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Plasma membrane calcium fluxes in intact rods are inconsistent with the "calcium hypothesis"

(phototransduction/rod photoreceptor/membrane electrode)

GEOFFREY H. GOLD

Department of Physiology, Yale University School of Medicine, New Haven, CT 06510

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ABSTRACT **The temporal relationship between the extracellular rod photovoltage and light-induced net Ca fluxes across the rod plasma membrane is investigated. The net Ca flux measurements are derived from extracellular Ca concentration measurements at the receptor surface of the isolated bullfrog retina. As reported previously, illumination leads to a net Ca efflux, which is followed by a net influx, during which the released Ca is taken back up. However, the net Ca flux has two characteristics that are inconsistent with the hypothesis that intracellular free Ca is the intracellular messenger for phototransduction in rods. First, during maintained photovoltage saturation, the net Ca efflux is transient, declining with a stereotypic time course that is independent of stimulus intensity and duration. Second, the significant rate of net influx during Ca uptake has no correlate in the photovoltage waveform. These observations are not consistent with the "Ca hypothesis." Rather, these data corroborate recent findings suggesting that light causes a decrease rather than an increase in intracellular free Ca concentration.**

The light-stimulated net Ca efflux from intact retinal rods has been interpreted to be the result of a light-induced increase in intracellular free Ca concentration (1, 2). This interpretation has been taken as strong evidence for the hypothesis (3) that Ca is the intracellular messenger for phototransduction in rods. According to the "Ca hypothesis," light releases Ca from an intracellular store and the released Ca blocks the plasma membrane conductance, thereby generating the rod's electrical response to light. In this report, I further test the Ca hypothesis by examining the temporal relationship between the net Ca flux across the plasma membrane and the extracellular rod photovoltage. The data reported here are not consistent with predictions of the Ca hypothesis. These data corroborate recent findings suggesting that light causes a decrease rather than in increase in intracellular free Ca concentration (4). A preliminary report of this work has appeared (5).

MATERIALS AND METHODS

Extracellular Ca concentration is measured by positioning a planar Ca-selective membrane electrode in contact with the receptor surface of the isolated bullfrog retina. The isolated retina is mounted receptor-side-up in a perfusion chamber, and the Ca electrode is positioned above the retina with a micromanipulator (Fig. 1). The Ca-selective membrane is glued over a 1-mm-diameter opening at the small end of a hollow, cone-shaped polyvinyl chloride support (electrode body, Fig. 1). The membrane is made as described previously (1) but contains the more selective Ca sensor, ETH1001 (6). The Ca electrode potential is measured between a Ca standard solution that fills the inside of the polyvinyl chloride cone and the Ringer's solution that surrounds the tip of the Ca electrode. The reference potential is that which exists in the annulus of solution surrounding the active area of the membrane. The improved symmetry of this configuration with respect to the previous design (1) virtually eliminates detection of the transretinal potential [electroretinogram (ERG)] by the Ca electrode. This improvement allows experiments to be performed in a normal Ringer's solution, whereas the previous measurements had to be performed in a low-Na/high-K Ringer's solution in order to reduce the ERG amplitude. The Ca electrode is mounted via fine screw threads on the front of a long-working-distance microscope objective (Leitz LL25). Because the polyvinyl chloride membrane is transparent, the retina can be viewed through the Ca electrode assembly. Infrared illumination, visualized with an IR-sensitive television camera, thus allows the position of the Ca electrode with respect to the retina to be controlled during an experiment. This arrangement also permits raising and lowering of the Ca electrode to change the composition of the solution trapped between the retina and the Ca electrode.

The retina is mounted receptor-side-up in a perfusion chamber located beneath the Ca electrode (Fig. 1). The isolated retina rests on a dome-shaped surface that is machined from Plexiglas in order to allow illumination from below. The curvature of the dome allows the retina to partially maintain its natural curvature, thus preventing the retina from buckling. The central portion of the dome is machined flat to allow coplanar contact with the Ca electrode. The transretinal potential is measured between the Ca reference electrode and a third electrode located beneath the perfusion chamber. Electrical access to the bottom surface of the retina is provided by a hole drilled through the bottom of the Plexiglas chamber; this hole is filled with a transparent polyacrylamide gel (10% by weight in Ringer's solution), in order to minimize distortion of the light stimulus. Pieces of glass are clamped over the ends of the hole during the acrylamide polymerization, to produce smooth, flat end surfaces.

Positioning of the Ca electrode with respect to the retinal surface is accomplished as follows. The Ca electrode is lowered through the superfusate until the retinal surface just begins to come into focus. At this point, the Ca membrane is located ≈ 50 μm above the retinal surface, and the Ca signal in response to a light stimulus is small and exhibits a delay of several seconds, due to diffusion of Ca from the retina to the Ca electrode. As the Ca electrode is lowered further, the rod outer segments come into sharper focus, and the Ca signal becomes larger and its latency decreases. When both the rod outer segments and the bottom of the Ca selective membrane are in focus, the Ca electrode is in direct contact with the retinal surface and the latency of the Ca signal is <200 msec.

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Abbreviation: ERG, electroretinogram.

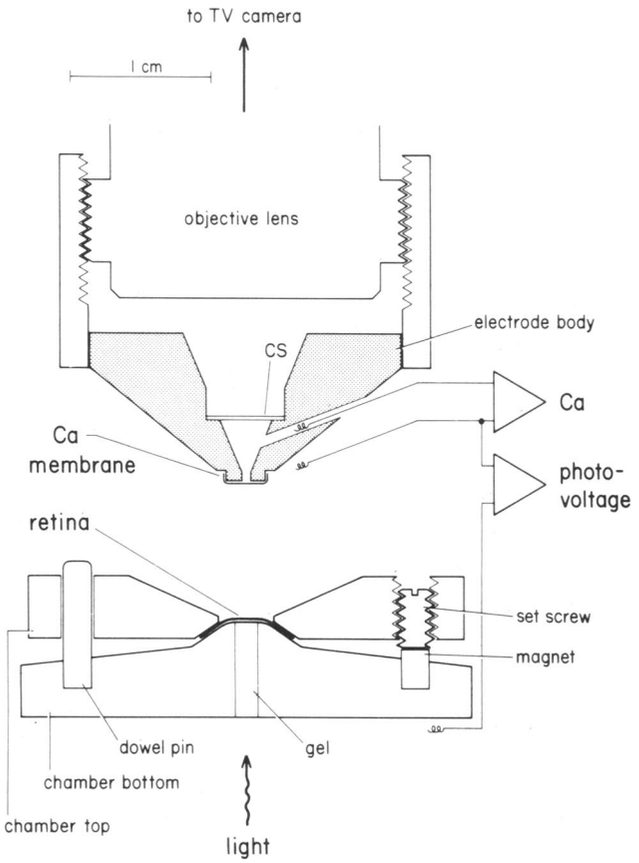

FIG. 1. Diagram of the Ca electrode (above) and perfusion chamber (below), in cross-section. In the Ca electrode, the space between the Ca membrane and a round coverslip (CS) is filled with the Ca standard solution (a Hepes-buffered Ringer's solution containing 1 mM $CaCl_2$). The coverslip is necessary to eliminate the optical distortion that would be caused by a meniscus at the air/water interface. Electrical access to the Ca standard solution is provided by a diagonal hole through the side of the electrode body. The coverslip is recessed from the top of the electrode body to minimize the optical path length through the saline solution and the resulting degradation of image quality. In the perfusion chamber, there are three dowel pins and three set screws/magnets located at 60° intervals around the perimeter of the perfusion chamber. The dowel pins serve to align the two halves of the chamber. Contact between the set screws and magnets provides adjustable and reproducible spacing between the halves of the chamber, providing uniform clamping around the edge of the retina.

Ca concentration measurements are ordinarily made in this position. Frequently, as the Ca electrode contacts the retinal surface, some compression of the retina occurs, as indicated by lateral movement of the rod outer segments. This compression is usually accompanied by the appearance of a small initial transient in the Ca signal (<20 μV), with the same latency as the photovoltage. This transient is presumably pick-up of the photovoltage by the Ca electrode. Removal of this artifact after the experiment can be accomplished by scaling and subtracting the photovoltage from the Ca signal so that the initial transient is eliminated. The Ca signal thus corrected is identical in time-course to the uncorrected Ca signal obtained just before the retina is compressed; the amplitude of the corrected Ca signal is ≈10% greater, due to the reduction in extracellular volume. The amplitude of the photovoltage artifact is only about 5% of the peak amplitude of the Ca signal. Therefore, this artifact-subtraction is a small correction to the raw data and was performed on the data in Figs. 2 and 3. The main advantage to using this correction is that it speeds up the experiment by allowing the experimenter to quickly ensure contact with the retina.

Pharmacological Isolation of Rod Photovoltage. The retina was superfused with a Ringer's solution, saturated with 98.5% O_2/1.5% CO_2 (pH ≈ 7.75) and containing 85 mM NaCl, 2.5 mM KCl, 1.0 mM $CaCl_2$, 1.0 mM $MgSO_4$, 10 mM $NaHCO_3$, 10 mM glucose, 25 mM sodium aspartate, and 0.01 mM $BaCl_2$. Aspartate and Ba were used to pharmacologically isolate the rod photovoltage from other components of the ERG (7, 8). Simultaneous measurements of the ERG and photovoltage [isolated by intraretinal recording (9)] demonstrate that the pharmacological isolation provided by these agents is excellent and that the presence of these agents has no significant effect on the photovoltage signal (unpublished results). At subsaturating intensities the intra- and transretinally recorded signals are indistinguishable in the presence of Ba and aspartate. At suprasaturating intensities, the recovery of the transretinal signal lags no more than 1 sec behind the recovery of the intraretinal signal. Bullfrogs were used for these experiments because the pharmacological isolation of the photovoltage was more complete than in toads, which were used in previous experiments (1).

Calculation of Net Ca Flux. The net Ca flux per rod outer segment is calculated by multiplying the rate of change of extracellular Ca concentration by the extracellular volume per rod (≈2.6 pl, measured from cryosectioned retinas; unpublished observation). The validity of this simple method is based on the following assumptions and supporting evidence.

The rod outer segments are the predominant source and sink for the extracellular Ca concentration changes. As shown previously (1), the initial latency and action spectrum of the Ca signal shows that the red-rod outer segments are the predominant source for the initial Ca efflux. The entire time course of the Ca signal is independent of stimulus wavelength (data not shown), indicating that all sources and sinks for the Ca changes are either part of the red rods or are driven by red-rod activity. Because synaptic transmission is saturated postsynaptically by the presence of aspartate, and the glial light response is blocked by the presence of Ba, all light-stimulated sources and sinks must be located within the red rods. Ca uptake by cells other than the red rods is also unlikely because of the small magnitude of the extracellular concentration changes (maximally a 5% increase); the light-induced increase in extracellular concentration should provide a small increase in the driving force for Ca entry into other cells when compared with that for entry into the rod outer segments caused by the concomitant loss of intracellular Ca. Voltage-dependent Ca currents at the rod synaptic terminal do not contribute to the observed Ca concentration changes, because blocking these currents with 0.5 mM Cd^{2+} has no significant effect on the Ca signal. Thus it appears that the red-rod outer segments are the dominant source and sink for the extracellular Ca concentration changes.

The extracellular measuring compartment is closed. The extracellular measuring compartment extends from the Ca electrode, which is located at the tips of the outer segments, to the external limiting membrane at the level of the rod inner segments. This compartment can be considered to be closed due to the presence of the Ca electrode, at the distal end, and the presence of glia, which occupy most of the extracellular volume, at the proximal end. Lateral diffusion of Ca is not expected to be significant, because the retina is illuminated uniformly. Loss of Ca into the superfusate is expected to be very slow because the 1-mm-diameter active area of the Ca membrane is separated from the flowing Ringer's solution by a 1-mm-wide annulus of inactive (glued) membrane. Experimental support for a closed compartment comes from the observation that continuous illumination produces a maintained rise in extracellular Ca concentration, which declines at a rate <5%/min; if a significant fraction of released Ca either diffused out of the measuring compartment or were

taken up by other structures, it is expected that Ca uptake by the rods would then reduce the extracellular concentration to below the dark level. However, extracellular Ca concentration recovers to the baseline without overshoot.

Diffusional equilibration of Ca in the extracellular measuring compartment is rapid compared with the time-course of the light-induced flux changes. This assumption is justified because the outer segments are separated by <10 μm from the Ca electrode and extend over 80% of the length of the measuring compartment (outer segment length = 80 ± 7 μm, inner segment length = 20 ± 2 μm; unpublished observation). Using a one-dimensional approximation to simulate diffusion in this compartment, the Ca concentration at the plane of the Ca electrode reaches 90% of its equilibrium value in ≈250 msec.

It follows from the above assumptions that the net Ca flux across the rod outer segment can be approximated by the rate of change of extracellular Ca concentration.

RESULTS

Fig. 2 illustrates the stimulus intensity and time dependence of the calculated net Ca flux (*A*), extracellular Ca concentration (*B*), and extracellular rod photovoltage (*C*). The baseline for the flux traces corresponds to zero net flux; an upward deflection indicates a net efflux, and a downward deflection, a net influx. Traces a–d are responses to light flashes of increasing intensity; trace e is in response to a step of light that is sufficiently bright to saturate the photovoltage during the entire trace. Extracellular Ca concentration and the rod photovoltage are measured simultaneously. The rod photovoltage is the extracellular voltage generated by the light-regulated current (photocurrent) that enters the outer segment (10). The extracellular photovoltage provides a fairly accurate measure of changes in the light-regulated membrane conductance, because the photocurrent does not change significantly with membrane hyperpolarization (11, 12). Thus, the data in Fig. 2 allow a direct comparison of the dynamics of Ca fluxes to the dynamics of the membrane conductance changes.

As reported previously (1), illumination causes a transient rise in extracellular Ca concentration. However, the present data, obtained in a normal Ringer's solution, containing 110 mM Na^+ and 2.5 mM K^+, differ both quantitatively and qualitatively from those obtained previously in a low-Na/high-K Ringer's solution. Quantitatively, the extracellular Ca concentration changes are ≈6 times larger than those in the modified Ringer's solution [maximal rate of extrusion = (7.7 ± 1.2) × 10^6 Ca per rod per sec (n = 4), and maximal Ca release = (7.9 ± 1.0) × 10^7 Ca per rod (n = 4)]. Qualitatively, the Ca concentration transients in normal Ringer's solution differ in that the rate of net Ca efflux (i.e., the slope of the concentration signal) is not maintained as it was in the low-Na/high-K Ringer's solution (cf. figure 2 of ref. 1) but declines immediately from its initial peak value. The transient nature of the net Ca efflux is more apparent in the calculated net flux traces (Fig. 2*A*); following illumination, the net flux trace rises to an initial peak rate of efflux and then declines immediately with a $t_{1/2}$ ≈ 4 sec. During photovoltage saturation, the net flux traces are all superimposable. Thus, the rate of net efflux declines along a stereotypic time-course that is independent of stimulus intensity and duration. The initial, net-efflux phase is followed by a net-influx phase during which all of the released Ca is taken back up; i.e., concentration returns to the pre-illumination level. The maximal rate of net influx is a significant fraction of the maximal rate of net efflux. Because the influx is most likely mediated by the light-regulated conductance (4, 12–15), these data show that light regulation of the inward Ca leak could make a significant contribution to the observed net fluxes.

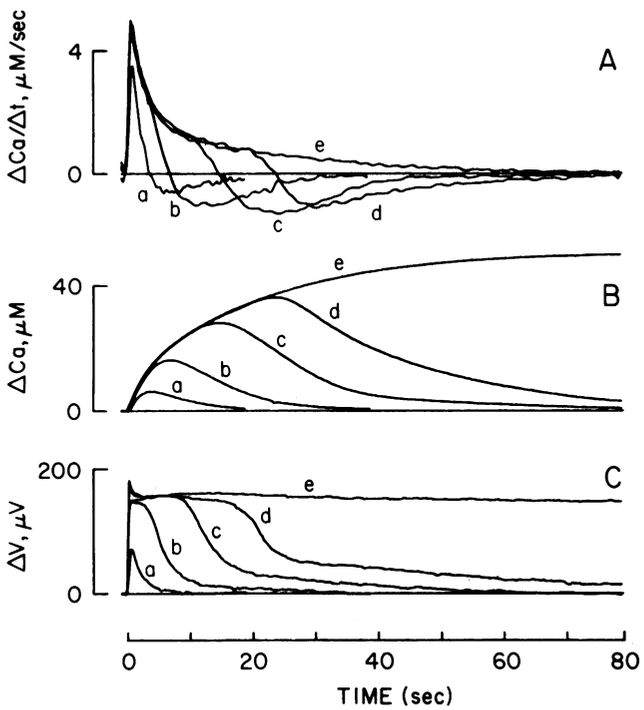

FIG. 2. Light-induced transients of calculated net Ca flux (*A*), extracellular Ca concentration (*B*), and extracellular rod photovoltage (*C*). The baseline in the flux trace corresponds to zero net flux; an upward deflection represents net efflux and a downward deflection represents net influx. The vertical calibration in the flux trace is expressed as the rate of change of concentration but can be converted to the net flux of Ca ions per rod per sec by multiplying by the extracellular volume per rod (2.6 pl) and Avogadro's number. A rate of 4.0 μM/sec corresponds to 6.2 × 10^6 Ca ions per rod per sec. Changes in extracellular Ca concentration are converted to net release or reuptake of Ca per rod in the same way; 40 μM corresponds to 6.2 × 10^7 Ca/rod. The baseline in the concentration trace is the extracellular Ca concentration that exists in the dark (≈1 mM). Changes in the Ca electrode potential are converted to changes in Ca concentration by multiplying by the slope of the Ca electrode calibration curve at 1 mM concentration. This linear scaling is adequate for the small (<5%) changes in concentration. The absolute voltage corresponding to the baseline in the photovoltage traces was not measured but, based on prior work (8), would be expected to be approximately −200 μV (vitreal minus scleral potential). Light stimuli were 20-msec flashes (traces a–d) which occurred at zero on the time axis. The flashes (λ = 499 nm) were calculated to bleach 9.0 × 10^1, 2.0 × 10^3, 5.4 × 10^4, and 1.0 × 10^6 rhodopsin molecules per rod, respectively. The stimulus for trace e was a step of light, which bleached 2.3 × 10^4 rhodopsin molecules per sec. The small initial transient in the photovoltage records (traces c and d) was contributed by cones. Signals were low-pass filtered (20 Hz) before being digitized (10-msec sample interval). Because of the slow time scale on which the data are displayed, the data were subsequently filtered digitally. In addition, the slow phase of the flux signals was further filtered for this figure to allow the superimposed traces to be distinguished more easily. None of these filtering operations caused detectable distortion of the time-course or amplitude of the signals.

Test of the Ca Hypothesis. What information does the net Ca flux across the plasma membrane provide about the role of intracellular free Ca in mediating transduction in rods? There are two mechanisms by which Ca crosses the outer segment plasma membrane: extrusion via Na/Ca exchange (1, 13) and an inward leak through the light-regulated conductance (4). Na/Ca exchange is well known to depend on intracellular free Ca concentration (16). According to the Ca hypothesis, the light-regulated conductance, and therefore the inward leak of Ca, also depends on intracellular free Ca

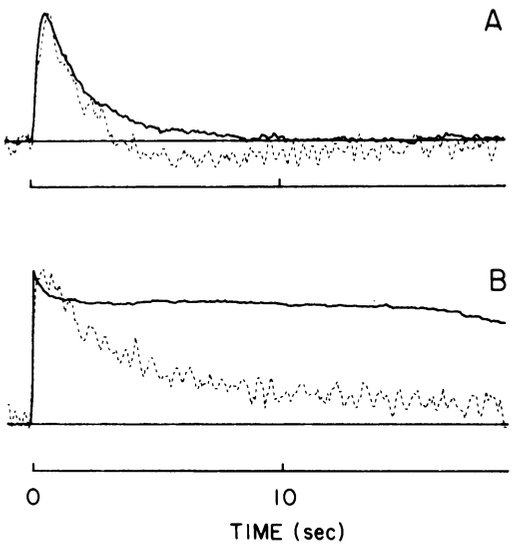

FIG. 3. The net flux (broken) and photovoltage (solid) traces from Fig. 2 are superimposed and scaled to the same peak amplitude. (*A*) Trace a from Fig. 2. (*B*) Trace d from Fig. 2. The flux traces are noisier than they are in Fig. 2 because the data were not filtered as extensively.

concentration. Thus, if the Ca hypothesis is correct, both the net flux of Ca and the membrane conductance depend on intracellular free Ca concentration. Specifically, increased intracellular free Ca concentration would both increase the rate of Na/Ca exchange and reduce the membrane conductance and, therefore, the inward leak. Thus, an increase in intracellular free Ca concentration should produce a net efflux of Ca, and a decrease in intracellular free Ca concentration should produce a net influx. Because the actions of intracellular free Ca on Na/Ca exchange and on the membrane conductance are both expected to be rapid,* the Ca hypothesis predicts a unique, instantaneous relationship between the net flux and the light-regulated conductance. Therefore, because of the close relationship between the photovoltage and the membrane conductance changes, the Ca hypothesis predicts that it should be possible to completely superimpose the flux and photovoltage waveforms by linear and appropriate nonlinear scaling. This superposition is attempted, for two flash intensities, in Fig. 3, in which the calculated net flux (broken lines) and photovoltage (solid lines) traces are linearly scaled to the same peak amplitude. The net Ca flux and photovoltage do indeed reach peak values simultaneously at both intensities, indicating that the flux measurements have sufficient time-resolution to follow the light-induced transients in net Ca flux. However, the net flux and photovoltage waveforms differ in two important respects. First, the overshoot of the net flux trace, below the baseline during the influx phase, has no correlate in the photovoltage waveform (Fig. 3*A*). As explained above, according to the Ca hypothesis, a net influx should result from a drop in intracellular free Ca concentration below the dark level; thus, if Ca controls the membrane conductance, a similar overshoot is expected in the photovoltage waveform. The lack of an overshoot in the photovoltage waveform cannot be explained by saturation of the membrane conductance, because the membrane conductance can be increased well above the dark level by lowering intracellular free Ca below the normal dark level (e.g., ref. 17). The second difference between the flux and photovoltage waveforms is that the net Ca efflux is transient during periods of maintained photovoltage saturation (Fig. 3*B*). It might be asserted that the transient net efflux is caused by a transient rise in intracellular free Ca concentration, which is large enough to saturate the photovoltage for a finite time interval. However, according to this view, the longer periods of photovoltage saturation, which occur as intensity is increased, should result from larger intracellular Ca transients. Yet the amplitude and time-course of the net Ca efflux, which must depend on the intracellular free Ca concentration, are identical at all intensities during photovoltage saturation (see Fig. 2*A*, traces b–d). The superimposability of the Ca flux traces during photovoltage saturation does not reflect saturation of the efflux mechanism, because the rate of net efflux declines continuously during photovoltage saturation. Maintained illumination (Fig. 2, trace e) also produces a transient net efflux with the same peak amplitude and time-course as that produced by saturating flash illumination. Thus, the transient nature of the net Ca efflux is not a consequence of the transient nature of flash illumination.

*A rapid effect of Ca on the membrane conductance is a requirement of the Ca hypothesis. If the rate of Na/Ca exchange does not respond rapidly to changes in intracellular Ca concentration, then the fact that the flux closely follows the initial rise of the photovoltage waveform would constitute additional evidence that the efflux is caused by reduced leak (see *Discussion*).

DISCUSSION

As outlined above, if intracellular free Ca predominantly controls both the rate of Na/Ca exchange and the light-regulated conductance in rods, then the net flux and photovoltage waveforms should be completely superimposable. Because the net flux and photovoltage waveforms are not superimposable, it is likely that substances other than Ca play a major role in controlling either the rate of Na/Ca exchange, or the light-regulated conductance, or both. Existing data indicate that the rate of Na/Ca exchange predominantly reflects the intracellular free Ca concentration in rods. For example, Yau and Nakatani (4) found that in Ca-loaded rods, the rate of Na/Ca exchange depends only on the amount of Ca loaded into a rod and is not altered following illumination. Light-induced changes in ATP and Na concentration might be expected to alter the rate of Na/Ca exchange (18, 19). For example, illumination causes a rapid decline in intracellular ATP concentration in rods (20). However, the ATP concentration is always well above the K_m for ATP regulation of the pump (19) and therefore should not significantly affect Na/Ca exchange. The Na gradient is also known to change following illumination (21). However, the magnitude of the Na gradient changes in the opposite direction of that needed to account for the differences between the flux and photovoltage waveforms. Therefore, the most likely conclusion from this work is that, contrary to the Ca hypothesis, substances other than intracellular Ca must control the light-regulated conductance in rods. This conclusion is consistent with recent evidence that the light-regulated conductance is controlled exclusively by cGMP; i.e., that cGMP, and not Ca, is the intracellular messenger for transduction in rods (22, 23).

Although it now appears that Ca is not the intracellular messenger for transduction in rods (4, 13, 22–25), the mechanism and physiological significance of these light-induced Ca fluxes remain to be explored. Previously, Gold and Korenbrot (1) had interpreted the net efflux as resulting from an increase in Ca extrusion caused by a light-induced increase in intracellular free Ca concentration. However, in view of the discrepancies between the observed fluxes and those expected if a light-induced rise in intracellular free Ca concentration controls the membrane conductance, this interpretation needs to be reexamined. In fact, the present data are entirely consistent with recent evidence that the net efflux is caused not by an increase in Ca extrusion, but rather by a

decrease in the inward leak through the light-regulated conductance (4). Using measurements of the membrane current caused by electrogenic Na/Ca exchange, Yau and Nakatani (4) estimated the rate of inward Ca leak in the dark and found it to be approximately equal to the maximal rate of Ca extrusion. Thus, they suggested that a steady state exists in the dark, in which the inward leak is equal to the rate of Ca extrusion by Na/Ca exchange. Because light reduces the outer segment membrane conductance, illumination should reduce the rate of inward leak, producing a net efflux. According to this model, illumination would have no immediate effect on Ca extrusion. However, in the absence of Ca release by the intracellular disc membranes, the net Ca efflux would cause a decrease in the intracellular free Ca concentration and therefore would lead to a decline in the rate of Na/Ca exchange. In support of this model, Yau and Nakatani observed a rapid decline in Na/Ca exchange current following illumination. Because they have also shown that the Na/Ca exchange mechanism is not inhibited following illumination, they have suggested that the decline in the rate of Na/Ca exchange reflects a light-induced decrease in intracellular free Ca concentration (4).

Several characteristics of the net Ca fluxes reported here provide additional support for Yau and Nakatani's model. For example, this model requires that the initial rate of efflux and the peak amplitude of the photovoltage should reach saturation at the same stimulus intensity. This is indeed the case (Fig. 2). During photovoltage saturation, the membrane conductance and, therefore, the inward leak are blocked completely (12, 26). Therefore, if the light-induced efflux is due solely to regulation of the leak, the amplitude and time-course of the Ca efflux should be independent of stimulus intensity and duration during photovoltage saturation. This is demonstrated by the superimposability of all of the flux traces during photovoltage saturation (Fig. 2*A*, traces b–e). If the light-induced net efflux is due solely to reduced inward leak, operating in parallel with a light-independent pump, then a net efflux should persist for as long as the photovoltage is saturated. Also, the beginning of the influx phase should be correlated with the recovery of the photovoltage from saturation. Both of these predictions are confirmed (compare traces in Fig. 2 *A* and *C*). However, note that the flux traces in Fig. 2*A* do not begin to recover from the limiting trajectory (trace e) until the photovoltage has recovered approximately one-third of the way back to the baseline. This suggests that the fraction of the light-regulated current that is carried by Ca varies with the total membrane current, perhaps due to an effect of membrane potential on the Na/Ca permeability ratio. Because the inward leak is completely blocked during photovoltage saturation, the net efflux observed during photovoltage saturation reflects only extrusion. Therefore, in agreement with Yau and Nakatani's Na/Ca exchange current measurements, these data show that the rate of extrusion declines continuously during photovoltage saturation, presumably due to the resulting decrease in intracellular free Ca concentration. Although Yau and Nakatani's Na/Ca exchange current declines more rapidly than the calculated flux reported here (their $\tau \approx 0.4$ sec vs. my $t_{1/2} \approx 4$ sec), this difference probably reflects differences between the two techniques used. For example, the better time resolution of the Na/Ca exchange current measurements may have allowed Yau and Nakatani to detect an initial fast component of the Ca efflux that is not resolved by the extracellular Ca concentration measurements. On the other hand, the better amplitude resolution of the extracellular Ca concentration measurements could reveal slow components of the Ca efflux that cannot be detected as a current.

CONCLUSION

The data reported here do not support the hypothesis that a rise in intracellular free Ca concentration mediates transduction in rods. Rather these data corroborate recent findings (4) suggesting that (*i*) the light-induced net Ca efflux from the rod outer segment is caused solely by a reduction of the inward leak of Ca through the light-regulated conductance and (*ii*) light reduces the intracellular free Ca concentration in rods. In view of the recent evidence that cGMP is the intracellular messenger for transduction in rods (22, 23, 27), the role of intracellular Ca in photoreceptor physiology must now be reexamined. For example, some of the well-known effects of Ca on the photoresponse can now be attributed in part to effects of Ca on the cGMP-controlling enzymes of the outer segment. It now remains to establish the existence of a light-induced decrease in intracellular free Ca concentration and to demonstrate the contribution that these concentration changes make to controlling the cGMP metabolism of the outer segment.

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