^{2+}). Note that the plateau potential was maintained throughout the background illumination. B, Response after 3 min of superfusion with 10^{-9} M Ca^{2+}. The resting membrane potential had depolarized to a new level. Amplitude of responses to background and superimposed flashes were increased over control. Plateau potential to background was still maintained. C, After 5 min of superfusion with 10^{-9} M Ca^{2+}, the resting membrane potential had further depolarized to a new level, and the plateau potential was no longer maintained throughout background illumination. In these experiments a range of increment flash intensities (0.3–300,000 photons absorbed per rod) was used, but only one representative flash is illustrated here. Fig. 7 presents the entire voltage-intensity function of the same rod. Qualitatively similar effects were obtained in 12 of 12 recordings.

observed in all such experiments and suggests that continuous light can partially oppose the effects of low Ca^{2+} perfusion, although the possibility remains that light and low Ca^{2+} could be acting on different systems.

EFFECTS OF PROLONGED EXPOSURE TO LOW CA^{2+} If superfusion of low Ca^{2+} Ringer's was continued for more than 6–7 min in the *dark-adapted* retina, the responses to brief flashes displayed a decrease in amplitude (V_{max}) and a very large alteration in σ. These effects are shown in Fig. 9 where V-log I curves taken between 6 and 16 min are plotted. With time, response amplitudes

decreased but, more interestingly, σ shifted laterally along the intensity scale. In this regard these effects are similar to those seen during light adaptation (Dowling and Ripps, 1972, 1976). That is, the gain or sensitivity (V_{max}/σ) of this dark-adapted receptor was decreased as if it were being light adapted, i.e. increasing time in this experiment was similar to increasing background light intensity in a control retina.

One might assume that the cell was merely dying in such an experiment and therefore losing responsiveness. However, when cells "die" or when the elec-

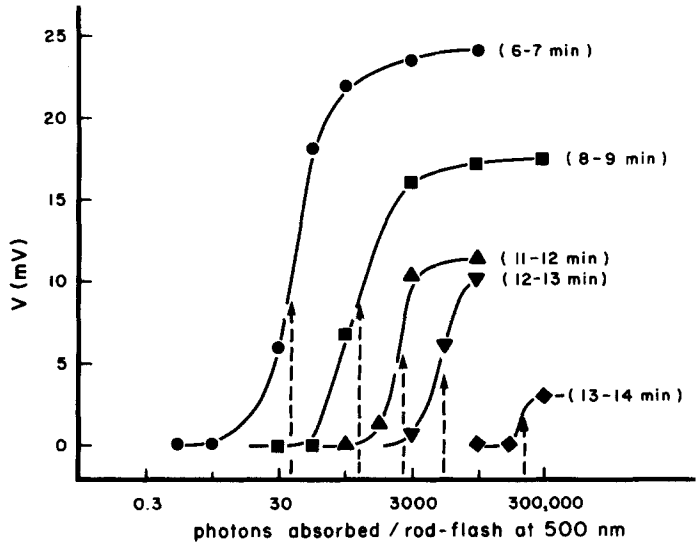

FIGURE 9. Plot of response amplitude vs. flash intensity for a single dark-adapted toad rod during superfusion in very long-term (6–15 min) 10^{-9} M Ca^{2+} Ringer's. Responses were to 200-ms flashes of 500-nm light; intensity represented on abscissa. The numbers in parentheses beside each curve give the time intervals of continuous addition of 10^{-9} M Ca^{2+} during which the data were obtained. With increasing time of low Ca^{2+} superfusion of the dark-adapted rod, voltage-intensity curves shifted to the right on the intensity scale, mimicking light adaptation. These curves are representative of the data collected in this type of experiment in 12 of 12 recordings; additional data points were obtained but are not shown for clarity.

trode is withdrawing from a cell, the response amplitudes decrease (i.e., at each intensity the response is diminished by a constant multiplicative factor), but σ does not change significantly. Here we found a large increase in σ, along with a decrease in response amplitude, indicating no constant factor of amplitude compression but, instead, a resetting of the sensitivity or range of intensities over which the photoreceptor would respond. Also, the membrane potential remained stable while desensitization of the rod progressed, thus providing evidence that the cell was well impaled by the electrode and that the electrode was not withdrawing from the rod. Thus, although light responsiveness was lost, the cells did not appear to die during this procedure since the membrane potential was maintained.

Why the penetrated cells did not recover after their return to control Ringer's is not understood. Normal rod responses were routinely recorded elsewhere in the retina after 15 min of control Ringer's washout. This implies that impaled cells did not recover as did their unimpaled neighbors. In the rat retina, Yoshikami and Hagins (1973) recorded receptor potentials extracellularly and found that effects caused by Ringer's containing 10^{-8} to 10^{-9} M Ca^{2+} were reversible. This finding further suggests that failure to recover after prolonged low Ca^{2+} exposure may be due to the electrode penetration of the cell and not simply to the low Ca^{2+} treatment.

In summary, we could distinguish two separate and sequential sets of effects of superfusing rods with 10^{-9} M Ca^{2+} Ringer's. For the first 6 min of superfusion the cells depolarized, and the maximum voltages elicited with test flashes increased. During this time there was little change in the sensitivity of the cells as a result of low Ca^{2+} superfusion in either the dark-adapted or partially light-adapted retina. Beyond 7 min of superfusion, however, the membrane potential of the cells stabilized, the maximum amplitude of responses declined, and the sensitivity of the rods significantly decreased, mimicking light adaptation.

Dark Adaptation—Responses to Flashes after a Bright Step of Light

After a bright flash of light, the sensitivity of a rod is severely diminished, and it recovers with time in the dark. In the experiments to be described here, a 12-s step of bright light was presented to the retina, and the membrane potential and sensitivity of the cell were subsequently monitored.

Fig. 10 shows the electrical response (upper left) of a typical rod superfused in control Ringer's during and after a 12-s light step which bleached $\leq 3\%$ of the photopigment. Other experiments were performed with adapting lights which produced less bleaching ($\ll 1\%$) of the visual pigment, and the results observed were very similar. After such an adapting exposure, the plateau phase of the electrical response of the receptor was maintained for many seconds, during which time no responses to brief flashes of any intensity could be elicited from the cell; i.e., the rod was saturated. After the period of saturation, the membrane potential of the rod rapidly returned (within 5–20 s) to base line. Also shown in Fig. 10 is the dark-adaptation curve of this rod, i.e. the recovery of sensitivity after the flash. In this figure, therefore, it is possible to compare the time course of recovery of sensitivity with recovery of membrane potential. In this experiment, rod sensitivity was determined by measuring the test light intensity necessary to evoke a small ($\sim$1 mV) criterion response from the rod. In other experiments V-log I curves were constructed continuously during dark adaptation to monitor sensitivity (i.e., σ was determined). Both gave similar results.

For the first 15 s or so after extinguishing the adapting flash (i.e., during the saturation period), no responses could be elicited from the rod. Thereafter, responses could be elicited and the sensitivity of the rod determined. Fig. 10 shows that the sensitivity of the rod increased rapidly during the time the membrane potential was rapidly returning to the dark-adapted level. About 3 min after the onset of the light step, the threshold intensity had returned to within 0.25 log units (1.3 photons absorbed per rod-flash) of the dark-adapted

value, and it remained there. The membrane potential had likewise recovered to dark-adapted levels by this time. Thus, under these essentially nonbleaching adapting conditions, the recovery of threshold ran roughly in parallel with the recovery of the membrane potential. However, we consistently observed that the membrane potential recovered completely to the dark-adapted level somewhat *before* recovery of final sensitivity (for example, see Figs. 10 and 11). Similar findings were reported by Kleinschmidt and Dowling (1975). In all

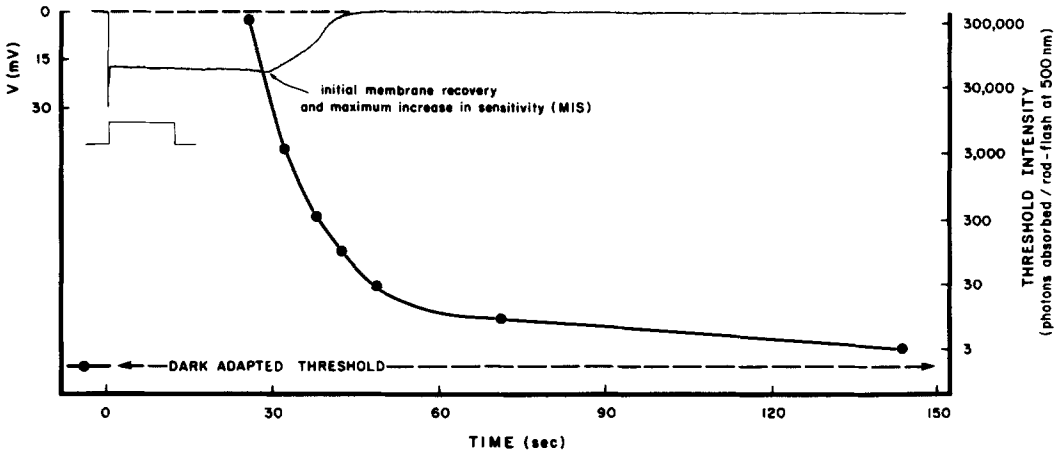

FIGURE 10. Comparison of the temporal changes in membrane potential (left ordinate scale) and threshold intensity (right ordinate scale) for a rod superfused in control Ringer's. The adapting procedure (step of light at time 0) consisted of a 12-s "yellow" (Corning cutoff filter 3484) unattenuated light with a retinal irradiance of 1.1 mW/cm^2 which bleached ≤3% of the pigment. The curve of threshold intensity (or dark adaptation) was determined by finding the flash intensity which elicited a criterion response of ~1 mV with increasing time after the light step. Thus the threshold intensity is an indication of the sensitivity of the receptor. Note that the initial recovery of the membrane potential coincided with the maximum increase in sensitivity. Also, the threshold intensity was still about 20 photons absorbed per rod-flash (0.83 log units) above its final value when the membrane potential had fully recovered to its dark-adapted level. This comparison of membrane potential and sensitivity could be made only for very stable recordings. Dark adaptation experiments of this kind were performed in 152 rods, superfused in control Ringer's solution. Results are quantitatively summarized in the text.

dark-adaptation experiments described in this paper (and in the next), the time of onset of rapid membrane potential recovery and the time of onset of rapid sensitivity recovery during dark adaptation were similar. If membrane potential recovery was speeded by a particular set of conditions, so was the recovery of sensitivity; if one was slowed, so was the other.

Among 152 rods bathed in control Ringer's and exposed to the same adapting light, the time when initial recovery of the membrane potential occurred during dark adaptation varied considerably (range: 17–100 s; 24–50 s in most

cases), but in any *one* cell the recovery time to successive adapting procedures was usually similar. During superfusion with control Ringer's, 33 rods were presented with at least three successive steps of adapting light at 3-min intervals. For 25 of these rods the time of onset of rapid membrane recovery was very constant (range: ±3 s). However, in 3 of 33 rods the onset of membrane recovery slowed after successive steps of light to the same rod. Also, in 5 of 33

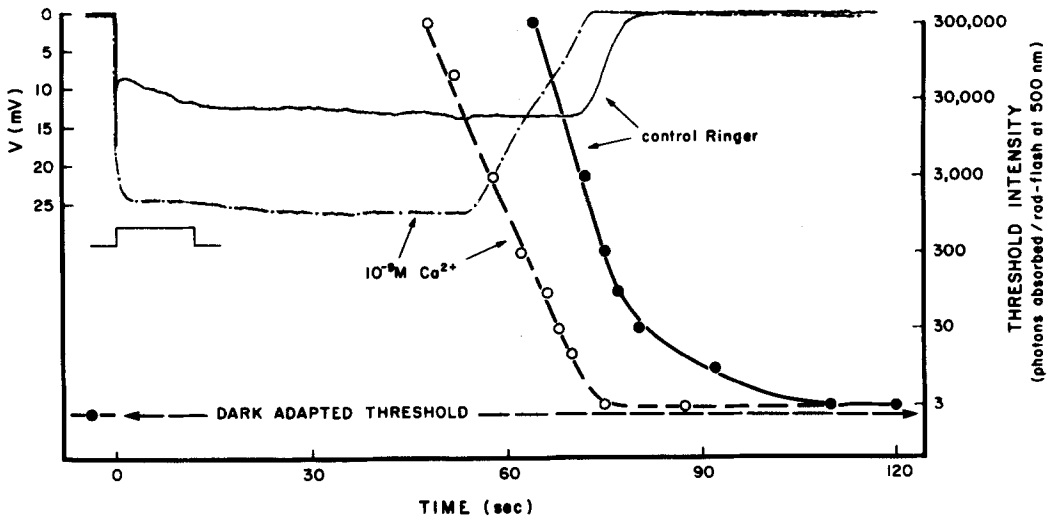

FIGURE 11. Comparison of the temporal changes in membrane potential (left ordinate scale) and threshold sensitivity (right ordinate scale) for a single rod superfused in control Ringer's and 10^{-9} M Ca^{2+}. See Fig. 10 for a description of the construction of this type of figure. Compared to control Ringer's, superfusion in 10^{-9} M Ca^{2+} resulted in an accelerated onset of recovery of the membrane potential. Qualitatively identical findings were observed in seven of eight intracellular recordings. In the seven cells, the onset of membrane recovery was accelerated to an average of 60% of the control (range 40–70%). These values were obtained by comparing the time of initial membrane recovery in control Ringer's and in 10^{-9} M Ca^{2+} for each intracellular recording; for seven cases acceleration was significant with $P < 0.01$. In the eighth cell an effect was seen but was not significant at the $P < 0.01$ level. It was not surprising that there was little effect of low Ca^{2+} on this cell because in control Ringer's alone the membrane potential returned very rapidly (within about 20 s, which is close to the limit observed in 152 cells). See text for details.

rods the onset of membrane recovery took longer after the first light step than after succeeding light steps; nevertheless, after subsequent light steps the onset of membrane recovery remained constant (±3 s). Moreover, for all of the experiments during multiple dark adaptation procedures in any single rod, a *reversible* change in the time of onset of membrane potential recovery without the addition of a test agent to the Ringer's was never observed.

Fig. 11 shows the effect of low Ca^{2+} Ringer's on the recovery of membrane

potential and sensitivity of a rod after an intense flash. The continuous lines represent the behavior of the rod in control Ringer's while the dashed and dotted lines refer to the same rod in low Ca^{2+} Ringer's after an identical flash. Before the adapting flash was presented, the retina was superfused in low Ca^{2+} Ringer's for about 4 min during which the amplitude of the response increased, and the initial transient disappeared. After the adapting flash, the rod in low Ca^{2+} Ringer's became responsive significantly sooner than it had while in control Ringer's, and the membrane potential and receptor sensitivity both achieved dark-adapted levels sooner. Note that the recovery of membrane potential and receptor sensitivity was essentially complete in low Ca^{2+} Ringer's before recovery of the rod in control Ringer's had even begun. Also, it appeared that the recovery of sensitivity paralleled the membrane potential more closely than in control Ringer's, i.e. by the time the membrane potential had recovered, the threshold intensity had also reached its final value. However, the *rate* of recovery of sensitivity did not appear to be greatly affected by low Ca^{2+} superfusion. The dark adaptation curves, although displaced on the time axis, are approximately parallel. That is, low Ca^{2+} appeared to affect primarily the duration of the saturation period after a bright adapting step, not the rate at which the rod is capable of increasing its sensitivity in the dark. In numerous other experiments we consistently observed these results.

In addition, a second presentation of the same adapting light to a rod produced similar effects; i.e. low Ca^{2+} would again decrease the period of receptor saturation. To avoid the occasional problem of membrane potential recovery accelerating spontaneously after the first adapting light step, we presented multiple light steps to most rods during superfusion with control Ringer's. After observing stable, reproducible recovery times in control Ringer's, low Ca^{2+} was introduced into the chamber, and results similar to those illustrated in Fig. 11 were noted. We conclude, therefore, that low extracellular Ca^{2+} accelerates the onset of time of recovery of the rod response after an adapting flash that bleaches negligible amounts of photopigment.

DISCUSSION

The primary assumption in the interpretation of these findings is that alterations in extracellular Ca^{2+} result in alterations of intracellular Ca^{2+} levels in the rod outer segment (Ebrey and Honig, 1975; Yoshikami and Hagins, 1973). Direct evidence for this assumption is lacking, but indirect evidence has been provided. For instance, changes in extracellular Ca^{2+} concentration and/or EGTA have been reported to influence intracellular calcium stores in isolated kidney cells (Borle, 1970, 1972; Rasmussen, 1970), fly salivary gland (Prince et al., 1972), liver mitochondria (Reed and Bygrave, 1975), and *Limulus* photoreceptors (Brown and Blinks, 1974). The addition of the calcium ionophore A23187 has also been used to affect intracellular Ca^{2+} levels in many tissues, including salivary glands (Prince et al., 1973), leukocytes (Zabucchi et al., 1975), artificial membranes (Hyono et al., 1975), and rat rods (Hagins and Yoshikami, 1974). In our experiments the addition of the ionophore A23187 hastened the onset of effects on rods of low or high calcium media, but the effects observed were identical to those seen when rods were exposed to low and high Ca^{2+} Ringer's

not containing ionophore. Thus, we believe that in all the experiments presented here intracellular Ca^{2+} levels were significantly changed.

It should be noted that agents such as 10^{-9} M Ca^{2+} (as well as several of the drugs discussed in the next paper) may directly or indirectly affect coupling between cells (for example, see DeMello, 1975), but we cannot conceive how uncoupling of photoreceptor cells can account for the effects described here. First, with the diffuse light stimuli used in these experiments, the rods are equipotential after all but the dimmest flashes; therefore, no net current flows between receptors, and alterations in coupling could not affect the results. In addition, on several occasions responses to repeated dim flashes (~1 photon absorbed per rod-flash at 500 nm) were studied, and the consistency of response amplitudes compared with a Poisson distribution of amplitudes indicated that receptors were pooling their signals equally in both control Ringer's and in low Ca^{2+} Ringer's superfused for up to 4 min (for statistical method see Fain, 1975*a*). It is likely, however, that penetrated rods which became unresponsive after long exposure to low Ca^{2+} Ringer's did uncouple from other photoreceptors. This almost certainly is the case since neighboring rods recovered after Ringer's washout. It is possible that the uncoupling contributed to the irreversible loss of responsiveness in these penetrated rods.

Ca^{2+}, Intracellular Messengers, and Visual Adaptation

If Ca^{2+} serves as an intracellular messenger in the rod outer segments, a primary requirement is that it regulate the Na^+ permeability of the plasma membrane, hence the membrane potential of the outer segment and the amplitude of the photoresponse. We have observed that varying extracellular Ca^{2+} levels around photoreceptors for relatively short time periods ($\leq$6 min) alters membrane potential and response amplitudes in an appropriate fashion. That is, lowering Ca^{2+} levels depolarizes the photoreceptor cell, presumably by opening more Na^+ channels in the outer segment membrane and thus increasing Na^+ permeability of the outer segment; increasing Ca^{2+} levels hyperpolarizes the receptor cell, presumably by closing Na^+ channels in the outer segment membrane and decreasing Na^+ permeability of the outer segment. Light-evoked responses increase in amplitude in low Ca^{2+} Ringer's presumably because there are more Na^+ channels available for blockade. In high Ca^{2+} Ringer's there are few Na^+ channels, hence smaller response amplitudes are observed.

It is interesting to note that increasing the extracellular concentration of protons also results in depolarization of the membrane and increased response amplitudes to photostimulation.[1] These effects, although small, are in the same direction as lowering the extracellular concentration of Ca^{2+}. These Ca^{2+} and H^+ effects may or may not be linked, and it is not known if the Ca^{2+}-H^+ exchange mechanism present in mitochondria exists in rod outer segments. These results may also suggest that Ca^{2+} can affect the activity of ions other than Na^+ in the rod outer segment.

The observation in Fig. 8 that after a 5-min perfusion in low Ca^{2+} the

[1] Pinto, L. H., and S. E. Ostroy. Submitted for publication.

membrane potential returned to the dark membrane potential level in the presence of a background light is of considerable interest when evaluated with the data of Fig. 7. There it was shown that rod sensitivity is strongly depressed by background illumination even when Ca^{2+} levels are lowered. Since lowering extracellular Ca^{2+} increased response amplitudes and negated the sustained effect of background light on the membrane plateau potential (Fig. 8C), it therefore appears that receptor sensitivity is not closely correlated either with membrane potential of the cell or with the amplitude of the light-evoked responses (Dowling and Ripps, 1972; Kleinschmidt and Dowling, 1975). The present experiments suggest further that the major determinant of receptor sensitivity (σ) does not appear to be regulated by cytosol Ca^{2+} levels. This is shown particularly clearly in those experiments in which Ca^{2+} levels were altered for relatively short time periods (4–6 min) in partially light-adapted receptors. Under such conditions, the sensitivity of the receptor was determined principally by the prevailing background light, not by Ca^{2+} levels (Figs. 3, 5, and 7). Thus, although cytosol Ca^{2+} concentrations are capable of regulating the membrane potential of the cell and its response amplitude, they affect only to a minor extent the sensitivity of the receptor cell in the dark-adapted or partially light-adapted state. This situation is predicted by a model such as that proposed by Baylor and Fuortes (1970), in which the internal transmitter is assumed to be Ca^{2+}. In terms of the sodium conductance, g_{Na}; the fixed dark conductance, G; and the ionic potential, E; the intensity response curve is of the form:

$$V = \frac{V_{max} I}{I + \sigma} \qquad \text{where } V_{max} = \frac{g_{Na} E}{g_{Na} + G}$$

$$\text{and} \qquad \sigma = \frac{G + g_{Na}}{G}.$$

If $G \gg g_{Na}$ (at least five times as large from results in Werblin, 1975; Fain et al., 1976), then a decrease in cytosol Ca^{2+} concentration (hence, an increase in g_{Na}) would increase V_{max} but have virtually no effect on σ (see Lipton, 1977, for a fuller development of these equations). On the other hand, some authors have argued that the light-sensitive conductance (g_{Na}) of the rod outer segment is similar to the fixed conductance (G) (Yoshikami and Hagins, 1973; Baylor et al., 1974; Korenbrot and Cone, 1972; footnote 1). If so, lowered cytosol Ca^{2+} may be affecting a K^+ conductance as well.

Light Adaptation

It has previously been proposed that receptor sensitivity is regulated mainly by a mechanism located close to the photochemical event (Dowling and Ripps, 1972; Kleinschmidt and Dowling, 1975). For example, it has been suggested that the sensitivity of the receptor might be determined by the *amount* of messenger released per photopigment molecule bleached (Cone, 1973; Kleinschmidt, 1974; Kleinschmidt and Dowling, 1975). If one assumes the Ca^{2+} hypothesis, upon light adaptation or during dark adaptation the amount of

Ca^{2+} released from the disks per rhodopsin molecule excited is reduced. This could occur if during light adaptation the amount of Ca^{2+} available for release is partially decreased. Thus more photopigment must be excited during light and dark adaptation to cause the release of a given (i.e. threshold) amount of Ca^{2+}. As a result the sensitivity of the receptor is decreased during light adaptation; i.e. the V-log *I* curve of the receptor is shifted toward higher intensities. An equation that simply states the case is the following:

$$\Delta[Ca^{2+}] = K_R Rh^*[Ca^{2+}]_d,$$

where ΔCa^{2+} is the transmitter released, K_R is a release constant, Rh* is photoactivated rhodopsin, and $[Ca^{2+}]_d$ is the amount of intradisk calcium available for release. Note that this model creates an intensity-dependent change in the rate of calcium release. Since the net effect of a decrease in the pool of Ca^{2+} available for release is the same as an intensity-dependent increase in the reuptake of messenger, the latter possibility is also tenable.

It may be possible to explain the loss of sensitivity of toad rods when they are exposed to low Ca^{2+} Ringer's for prolonged periods (>6–7 min) in view of this hypothesis. Liebman (1974) has shown by atomic absorption spectrometry that prolonged exposure to very low Ca^{2+} levels results in depletion of Ca^{2+} from the disks, and many of Liebman's findings have been confirmed by Hess (1975). Thus, it would appear that after prolonged exposure of a rod to low Ca^{2+} Ringer's, the amount of stored Ca^{2+} in the rod has decreased, and presumably the amount of Ca^{2+} that can be released per flash is reduced. Under such conditions, we have observed that the sensitivity (V_{max}/σ) of the rod is decreased significantly, and the rods behave as if they were light adapted. Equations that describe an intradisk decrease in Ca^{2+} concentration, producing changes in rod sensitivity, fit the empirical results quite well (Kleinschmidt, 1974; Lipton, 1977).

Dark Adaptation

We have also observed that lowering the extracellular Ca^{2+} decreases the period of receptor saturation (i.e., the time during which no light reponses can be evoked) after a bright adapting flash. Thus, the time required for both the sensitivity and membrane potential of the rod to recover to dark-adapted levels is shortened. These observations are understandable if such an adapting flash increases cytosol Ca^{2+} levels to the point that all Na^+ channels in the outer segment membrane are blocked. Hence, the receptor is "saturated" for a time and unable to respond to flashes. That is, any Ca^{2+} released in response to a flash cannot exert its effect. By reducing extracellular Ca^{2+}, we may be assisting the cell in decreasing cytosol Ca^{2+} levels. As cytosol Ca^{2+} is lowered, messenger (e.g., Ca^{2+}) released from the disks in response to a flash is effective in hyperpolarizing the outer segment membrane since there are Na^+ channels available to block; thus, the onset of dark adaptation is accelerated. Our experiments show, however, that the *rate* of dark adaptation is not significantly affected by exposure of the rod to low Ca^{2+}. The onset of dark adaptation is shortened as is the time required for its completion, but the time between the

onset of dark adaptation and its completion is the same as in control Ringer's. The effects of altered Ca^{2+} appear to be explained in terms of its action on the plasma membrane, although we cannot exclude some effect of altered Ca^{2+} on the release mechanism.

In summary, the results of the present experiments are consistent with the notion that Ca^{2+} is an intracellular messenger in the rod outer segment, but they do not prove the hypothesis. The experiments do indicate that in a partially light-adapted rod the membrane potential and cytosol Ca^{2+} levels do not regulate the major determinant of sensitivity (σ), but rather suggest, as did earlier experiments, that receptor sensitivity is mostly regulated by a mechanism more closely related to the photochemical event, e.g., the amount of intradisk Ca^{2+} that is released after photopigment excitation.

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