Calcium Regulates Some, but Not All, Aspects of Light Adaptation in Rod Photoreceptors

G. D. NICOL and M. D. BOWNDS

From the Laboratory of Molecular Biology, University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT   The role of calcium as a regulator of light adaptation in rod photoreceptors was examined by manipulation of the intracellular Ca^{2+} concentration through the use of the calcium ionophore A23187 and external Ca^{2+} buffers. These studies utilized suspensions of isolated and purified frog rod outer segments that retain their mitochondria-rich inner segments (OS-IS). Three criteria of the dark- and light-adapted flash response were characterized as a function of the Ca^{2+} concentration: (a) the time to peak, (b) the rate of recovery, and (c) the response amplitude or sensitivity. For all Ca^{2+} concentrations examined, the time to peak of the flash response was accelerated in the presence of background illumination, suggesting that mechanisms controlling this aspect of adaptation are independent of the Ca^{2+} concentration. The recovery kinetics of the flash response appeared to depend on the Ca^{2+} concentration. In 1 mM Ca^{2+}-Ringer's and 300 nM Ca^{2+}-Ringer's + A23187, background illumination enhanced the recovery rate of the response; however, in 10 and 100 nM Ca^{2+}-Ringer's + A23187, the recovery rates were the same for dark- and light-adapted responses. This result implies that a critical level of Ca^{2+} may be necessary for background illumination to accelerate the recovery of the flash response. The sensitivity of the flash response in darkness (S_F^D) was dependent on the Ca^{2+} concentration. In 1 mM Ca^{2+}-Ringer's, S_F^D was 0.481 pA per bleached rhodopsin (Rh*); a background of four Rh*/s decreased S_F^D by half (I_o). At 300 nM Ca^{2+} + A23187, S_F^D was reduced to 0.0307 pA/Rh* and I_o increased to 60 Rh*/s. At 100 nM Ca^{2+} + A23187, S_F^D was reduced further to 0.0025 pA/Rh* and I_o increased to 220 Rh*/s. In 10 nM Ca^{2+} + A23187, S_F^D was lowered to 0.00045 pA/Rh* and I_o raised to 760 Rh*/s. Using these values of S_F^D and I_o for each respective Ca^{2+} concentration, the dependence of the flash sensitivity on background intensity could be described by the Weber-Fechner relation. Under low Ca^{2+} conditions + A23187, bright background illumination could desensitize the flash response. These results are consistent with the idea that the concentration of Ca^{2+} may set the absolute magnitude of response sensitivity in darkness, and that there exist mechanisms capable of adapting the photoresponse in the absence of significant changes in cytoplasmic Ca^{2+} concentration.

INTRODUCTION

The presence of adapting background illumination causes vertebrate rod photoreceptors to desensitize so that a greater flash intensity is required to elicit a response of criterion amplitude (Kleinschmidt, 1973; see review by Shapley and Enroth-Cugell, 1984). Furthermore, the response to a flash of light in the presence of background illumination undergoes a change in waveform kinetics; both the time to peak and the recovery phase are accelerated (Baylor and Hodgkin, 1974; Fain, 1976; Baylor et al., 1979). It has been suggested that intracellular Ca^{2+} levels may regulate the state of adaptation since lowering the external concentration of Ca^{2+} desensitizes the rod (Yoshikami and Hagins, 1973) and the effects of increased external Ca^{2+} are similar to background illumination in reducing the response amplitude (Brown and Pinto, 1974). However, the changes in waveform kinetics observed for the light-adapted photoresponse are not similar to those kinetic changes observed for increased or decreased levels of external Ca^{2+}, suggesting that Ca^{2+} and background illumination may desensitize the rod via different mechanisms (Bastian and Fain, 1979, 1982; Greenblatt, 1983).

A suggestion that the intracellular concentration of Ca^{2+} is highest in the dark and falls upon illumination (Bownds, 1980; Yau and Nakatani, 1985) has been demonstrated experimentally to be the case (McNaughton et al., 1986; Ratto et al., 1988). Torre et al. (1986) have shown that intracellular perfusion of the Ca^{2+} buffer BAPTA into a rod photoreceptor causes slowing and sensitization of the light response and an undershoot during recovery. In the presence of background illumination the recovery of the light response is accelerated even though the overall time course of the response is slowed. Similar results have been described for rods loaded with the Ca^{2+} indicator/buffer, quin 2 (Korenbrot and Miller, 1986).

Two recent reports have suggested that the changes in Ca^{2+} concentration occurring upon illumination are directly involved in light adaptation in both rod and cone photoreceptors. These studies utilized a low Ca^{2+}-Ringer's (nominally micromolar levels) to prevent the influx of Ca^{2+} through the light-regulated ion channels and replaced external sodium with guanidinium to inhibit Na/Ca exchange, thus blocking the efflux of Ca^{2+} from the rod. Matthews et al. (1988) found that, when changes in cytoplasmic Ca^{2+} concentration were prevented, light-induced changes in response sensitivity were subsequently abolished. The dependence of response sensitivity on the intensity of background illumination was no longer described by the Weber-Fechner relation. Nakatani and Yau (1988) observed that the relaxation from peak to plateau of the photoresponse elicited by a step of light was suppressed under those conditions that prevented changes in cytoplasmic Ca^{2+}. These findings are consistent with the idea that Ca^{2+} may be the adaptation transmitter in photoreceptors.

We have investigated the role of Ca^{2+} in regulating the adaptational state of the rod photoreceptor by utilizing the Ca^{2+} ionophore A23187 and Ca^{2+} buffers to perturb any light-induced changes in the intracellular Ca^{2+} concentration while examining the effects of background illumination on three aspects of the flash response: (a) the time to peak, (b) the kinetics of the response recovery, and (c) the response amplitude or sensitivity. We find that the response sensitivity in darkness is correlated with the Ca^{2+} concentration, but that some aspects of light adaptation, namely

response desensitization and acceleration of the time to peak, still occur in isolated rod photoreceptors that have been depleted of intracellular Ca^{2+}. A discrete or critical level of Ca^{2+} rather than a change in the cytoplasmic concentration appears to be required for acceleration of the recovery phase of the light-adapted photoresponse.

METHODS

Preparation of Rod Photoreceptors

Rod photoreceptors were prepared according to the method described by Biernbaum and Bownds (1985) with slight modifications. Briefly, isolated dark-adapted retinas from the bullfrog, *Rana catesbeiana*, were gently shaken in oxygenated Ringer's containing (in millimolar), 105 NaCl, 2 KCl, 1 $CaCl_2$, 2 $MgCl_2$, $5NaHCO_3$, 5 glucose, 10 HEPES, 5% isosmotic Percoll (Pharmacia Fine Chemicals, Piscataway, NJ), pH 7.6, adjusted with NaOH. Retinas were then shredded into fine particles and gently vortexed. The rod suspensions were layered onto a linear Percoll density gradient (30–70% Percoll-Ringer's). After centrifugation at 10,000 rpm for 1 min, the lower band containing osmotically intact rod outer segments and intact rod outer segments that retain the mitochondria-rich ellipsoid portion of their inner segments (OS-IS) was removed.

Electrophysiology

The electrophysiological recording technique and apparatus has been described in detail elsewhere (Nicol et al., 1987). Briefly, an aliquot (3–5 µl) from a suspension of purified OS-IS was placed into a recording chamber containing 5% Percoll-Ringer's with the appropriate Ca^{2+} concentration. The light-regulated current was measured with the suction electrode technique (Baylor et al., 1979). The inner segment of an OS-IS was drawn into a suction electrode under visual control in darkness using an infrared video system. During the measurements of the dark sensitivity and the effects of background illumination on the flash sensitivity the microscope illuminator was turned off to insure that the rod was dark-adapted.

The rod was illuminated with a small focused spot (~75 µm diameter) whose intensity was controlled with a series of calibrated neutral density filters. The duration of the flash was 12 ms. Background illumination was presented to the OS-IS suspension via a fiber optic bundle that took the place of one of the ocular lens of the microscope and was focused through the 20× objective. The intensity of the background light was attenuated with a series of calibrated neutral density filters. All current records were low-pass filtered at 20 Hz with an eight-pole active filter. Current traces were recorded on either a chart recorder or digitized (sampling interval 10 ms) and stored on computer disk for later analysis. The recording chamber was perfused with Ringer's solutions so that the calcium ionophore A23187 could be introduced to the OS-IS suspension at the appropriate time. The turnover time for the recording chamber was between 10 and 15 s.

Flash and Background Illumination Protocols

In experiments that examined the effects of background illumination on the sensitivity and kinetics of the flash response the protocol was as follows. A dim flash was presented to the OS-IS that elicited a response between 1 and 3 pA in amplitude (in the linear portion of the intensity–response curve); this test flash was then used to determine the dark sensitivity (S_F^D, defined as picoamperes per bleached rhodopsin [Rh*]) of the OS-IS. Typically between 5 and 10 responses were averaged to determine the dark sensitivity (see subsequent figure legends

for specific numbers). At this point a saturating test flash was presented to determine the maximal response amplitude. When the membrane current had recovered to its dark base-line, the background light was turned on and remained on for at least 30 s before any test flashes were presented. This enabled the membrane current and response sensitivity of the OS-IS to come to equilibrium (see Copenhagen and Green, 1985). Experiments began with the dimmest background intensity and were followed by a series of progressively brighter backgrounds until the dark current was completely suppressed by the background and no response could be elicited by the test flash. At the conclusion of an experiment the recording chamber was thoroughly washed with ethanol to remove any traces of the ionophore, the chamber filled with the appropriate Ringer's solution, and a new aliquot of dark-adapted OS-IS added.

In experiments that examined the effects of Ca^{2+} concentration on the flash sensitivity and kinetics, OS-IS were incubated in the recording chamber containing a Ringer's solution of the appropriate Ca^{2+} concentration for ~10 min. The light-regulated current was recorded from an OS-IS and if the photoresponse to a saturating flash was at least 10 pA in amplitude, the perfusate was changed to one containing 20 μM A23187 with the identical Ca^{2+} concentration. The suspension was incubated under these conditions for at least 10 min before examining the effects of background illumination on the sensitivity and kinetics of the flash response as described above.

Rates of Response Recovery

The recovery rates for the photoresponses were determined in the following manner. The flash responses were all normalized to the same peak amplitude and then plotted on an expanded time scale similar to that shown for Fig. 10. A line was drawn through the data points by eye so as to give a best fit to the recovery phase of the response. The slope of this line, i.e., the rate at which the membrane current returns to its dark baseline, was then determined. The value for the slope is given in arbitrary units (au) per second; the larger the number, the faster the rate of recovery.

Solutions

The calcium ionophore A23187 was dissolved in ethanol as a 5 mM stock solution and added to the Ringer's solution just before use. The 10 nM Ca^{2+}-Ringer's was obtained by adding 0.39 mM EGTA to 0.1 mM Ca^{2+} Percoll-Ringer's, pH 7.6 (Caldwell, 1970). For experiments involving 100 nM Ca^{2+}, a 0.1 mM Ca^{2+}-Ringer's was buffered nominally with 0.13 mM EGTA. For 300 nM Ca^{2+}, a 0.1 mM Ca^{2+}-Ringer's was buffered nominally with 0.11 mM EGTA. A Ca^{2+}-sensitive macroelectrode of the type described by Tsien and Rink (1980) was used to verify the Ca^{2+} concentration of both the 100 and 300 nM Ca^{2+}-Ringer's; the solution was either titrated with Ca^{2+} or EGTA to achieve the desired Ca^{2+} concentration.

RESULTS

We sought to characterize the properties of light adaptation in frog OS-IS to establish whether these rods are similar to rod photoreceptors that remain attached to the retina. The value of the OS-IS preparation is that both electrophysiological and biochemical measurements can be made during light adaptation using the same cellular suspension.

Light Adaptation in OS-IS at Normal Ca^{2+} Levels

The sensitivity of the OS-IS flash response in 1 mM Ca^{2+}-Ringer's was decreased by increasing intensities of background illumination; these results are illustrated in

Fig. 1. The flash sensitivity of OS-IS in the dark (S_F^D) was 0.481 ± 0.119 pA/Rh* (mean ± standard deviation) and ranged in value from 0.725 to 0.317 pA/Rh* (n = 12). Background illumination of only four Rh*/s was sufficient to reduce S_F^D by half and is defined as I_o. For bright background illumination (1,060 Rh*/s) the flash sensitivity S_F was reduced to 0.0027 ± 0.0009 pA/Rh*; this corresponds to about a 180-fold reduction in S_F by the background. The dependence of response sensitivity on the intensity of background illumination was described by the Weber-Fechner relation: $S_F = S_F^D/[1 + (I_{BK}/I_o)]$, where S_F is the flash sensitivity in pA/Rh*, S_F^D is the
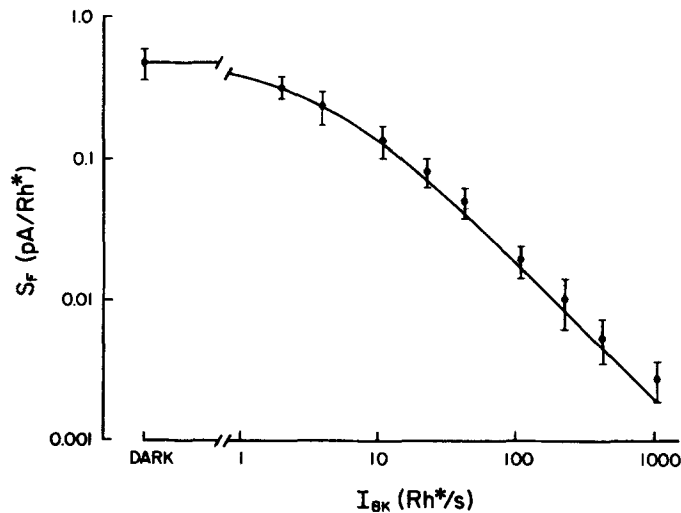

FIGURE 1.   The flash sensitivity of OS-IS and its dependence on background intensity can be described by the Weber-Fechner relation. The magnitude of dark current suppression by dim flashes and its dependence on background intensity was determined in 1 mM Ca^{2+}-Ringer's. In the absence of background illumination, the OS-IS flash sensitivity (S_F^D) was 0.481 pA/ Rh*. The flash sensitivity, S_F, was plotted as a function of the intensity of background illumination, I_{BK} (Rh*/s). For the brightest background (1,060 Rh*/s), S_F was reduced to 0.0027 pA/Rh*, a light-induced desensitization of ~ 180-fold. S_F^D was reduced by half at a background intensity (I_o) of four Rh*/s. Using the values of 0.481 pA/Rh* and four Rh*/s for S_F^D and I_o, respectively, the data can be fit by the Weber-Fechner relation, which is the *solid line* drawn through the experimental points. Each point represents the mean ± standard deviation; for the dark (n = 12), for I_{BK} of two Rh*/s (n = 8), and for the remaining points (n = 6).

flash sensitivity measured in the dark, I_{BK} is the intensity of the background (Rh*/s), and I_o is the background intensity that reduces S_F^D half. This equation has been used to describe light adaptation in turtle cones (Baylor and Hodgkin, 1974) and toad rods (Fain, 1976; Baylor et al., 1980). The results obtained for frog OS-IS were well fit by the Weber-Fechner relation, where S_F^D is 0.481 pA/Rh* and I_o is four Rh*/s; this is the solid line drawn through the data points. This value for I_o is identical to those observed by others (four Rh*/s Fain, 1976; six Rh*/s Baylor et al., 1980; three Rh*/s Matthews et al., 1988). Thus, the response sensitivity of frog OS-IS is similar to measurements obtained by others in different rod preparations.

In the frog OS-IS, background illumination results in desensitization of the flash response along with acceleration of the waveform kinetics. Fig. 2 illustrates the kinetics of the photoresponse to flashes in darkness and in the presence of background illumination. In Fig. 2 a, a six-Rh* flash in darkness elicited a photoresponse
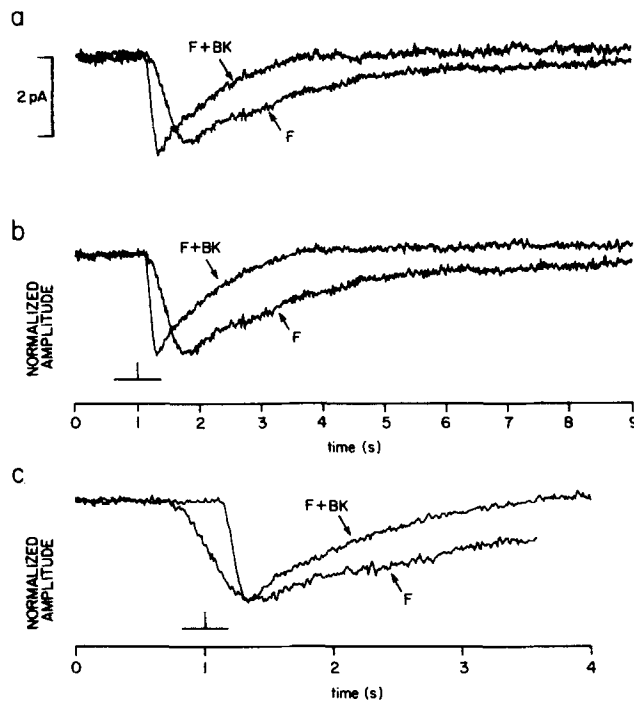

FIGURE 2. Background illumination accelerates the time to peak and the recovery of the OS-IS flash response in 1 mM Ca^{2+}-Ringer's. In a, a 6-Rh* flash produced a 2.4-pA suppression of the dark current (trace labeled F). In the presence of background illumination (1,060 Rh*/s), a 609-Rh* flash was required to elicit a 2.5-pA photoresponse (trace labeled $F + BK$). Each trace is the average of 14 responses. This OS-IS had a dark current of 19.4 pA as determined by a saturating flash of light. The traces shown were obtained from cell E listed in Table I. In b, these flash responses have been normalized to the same peak amplitude to illustrate the acceleration of the response kinetics. Both the time to peak (t_p) and rate of recovery for the flash response were accelerated by the background. The t_p for the response in darkness was 0.75 s, whereas in the presence of background illumination t_p was shifted to 0.35 s. The vertical marker at 1 s indicates the timing of the light flash; the time scale is the same for a and b. In c, the responses illustrated in b have been normalized to the same point in time, t_p, and are shown on an expanded time scale. This demonstrates that the recovery of the flash response is significantly accelerated in the presence of background illumination. The flash in darkness had a recovery rate of 19.5 au/s, whereas in the presence of background illumination the recovery rate was accelerated to 38.4 au/s. The time scale and the flash marker are for trace $F + BK$ while trace F has been shifted to the left by 0.40 s.

2.4 pA in amplitude (trace labeled F, $S_F^D = 0.400$ pA/Rh*), whereas in the presence of a 1,060-Rh*/s background, a 609-Rh* flash produced a photoresponse 2.5 pA in amplitude (trace labeled F + BK, $S_F = 0.0041$ pA/Rh*). This background intensity produced nearly complete suppression of the dark current; corresponding to a sup-

pression of 16 pA from the 19-pA dark current (see Table I for more details). In Fig. 2 *b*, the photoresponses were normalized to the same peak amplitude. Both the time to peak and the rate of recovery for the flash response were accelerated in the presence of background illumination as compared with the flash in the dark. In five OS-IS the average time to peak for the flash in darkness was 1.00 ± 0.17 s and in the presence of bright background illumination the time to peak was reduced to 0.41 ± 0.08 s (mean ± SD). This corresponds to a 2.5-fold decrease in the time to peak and is similar to the three-fold decrease observed in toad rods by Baylor et al. (1980). The accelerated rate of recovery is shown on an expanded time scale in Fig. 2 *c*. Here, the flash in darkness and the flash in the presence of a background have been normalized to the same point in time, their times to peak. For the flash in darkness the average rate of recovery was 21.8 ± 3.5 au/s, whereas in background light the rate was increased to 32.1 ± 5.7 au/s (mean ± SD, $n = 5$). This clearly shows that the recovery kinetics for the light-adapted photoresponses were faster than that for the dark-adapted flash responses.

TABLE I

Sensitivity and Kinetic Parameters in 1 mM Ca^{2+}

Cell	S_F^D	I_{max}	I_{BK}	t_p	$t_p + BK$
	pA/Rh^*	pA	pA	s	s
A	0.450	16.3	12.8	0.99	0.30
B	0.525	19.4	15.0	1.22	0.44
C	0.466	15.0	11.9	0.99	0.48
D	0.667	18.8	17.5	1.04	0.47
E	0.333	19.4	16.3	0.75	0.35
Mean	0.488	17.8	14.7	1.00	0.41
±SD	±0.122	±2.0	±2.3	±0.17	±0.08

S_F^D, dark sensitivity; I_{max}, maximum photoresponse; I_{BK}, dark current suppression by background illumination (1,060 Rh*/s); t_p, time to peak; $t_p + BK$, time to peak during background illumination.

The effects of background illumination of the response sensitivity and kinetic parameters in 1 mM Ca^{2+}-Ringer's are summarized in Table I.

Light Adaptation in Ca^{2+}-depleted OS-IS

Modulation of the intracellular Ca^{2+} concentration has been suggested to regulate the adaptational state of the rod (Yoshikami and Hagins, 1973; Brown and Pinto, 1974; Matthews et al., 1988; Nakatani and Yau, 1988). To test this hypothesis, the flash sensitivity and its dependence on background intensity were examined in OS-IS depleted of their intracellular Ca^{2+}. In order to deplete the rod of intracellular Ca^{2+}, endogenous Ca^{2+} transport mechanisms must be overcome with a transporter of greater flux capacity. We have compared previously the maximum flux capacity of the calcium ionophore A23187 with the Na/Ca exchange mechanism in rod photoreceptors and found that A23187 has about a 10-fold greater flux capacity than does the Na/Ca exchanger (see Nicol et al., 1987). Thus, the calcium ionophore A23187 in combination with Ca^{2+} buffers (EGTA, yielding 10 nM free Ca^{2+}) can remove nearly 95% of the intracellular Ca^{2+} content in frog OS-IS (Nicol et al.,

1987). A23187 and extracellular Ca^{2+} buffers can then be utilized to shunt native Ca^{2+} transport mechanisms of the rod membrane and abolish or at least greatly diminish any light-induced changes in the cytosolic Ca^{2+} concentration. The effects of background illumination on the flash sensitivity and response kinetics under these low-Ca^{2+} conditions are presented below.
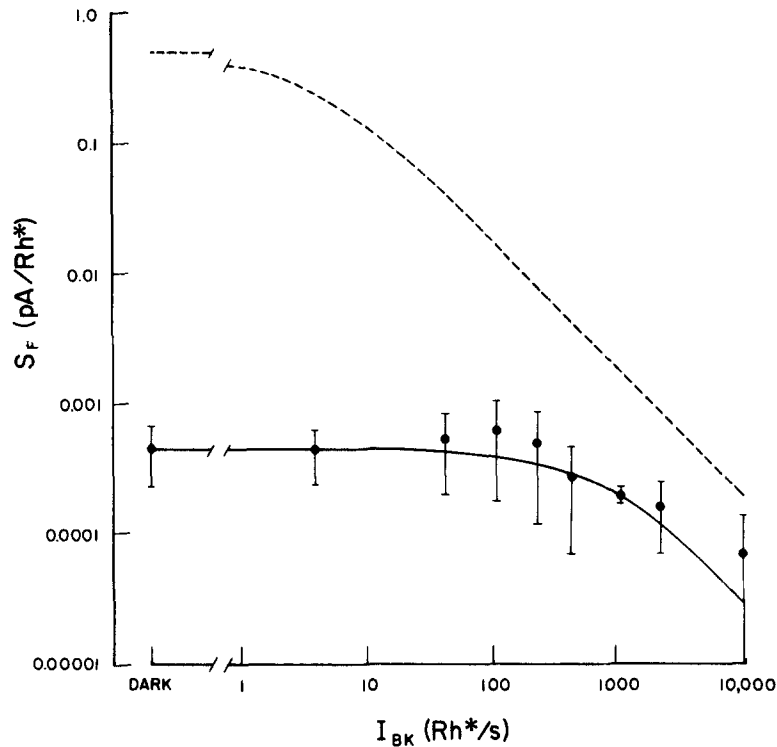

FIGURE 3. OS-IS flash sensitivity and its dependence on background illumination in 10 nM Ca^{2+}-Ringer's + A23187. Under these low-Ca^{2+} conditions S_F^D was reduced to 0.00045 pA/ Rh*, corresponding to a 1,069-fold reduction when compared with S_F^D obtained for 1 mM Ca^{2+}. For the brightest background (9,890 Rh*/s), S_F was reduced to 0.00007 pA/Rh*, a light-induced desensitization of only about sixfold. The relation between the flash sensitivity S_F and intensity of the background light I_{BK} is no longer described by the Weber-Fechner relation using the parameters obtained for 1 mM Ca^{2+} (the *dashed line* is redrawn from Fig. 1). When the values for the parameters of S_F^D and I_o were changed to those determined experimentally, 0.00045 pA/Rh* and 760 Rh*/s (see Fig. 9), respectively, the points were fit by the Weber-Fechner relation; this is the *solid line* through the experimental points. OS-IS were incubated in 10 nM Ca^{2+}-Ringer's + 20 μM A23187 as described in Methods. Each point represents the mean ± standard deviation; for the dark ($n = 11$) and for the I_{BK} points (n ranged between 4 and 7).

As illustrated in Fig. 3, the dark-adapted flash sensitivity (S_F^D) of OS-IS in 10 nM Ca^{2+}-Ringer's + A23187 was reduced by ~3 log units from that observed in 1 mM Ca^{2+}-Ringer's. Under these low-Ca^{2+} conditions, S_F^D was reduced to 0.00045 ± 0.00022 pA/Rh* and ranged from 0.00075 to 0.00024 pA/Rh* ($n = 11$). Fig. 3

demonstrates that the photoresponse could be desensitized by background illumination, but very bright backgrounds were required (>1,000 Rh*/s). The value of S_F was relatively constant for backgrounds up to ~1,060 Rh*/s, above this intensity S_F was reduced by only sixfold. The dependence of flash sensitivity S_F on the intensity of background illumination was no longer described by the Weber-Fechner relation using the parameters obtained for 1 mM Ca^{2+} (redrawn from Fig. 1 for reference: dashed line). However, if the values for S_F^D (0.00045 pA/Rh*) and I_o (760 Rh*, see Fig. 9 for the determination of I_o) obtained in 10 nM Ca^{2+}-Ringer's are now used, then the experimental points can be described by the Weber-Fechner relation (given in the preceding subsection); this is drawn as the solid line through the experimental points. The fit is rather good except for the highest background intensity where the Weber-Fechner relation begins to fall off quickly. The large value of I_o accounts for the flat shape of the curve, i.e., very little desensitization for I_{BK}'s up to 432 Rh*/s. Also, for I_{BK}'s >3,000 Rh*/s the slope of the curve becomes -1 indicating that the sensitivity is inversely proportional to I_{BK}. A more satisfactory fit of the experimental points for $I_{BK} > 1,060$ Rh*/s was obtained when the quantity (I_{BK}/I_o) was raised to the 0.7 power, but this has the drawback of decreasing the fit for less intense backgrounds. These results suggest that under conditions where the rods have been depleted of Ca^{2+} some desensitization of the photoresponse by background illumination does persist.

The kinetics of the flash responses in the absence and presence of background illumination recorded from Ca^{2+}-depleted OS-IS are illustrated in Fig. 4. In contrast to the flash response recorded in 1 mM Ca^{2+}-Ringer's, Ca^{2+}-depleted OS-IS do not exhibit acceleration of the response recovery in the presence of bright background illumination. Fig. 4 *a* shows that in darkness a 5,068-Rh* flash elicited a response of ~1.5 pA in amplitude (trace labeled F, $S_F^P = 0.00030$ pA/Rh*). In the presence of nearly saturating background illumination (38,476 Rh*/s) a 27,717-Rh* flash was required to elicit a 3.2-pA response (trace labeled F + BK, $S_F = 0.00012$ pA/Rh*), corresponding to a 2.5-fold reduction in sensitivity. In Fig. 4 *b*, the two flash responses have been normalized to the same peak amplitude; this clearly demonstrates that background illumination still produces acceleration of the time to peak for the photoresponse. In four other OS-IS the average time to peak for the flash response in darkness was 1.23 ± 0.39 s and in bright background illumination the time to peak was reduced to 0.47 ± 0.03 s (mean ± SD), a reduction of 2.6-fold. These values are not significantly different from those obtained in 1 mM Ca^{2+}. When the above photoresponses were normalized to the same point in time (the time to peak) as shown in Fig. 4 *c*, the recovery rate for the light-adapted response was identical to that for the dark-adapted response. Thus, acceleration of the recovery phase for the flash response by background illumination is no longer observed in the Ca^{2+}-depleted OS-IS.

In summary, it appears that two aspects of light adaptation persist in OS-IS depleted of intracellular Ca^{2+}: the flash response can be desensitized by bright background illumination (although this is a small effect) and the time to peak of the flash response is accelerated by the background light. These results are consistent with the notion that changes in Ca^{2+} concentration may not be obligatory for desensitization of the flash response. The intracellular Ca^{2+} concentration does, however,

greatly influence the absolute magnitude of the flash sensitivity in darkness and the recovery kinetics of the flash response.

Light Adaptation at Intermediate Ca^{2+} Levels

Recent studies have determined that under normal physiological conditions the intracellular free Ca^{2+} concentration is between 200 and 600 nM in the dark (Korenbrot and Miller, 1986; McNaughton et al., 1986). In experiments utilizing the
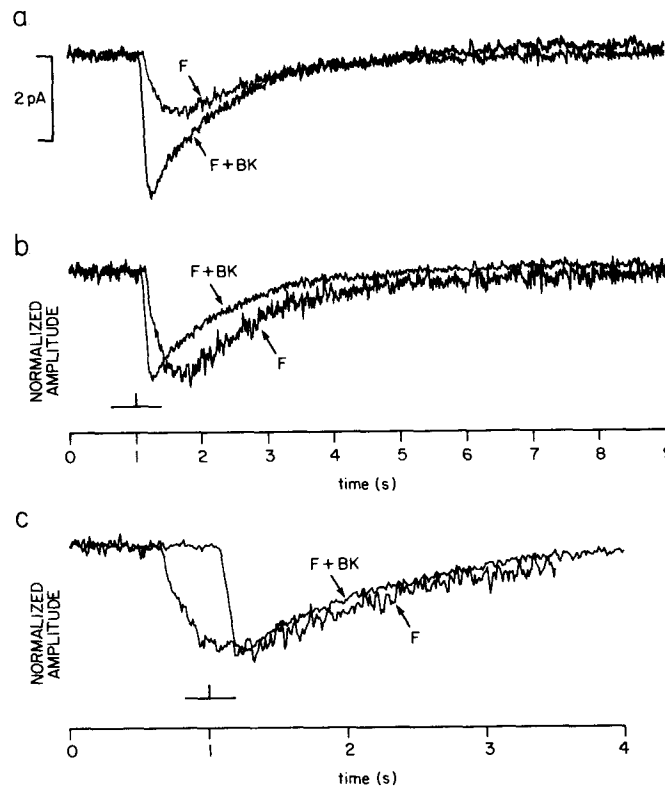

FIGURE 4.   Kinetics of the OS-IS flash response in the absence and presence of background illumination in 10 nM Ca^{2+}-Ringer's + A23187. In a, a 5,068-Rh* flash elicited a 1.4-pA photoresponse (trace labeled F), yielding a value for S_F^D of 0.00028 pA/Rh*. In the presence of a nearly saturating background (38,476 Rh*/s) a flash of 27,717 Rh* was required to produce a 3.2-pA response (trace labeled $F + BK$), this corresponds to a value for S_F of 0.00012 pA/Rh*. For this particular OS-IS the maximum amplitude of the photoresponse was 13.4 pA. Trace F is the average of 8 responses, trace $F + BK$ the average of 10 responses. In b, the responses have been normalized to the same peak amplitude for a comparison of the response kinetics. Under the low-Ca^{2+} conditions, t_p was accelerated in the presence of background illumination. The time scale and flash marker are the same for both a and b. In c, the responses shown in b have been normalized to their t_p's. Under the low-Ca^{2+} condition, the rates of recovery were nearly identical for both the flash in darkness and in the presence of background illumination. The time scale and flash marker are for trace $F + BK$; trace F has been shifted to the left by 0.50 s.

calcium-indicating dye fura 2, the intracellular concentration of free Ca^{2+} in the dark was determined to be $\sim$220 nM and during illumination this value fell to $\sim$140 nM (Tsien and Poenie, 1987; Ratto et al., 1988). If Ca^{2+} plays a role in the regulation of light adaptation, then its physiological action must occur over a narrow window of concentrations.

In OS-IS depleted of intracellular Ca^{2+} the flash sensitivity is greatly reduced and the capacity of background illumination to accelerate the recovery of the flash response appears to be either blocked or lost. Thus it was of interest to examine the effects of background illumination on the sensitivity and response kinetics of OS-IS at intermediate Ca^{2+} concentrations that may be of physiological importance. We have examined the effects of background illumination at concentrations of Ca^{2+} that are slightly below (100 nM) and slightly above (300 nM) those values reported for the lower and upper limits of the normal intracellular free Ca^{2+} concentration as determined by fura 2.

Light Adaptation at 100 nM Ca^{2+} + A23187

A Ca^{2+} concentration of 100 nM is slightly lower than the 140 nM value reported for normal intracellular free Ca^{2+} during illumination. At this Ca^{2+} concentration the dark flash sensitivity was reduced and background illumination produced some desensitization of the photoresponse. However, the recovery of the flash response was not accelerated in the presence of background illumination. These results are shown in Figs. 5 and 6.

After a 10-min incubation in 100 nM Ca^{2+}-Ringer's containing 20 μM A23187, the dark sensitivity of the OS-IS flash response was determined to be 0.00251 $\pm$ 0.00182 pA/Rh* and is shown in Fig. 5. The value of S_F^D ranged from 0.00705 to 0.00095 pA/Rh* ($n = 17$). On average, S_F^D was reduced by 192-fold from that obtained at 1 mM Ca^{2+}. The magnitude of desensitization was not as great as that observed in 10 nM Ca^{2+} + A23187, being about sixfold greater. Like the results obtained in 10 nM Ca^{2+}, S_F was relatively constant as the intensity of the background illumination was increased. The experimental points were not fit by either the Weber-Fechner relation using the parameters obtained for 1 mM Ca^{2+} (dashed line redrawn from Fig. 1) or using the values of S_F^D (0.00251 pA/Rh*) and I_0 (220 Rh*/s, see Fig. 9 for the determination of I_0) obtained at 100 nM Ca^{2+} + A23187; this is the solid line drawn through the data. The values for the response sensitivity at backgrounds $<\sim$2,000 Rh*/s could be described by the Weber-Fechner equation, but for greater I_{BK}'s this fit was lost. These results are consistent with the idea that in the absence of light-induced changes in intracellular Ca^{2+} concentration background illumination can to some extent desensitize the photoresponse.

It is important to point out that the value for S_F^D at 100 nM Ca^{2+} + A23187 (0.00251 pA/Rh*) is nearly identical to the value obtained for S_F at 1 mM Ca^{2+} (0.0027 pA/Rh*) in the presence of bright background illumination (1,060 Rh*/s). If one assumes that the flash sensitivity correlates with the intracellular Ca^{2+} content then the similar values for S_F in bright background light (in normal Ca^{2+}) and S_F^D (in 100 nM Ca^{2+} + A23187) indirectly corroborate the findings obtained by Ratto et al. (1988) that the Ca^{2+} concentration is reduced to $\sim$140 nM during bright illumination. The nearly identical values for S_F^D and S_F also demonstrate the effectiveness of

A23187 in combination with the Ca^{2+} buffer in controlling the intracellular Ca^{2+} concentration of the OS-IS.

In 100 nM Ca^{2+} + A23187 background illumination produces acceleration of the time to peak but not the recovery kinetics for the flash response. As illustrated in Fig. 6 a, a 1,109-Rh* flash elicited a response of ~1.2 pA in amplitude (trace labeled F, $S_F^D = 0.0011$ pA/Rh*). In the presence of a nearly saturating background (38,476
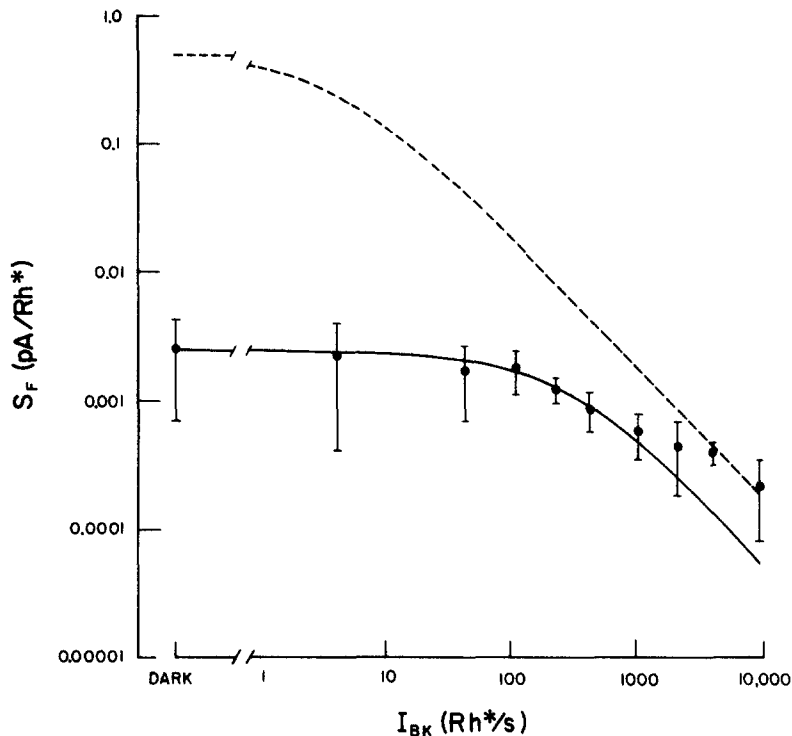

FIGURE 5.   OS-IS flash sensitivity and its dependence on background intensity for 100 nM Ca^{2+}-Ringer's + A23187. At this Ca^{2+} concentration the value of S_F^D was reduced to 0.00251 pA/Rh*; this is a 192-fold reduction in sensitivity when compared to 1 mM Ca^{2+}. For the brightest background (9,890 Rh*/s), S_F was reduced to 0.00021 pA/Rh*, corresponding to a 12-fold desensitization. The dependence of S_F on I_{BK} is no longer described by the Weber-Fechner relation using the parameters obtained for 1 mM Ca^{2+}, which is shown as the *dashed line*. The values of S_F^D and I_o in the Weber-Fechner relation were adjusted to those determined experimentally, 0.00251 pA/Rh* and 220 Rh*/s, respectively (see Fig. 9). This yielded the *solid line*, which also did not fit all the data points. Each point represents the mean ± standard deviation; for the dark ($n = 17$), for I_{BK} of 4 and 43 Rh*/s ($n = 14$), and for the remaining points (n ranged between 4 and 7).

Rh*/s) a 14,957-Rh* flash was required to produce a 2.9-pA photoresponse (trace labeled F + BK, $S_F = 0.00019$ pA/Rh*). This corresponds to about a sixfold reduction in response sensitivity by the background light. In Fig. 6 b, the photoresponses shown in Fig. 6 a were normalized to the same peak amplitude. The time to peak for the flash response in the presence of background illumination was accelerated when

compared with the flash in darkness. In four OS-IS the average time to peak for the flash response in darkness was 1.11 ± 0.10 s whereas in the presence of bright background illumination the time to peak was reduced to 0.26 ± 0.04 s (mean ± SD). The time to peak for the flash response in darkness was not significantly different
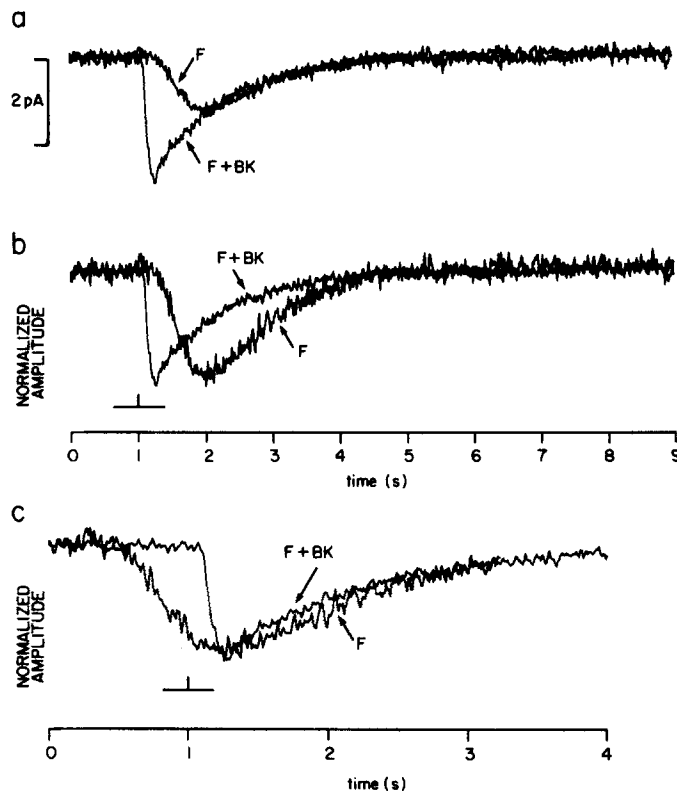

FIGURE 6. Kinetics of the flash response in the absence and presence of background illumination for 100 nM Ca^{2+}-Ringer's + A23187. In a, a 1,109-Rh* flash produced a 1.2-pA photoresponse (trace labeled F), giving a value for S_F^D of 0.0011 pA/Rh*. In the presence of bright background illumination (9,890 Rh*/s), a 14,957-Rh* flash was required to elicit a 2.9-pA response (trace labeled $F + BK$), yielding a value for S_F of 0.00019 pA/Rh*. In b, these photoresponses have been normalized to the same peak amplitude to demonstrate that t_p was significantly accelerated in the presence of background illumination. The t_p for trace F was 1.02 s, and for trace $F + BK$ t_p was 0.28 s, about a four-fold decrease in t_p. The time scale and flash marker are the same for both a and b. In c, the responses shown in b have been normalized to their t_p's; the rates of recovery for both F (34.2 au/s) and $F + BK$ (34.6 au/s) were the same. The time scale and flash marker are for trace $F + BK$; trace F has been shifted to the left by 0.78 s.

from that recorded in 1 mM Ca^{2+}, but the time to peak for the flash in background illumination was slightly faster than that obtained in both 1 mM and 10 nM Ca^{2+}-Ringer's. It is not clear why the time to peak for this condition is faster and may reflect deviations in the small sample size. When these flash responses were normal-

ized to the same point in time (the time to peak) as shown in Fig. 6 c, the recovery kinetics were the same for both photoresponses. For four OS-IS, the average rate of recovery for the flash in darkness was 34.1 ± 6.7 au/s, whereas in the presence of background illumination the recovery rate was 35.5 ± 3.8 au/s (mean ± SD). (For more details see the results presented for Fig. 10.) Greenblatt (1983) has also observed no acceleration of the recovery kinetics by background illumination in 100 nM Ca^{2+}-Ringer's. The above results for the response kinetics in 100 nM Ca^+ + A23187 are similar to those obtained in 10 nM Ca^{2+} + A23187. These results imply that when the Ca^{2+} concentration is held fixed near the physiological range, background illumination does not accelerate the recovery phase of the photoresponse. This is consistent with the idea that guanylate cyclase activity is responsive to changes in the intracellular Ca^{2+} level and controls the recovery phase in a regulatory feedback mechanism.

Light Adaptation at 300 nM Ca^{2+} + A23187

A Ca^{2+} concentration of 300 nM is slightly above the value of 220 nM reported for the normal intracellular free Ca^{2+} concentration in the dark (Ratto et al., 1988). The effects of background illumination of flash sensitivity and response kinetics at this Ca^{2+} are illustrated in Figs. 7 and 8.

At 300 nM Ca^{2+} + A23187, the dark sensitivity for the flash response was 0.03066 ± 0.02120 pA/Rh* and ranged in value from 0.06061 to 0.01058 pA/Rh* ($n = 17$). These results are illustrated in Fig. 7. This value for S_F^D was ~15-fold lower than that obtained for 1 mM Ca^{2+}-Ringer's. It is not clear why the dark sensitivity for 300 nM Ca^{2+} + A23187 should be so different from that in 1 mM Ca^{2+} where the presumed intracellular free Ca^{2+} is ~220 nM. One possible explanation may be that this Ca^{2+} concentration is sufficiently high to inhibit the efficacy of the enzymatic cascade controlling the magnitude of the light-regulated current (see Discussion). Should this be the case, then it would imply that the activity of the guanylate cyclase or the phosphodiesterase is quite sensitive to the Ca^{2+} concentraiton. The activity of guanylate cyclase appears to be strongly dependent on the Ca^{2+} concentration (Koch and Stryer, 1988); however, the activity of the phosphodiesterase is only moderately affected by changes in Ca^{2+} concentration (Robinson et al., 1980).

For I_{BK}'s > 227 Rh*/s, the values obtained for S_F could be fit by the Weber-Fechner relation using the parameters obtained for 1 mM Ca^{2+} (dashed line redrawn from Fig. 1). The overall fit was enhanced when the values obtained for S_F^D (0.03066 pA/Rh*) and I_o (36 Rh*/s, see Fig. 9 for the determination of I_o) were used in the Weber-Fechner relation; however, increasing I_o to 60 Rh*/s greatly improved the fit as the falloff of the curve was reduced for an I_o of 60 Rh*/s. Given the error for the mean values of S_F at 4 and 43 Rh*/s, the difference between 36 and 60 Rh*/s as the value of I_o is not likely to be significant. The Weber-Fechner relation is drawn as the solid line through the experimental points. The response sensitivity became inversely proportional to the background intensity for I_{BK}'s > 200–300 Rh*/s.

In 300 nM Ca^{2+}-Ringer's + A23187, both the time to peak and the recovery phase of the flash response were accelerated in the presence of background illumi-

nation. As shown in Fig. 8 a, a 106-Rh* flash elicited a 1.2 pA response (trace labeled F, $S_F^D = 0.0113$ pA/Rh*). In the presence of background illumination (9,890 Rh*/s) a 5,860-Rh* flash was required to elicit a response of ~2.2 pA in amplitude (trace labeled F + BK, $S_F = 0.00038$ pA/Rh*). This corresponds to a 30-fold desensitization of the flash response by background illumination. The initial on-kinetics of
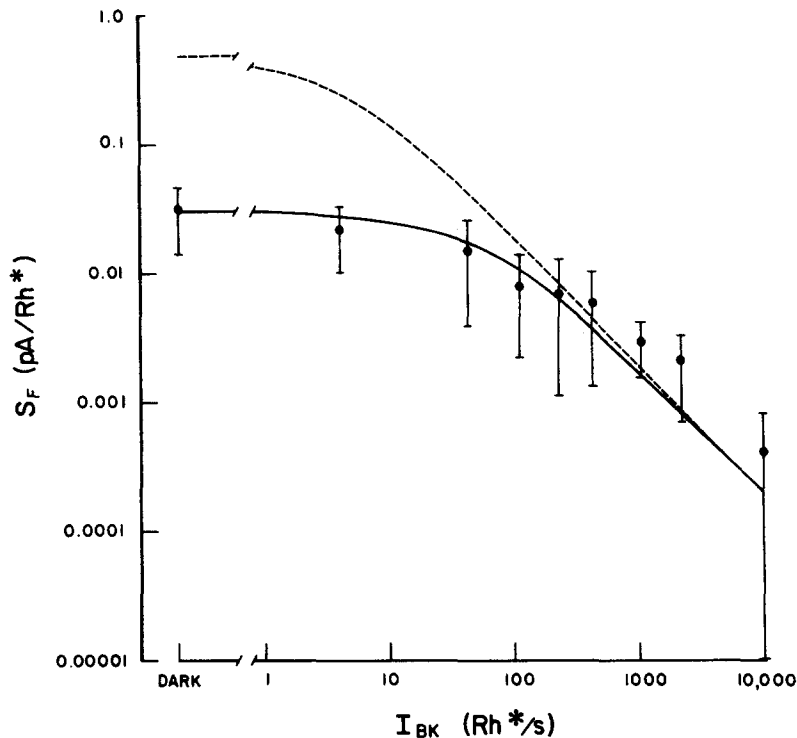

FIGURE 7. OS-IS flash sensitivity and its dependence on background intensity for 300 nM Ca^{2+}-Ringer's + A23187. For this Ca^{2+} concentration the value of S_F^D was reduced to 0.03066 pA/Rh*, a 15-fold reduction compared with S_F^D obtained for 1 mM Ca^{2+}. For the brightest background (9,890 Rh*/s), S_F was reduced to 0.0004 pA/Rh*, a light-induced desensitization of 77-fold. Using the values for S_F^D and I_0 for 1 mM Ca^{2+} the experimental points were fit reasonably well by the Weber-Fechner relation but only for I_{BK}'s > 200 Rh*/s. However, if the values for S_F^D and I_0 were changed to those determined experimentally 0.03066 pA/Rh* and 36 Rh*/s (see Fig. 9), respectively, the Weber-Fechner fit was improved. A much better fit was obtained with $I_0 = 60$ Rh*/s (see text). Each point represents the mean ± standard deviation; for the dark ($n = 17$), for I_{BK}'s between 4 and 2,164 Rh*/s (n ranged from 7 to 12), and for 9,890 Rh*/s ($n = 3$).

the photoresponse recorded in 300 nM Ca^{2+} + A23187 appear to be slower than that observed in 1 mM Ca^{2+}-Ringer's; compare the two traces labeled F in Figs. 2 and 8. This slower onset of current suppression was observed in 12 other OS-IS for the 300 nM Ca^{2+} + A23187 (data not shown), but the recovery kinetics under the two conditions were nearly identical (see discussion of Fig. 10). When the photore-
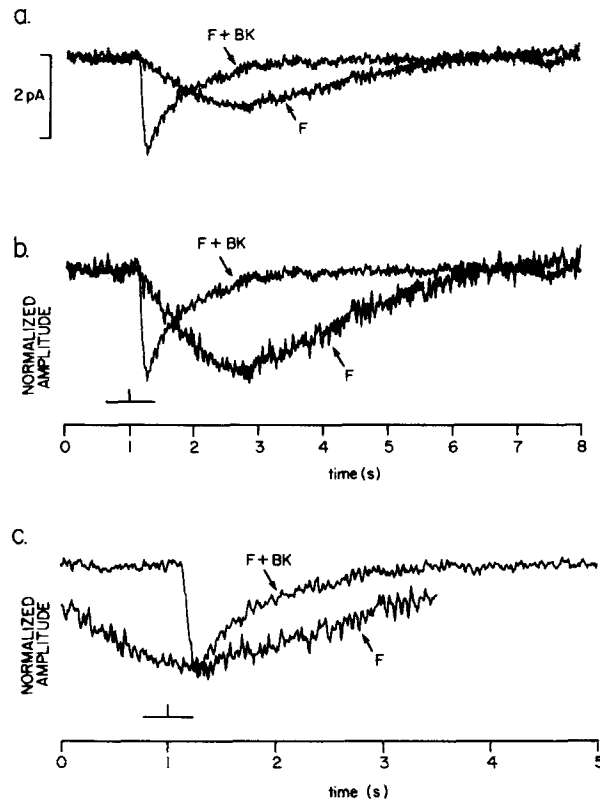
FIGURE 8.   Kinetics of the flash response in the absence and presence of background illumination for 300 nM Ca^{2+}-Ringer's + A23187. In a, a 106-Rh* flash elicited a photoresponse of 1.2 pA in amplitude (trace labeled F), yielding a value for S_F^D of 0.0113 pA/Rh*. In the presence of bright background illumination (9,890 Rh*/s) a 5,068-Rh* flash was necessary to produce a 2.2-pA response (trace labeled $F + BK$), corresponding to a vlaue for S_F of 0.00043 pA/Rh*. Note the very slow onset of current suppression; this phenomenon was observed in 12 other OS-IS. Each trace is the average of 10 responses. In b, these photoresponses have been normalized to the same peak amplitude and illustrate the large shift in t_p for the flash response with background illumination. For this cell the t_p in darkness (trace F) was 1.68 s, whereas in the presence of background illumination t_p (trace $F + BK$) was reduced to 0.28 s. The time scale and flash marker are the same for both a and b. In c, the responses shown in b were normalized to their t_p's. In the presence of background illumination, the rate of recovery for the flash response was accelerated when compared with the flash in darkness. The time scale and flash marker are for trace $F + BK$, trace F has been shifted to the left by 1.5 s.

sponses shown in Fig. 8 a were normalized to the same peak amplitude, the acceleration of the time to peak by background illumination is clearly demonstrated (Fig. 8 b). In two OS-IS, the average time to peak for the flash response in darkness was 1.44 s, whereas in the presence of bright background illumination the time to peak was reduced to 0.32 s; these values are similar to those obtained for 1 mM Ca^{2+}. Fig. 8c demonstrates that, when the photoresponses shown in Fig. 8 b were normalized

to the same point in time (the time to peak), the recovery for the flash in the presence of a background recovered much more quickly than did the flash in darkness. The average rate of recovery for the flash in darkness was 26.9 au/s whereas in the presence of background illumination the recovery rate was 46.5 au/s ($n = 2$). The rate of recovery for the flash in darkness was similar to that obtained for 1 mM Ca^{2+}, but the recovery rate in the presence of background illumination was faster than that observed in 1 mM Ca^{2+}. This difference may be partially accounted for by the fact that brighter background intensities were used in the experiments involving 300 nM Ca^{2+} + A23187. Similar results were observed in the recordings from five other OS-IS. These results in combination with those shown in Fig. 6 c suggest that the level of intracellular Ca^{2+} is critical in order for background illumination to
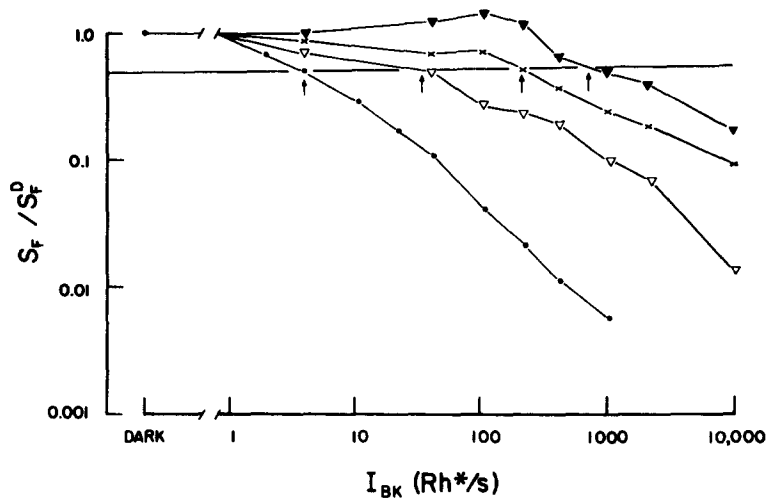

FIGURE 9.   Normalized flash sensitivities S_F/S_F^D and their dependence on I_{BK} and Ca^{2+} concentration. The flash sensitivities have been normalized to their respective S_F^D for each Ca^{2+} concentration. The mean values for each S_F have been used for the normalization. The line through the value 0.5 on the ordinate is the point where S_F^D is reduced by half; the corresponding intensity of I_{BK} gives the value of I_o for each Ca^{2+} concentration and is shown by the arrows. The different symbols represent 1 mM Ca^{2+}-Ringer's (●), 300 nM Ca^{2+}-Ringer's + A23187 (∇), 100 nM Ca^{2+}-Ringer's + A23187 (X), and 10 nM Ca^{2+}-Ringer's + A23187 (▼).

accelerate the recovery phase of the photoresponse; however, acceleration of the time to peak is consistently reduced by background illumination regardless of the Ca^{2+} concentration.

Sensitivity and Ca^{2+} Concentration

The effects of Ca^{2+} concentration on the relation between flash sensitivity S_F and background intensity are summarized in Fig. 9. The flash sensitivity for each Ca^{2+} concentration has been normalized to its respective value in the dark (S_F/S_F^D) and is plotted as a function of I_{BK} intensity. As the Ca^{2+} concentration was lowered or

raised from the normal level, the sensitivity curve became broader in shape and had a slower falloff as I_{BK} increased. The horizontal line is drawn for $S_F/S_F^D = 0.5$ and when extrapolated to the abscissa gives the value of I_o for each sensitivity curve in the different Ca^{2+} concentrations. The values for I_o are 4, or 36, 220, and 760 Rh^*/s in 1 mM Ca^{2+}-Ringer's, or 300, 100, and 10 nM Ca^{2+}-Ringer's + A23187, respectively. The values for I_o have been used to fit the experimental data shown in Figs. 1, 3, 5, and 7. These results indicate that as the intracellular Ca^{2+} concentration deviates, either higher or lower, from the normal level (1 mM external Ca^{2+} concentration) the response sensitivity in the dark is reduced. As the Ca^{2+} concentration is lowered below the normal value the flash sensitivity becomes less depen-
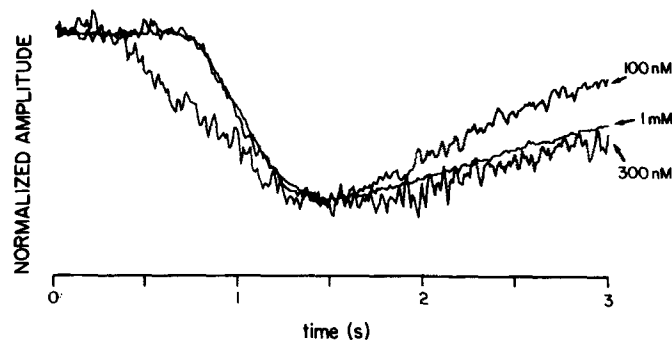

FIGURE 10.   Kinetics of the flash response in darkness and its dependence on Ca^{2+} concentration. The photoresponses illustrated represent the average of 5, 2, and 4 OS-IS for 1 mM Ca^{2+}-Ringer's, 300 nM, and 100 nM Ca^{2+}-Ringer's + A23187, respectively. The response for each individual OS-IS represents the average of between 8 and 14 single photoresponses; in a sense the traces shown are the grand means for their respective conditions. These photoresponses have been normalized to the same peak amplitude as well as to the same point in time, their t_p's. These results demonstrate that the rate of recovery for the flash response in 1 mM Ca^{2+} and 300 nM Ca^{2+} + A23187 were nearly the same. However, the recovery rate for the response in 100 nM Ca^{2+} + A23187 was much faster and was identical to the recovery rate observed for the flash response in bright background light for 1 mM Ca^{2+}. These results also illustrate that the initial light-induced suppression of the dark current was nearly the same for 1 mM Ca^{2+} and 100 nM Ca^{2+} + A23187 whereas it was much slower for the response in 300 nM Ca^{2+} + A23187.

dent on the background intensity so that the rod is less likely to show adaptive behavior.

Recovery Rates and Ca^{2+}

Fig. 10 illustrates the recovery rates for flash responses in darkness and their dependence upon Ca^{2+} concentration. In 1 mM Ca^{2+}-Ringer's and 300 nM Ca^{2+}-Ringer's + A23187, the rates of recovery for the flash responses were nearly identical while the response recovery in 100 nM Ca^{2+} + A23187 was significantly faster ($P < 0.01$). In 1 mM Ca^{2+}-Ringer's, the average rate of recovery for the flash response in darkness was 21.8 ± 3.5 au/s, whereas in the presence of bright background illumination the rate of recovery was 32.1 ± 5.7 au/s (mean ± SD, $n = 5$). For 300 nM Ca^{2+}-

Ringer's + A23187, the average recovery rate for the photoresponse in darkness was 26.9 au/s (n = 2) and is quite similar to that obtained for 1 mM Ca^{2+}. For 100 nM Ca^{2+}-Ringer's + A23187, the average rate of recovery for the flash response in darkness was 34.1 ± 6.7 au/s and in a bright background that rate was 35.5 ± 3.8 (mean ± SD, n = 4). In 100 nM Ca^{2+}-Ringer's + A23187 the rate of recovery for the flash response was the same whether or not the background was present. Also, the recovery rate in darkness was the same as that for the flash + background illumination in 1 mM Ca^{2+}-Ringer's (both are significant at $P < 0.01$). These results are consistent with the notion that in 100 nM Ca^{2+}-Ringer's + A23187 the mechanism(s) controlling the recovery of the flash response in darkness is (are) already accelerated, i.e., light-adapted. This suggests that the concentration of intracellular Ca^{2+} may be an important control factor in light adaptation that determines the recovery rate of the flash response (Torre et al., 1986).

Fig. 10 also demonstrates that the initial on-kinetics for the flash responses in darkness may be influenced by the intracellular Ca^{2+} content. When these flash responses were all normalized to their times to peak, the initial light-induced suppression of the dark current (the downward deflection of the current trace) was the same for both the 1 mM Ca^{2+}-Ringer's and the 100 nM Ca^{2+}-Ringer's + A23187, but the light-induced suppression was slower in 300 nM Ca^{2+}-Ringer's + A23187. The current suppression in 1 mM Ca^{2+} and 100 nM Ca^{2+} + A23187 may be the same because these two conditions are in the normal operating range for intracellular Ca^{2+}, but for the 300 nM Ca^{2+} + A23187 condition one would expect the intracellular Ca^{2+} level to be higher than normal; this may reflect the capacity of excess Ca^{2+} to influence or modulate the processes, i.e., the enzymatic cascade, controlling the closure of the light-regulated channels. (There are several indications that intracellular Ca^{2+} has been elevated. First, S_F^D is ~12-fold higher than that for 100 nM Ca^{2+}, suggesting that the intracellular Ca^{2+} level is closer to 220 nM. Secondly, the dependence of S_F on I_{BK} is fit fairly well by the Weber-Fechner relation using those parameters obtained for 1 mM Ca^{2+}. Thirdly, the rate of recovery for the flash response in darkness is nearly identical to that for 1 mM Ca^{2+} [refer to Fig. 10]. Fourthly, the initial on-kinetics of the photoresponse in darkness was slower than the response in either 1 mM Ca^{2+}-Ringer's or 100 nM Ca^{2+}-Ringer's + A23187, suggesting an altered level of Ca^{2+}.)

DISCUSSION

This paper describes several properties of light adaptation in rod outer segments that retain the ellipsoid portion of their inner segments. These OS-IS are shown to light-adapt and demonstrate parameters of adaptation that are similar to those described by others for different retinal rod preparations (Kleinschmidt, 1973; Fain, 1976; Baylor et al., 1980). Since OS-IS can be isolated and purified in large numbers, they can serve as a useful preparation in which to study the electrophysiological and biochemical correlates of light adaptation.

A23187 and the Intracellular Ca^{2+} Content

An advantage of isolated and purified OS-IS as opposed to rods that remain attached to the retina is that the intracellular Ca^{2+} concentration can be perturbed

by the calcium ionophore A23187 (cf. Hagins and Yoshikami, 1974; Nicol et al., 1987). We have previously shown that the endogenous Ca^{2+} transport mechanisms in the rod membrane can be shunted effectively by A23187, thereby preventing or greatly diminishing any light-induced changes in Ca^{2+} concentration (Nicol et al., 1987). In rod photoreceptors the highest flux of intracellular Ca^{2+} is presumed to occur in the dark where the influx of Ca^{2+} through the light-regulated channels is balanced by the efflux of Ca^{2+} through the Na/Ca exchange mechanism (MacLeish et al., 1984; Yau and Nakatani, 1984; Hodgkin et al., 1985). If the ionophore can shunt the endogenous Ca^{2+} transport mechanisms in the rod membrane then one might expect the addition of A23187 in 1 mM Ca^{2+}-Ringer's to fill the rod with excess Ca^{2+} and lead to a rapid and reversible suppression of the light-regulated current. This in fact is what is observed (see Figs. 8 and 9 in Nicol et al., 1987). Calculations suggest that the maximum flux of Ca^{2+} through the light-regulated channel (for a dark current of 20 pA) is ~100-fold less than the maximum flux capacity of the ionophore, whereas the maximum efflux of Ca^{2+} through the Na/Ca exchanger is ~10-fold less (see Nicol et al., 1987). It has been demonstrated that A23187 can effectively manipulate and control the intracellular Ca^{2+} content of bovine rod outer segments (see Kaupp et al., 1979; Fig. 2 in Schnetkamp, 1985; Figs. 6 and 8 in Schnetkamp, 1986). The ability of calcium ionophores to manipulate intracellular Ca^{2+} has been demonstrated in other cell types by monitoring the concentration of intracellular Ca^{2+} with the Ca^{2+} indicators, fura 2 or quin 2 (Rink and Sage, 1987; Simpson and White, 1988). However, the control of intracellular Ca^{2+} by ionophores in rod photoreceptors has not been established experimentally with direct measurements of intracellular Ca^{2+} levels utilizing optical indicators of Ca^{2+} concentration.

Does the Concentration of Ca^{2+} Set the Flash Sensitivity in Darkness and during Light Adaptation?

The results presented in this paper are consistent with the idea that the intracellular concentration of Ca^{2+} may be directly involved in setting or determining the magnitude of the flash sensitivity in darkness. As the Ca^{2+} concentration is lowered from or raised above the normal dark level the response sensitivity falls off dramatically. Over the presumed narrow window of light-induced changes in Ca^{2+} concentration that are of physiological importance, the flash sensitivity shows a strong dependence on the Ca^{2+} concentration. Under normal conditions one measures an S_F^D of 0.481 pA/Rh*, where we assume an intracellular Ca^{2+} concentration of ~220 nM. As the Ca^{2+} content is raised slightly to 300 nM, S_F^D falls by 15-fold to 0.03066 pA/Rh*. If the Ca^{2+} concentration is lowered to 100 nM, slightly below the lower limit of this Ca^{2+} window, S_F^D is reduced by 190-fold. This sort of behavior has been observed by others (Bastian and Fain, 1979; 1982; Sather et al., 1988). These results are consistent with the idea that there is an optimal concentration of free Ca^{2+} that sets the peak sensitivity of the rod's response to illumination. An optimal window of Ca^{2+} concentrations (140–370 nM) has also been observed in the glucose uptake response of rat adipocytes to insulin (Draznin et al., 1987).

In the presence of bright illumination the intracellular Ca^{2+} concentration falls from a value of ~220 to ~140 nM (Ratto et al., 1988). If the Ca^{2+} concentration is important in setting the response sensitivity of the rod then one might expect that

under normal conditions the value of S_F during bright background illumination should be equivalent to the value of S_F^D at that Ca^{2+} concentration attained during illumination, i.e., ~140 nM Ca^{2+}. This contention is strongly supported by the fact that under normal conditions the value of S_F in the presence of bright background illumination (0.0027 pA/Rh*) is nearly identical to the value of S_F^D (0.00251 pA/Rh*) in 100 nM Ca^{2+}-Ringer's + A23187. Thus, the extent of desensitization of the flash response produced by background illumination is equivalent to that caused by lowering the Ca^{2+} concentration. These results also argue for the effectiveness of the calcium ionophore in manipulating the intracellular Ca^{2+} concentration of the OS-IS.

A further observation in these current experiments is that background illumination can cause light adaptation when the OS-IS is depleted of >90% of its intracellular Ca^{2+} (10 nM Ca^{2+} + A23187). At 10 and 100 nM Ca^{2+} + A23187 the light-induced desensitization caused 6- and 12-fold reductions in S_F, respectively. This suggests that some light adaptation can occur in the absence of changes in intracellular Ca^{2+} concentration. It is possible that this desensitization occurring in low Ca^{2+} may result from saturation of the light-activated phosphodiesterase. In the presence of bright background illumination a sufficient number of phosphodiesterase molecules could already be in the active state, so that brighter flashes are now required for any additional activation. The dependence of S_F on the intensity of background illumination can be described by the Weber-Fechner relation for most of the Ca^{2+} concentrations examined, with the parameters S_F^D and I_o being adjusted from those values observed in 1 mM Ca^{2+}-Ringer's. Lowering the intracellular Ca^{2+} concentration appears to have the effect of compressing the sensitivity curves through the reduction of the sensitivity of the flash response in the dark as well as reducing the efficacy of background illumination in adapting the rod (shifting I_o to higher intensities).

Supralinearity and Response Desensitization

When rods are incubated in low Ca^{2+}-Ringer's, the flash intensity–response relation for dim stimuli becomes supralinear, i.e., a twofold increase in the flash intensity produces a greater than twofold increase in the response amplitude (Yau et al., 1981). An electrical model has been proposed to explain this behavior (Owen and Torre, 1983). Under normal conditions, the magnitude of the photoresponse is determined by the ratio of the outer and inner segment conductances, and the conductance of the inner segment exceeds that of the outer segment. However, in low Ca^{2+} the conductance of the outer segment is enhanced greatly, in effect shunting the inner segment conductance. These electrical effects in low Ca^{2+} will produce an apparent desensitization of the photoresponse because brighter lights are now required to overcome this shunting effect, subsequently reducing the conductance of the outer segment. Thus it might be possible to observe desensitization of the photoresponse due to these electrical factors rather than light-induced changes in the transduction cascade. This model potentially explains why the sensitivity curves we observed in 10 and 100 nM Ca^{2+}-Ringer's + A23187 are relatively flat until attaining bright enough background levels that are sufficient to reduce the outer segment conductance.

This electrical model of supralinearity, however, does not account fully for the

desensitization of the photoresponse observed in low Ca^{2+} (see also Matthews, 1985). First, the large increase in the dark current upon lowering the Ca^{2+} occurs within seconds (see Fig. 3 in Yau et al., 1981; Fig. 8 in Baylor and Nunn, 1986) while response desensitization may take minutes to reach a steady-state value (see Fig. 3 in Bastian and Fain, 1982). Secondly, background illumination that has little, if any, effect on the membrane voltage or current can potentiate the amplitude of the flash response when compared with an identical flash in darkness; this potentiation is not observed with the onset of the background and only develops slowly (see Fig. 9 in Bastian and Fain, 1982; Fig. 4 in Matthews, 1985). Thirdly, we find that when a series of flashes are presented the response amplitude is progressively enhanced even though the membrane current returns to its dark baseline between flashes, and thus the previous flash can facilitate the effects of the next flash with no apparent change in the membrane current (i.e., a lowering of the outer segment conductance). Similar results have been observed by Greenblatt (1983) and Matthews (1985). These findings are consistent with the notion that the decreased flash sensitivity occurring in lowered concentrations of Ca^{2+} reflects at least in part a direct role of intracellular Ca^{2+} in the regulation of response sensitivity, rather than simply increasing the outer segment conductance.

Ca^{2+} and the Kinetics of the Flash Response

The light-adapted photoresponse can be characterized by two prominent changes in the kinetics of the flash response: (a) an acceleration of the time to peak and (b) a more rapid recovery to the baseline. In these experiments background illumination caused acceleration of the time to peak of the flash response by about threefold, independent of the concentration of intracellular Ca^{2+}. This finding is consistent with the notion that the control mechanisms of the transduction cascade responsible for this aspect of the light-adapted flash response are not influenced by Ca^{2+} and persist in the absence of changes in the intracellular concentration of Ca^{2+}. The mechanisms controlling the rate of current suppression, which in effect set the time to peak, very likely reside in the enzymatic transduction cascade. As the photoresponse is believed to be generated by a light-induced decrease in the intracellular concentration of cyclic GMP, a good candidate for control of the initial kinetics of the photoresponse would be the rate at which phosphodiesterase is activated by the G protein, transducin. In the presence of background illumination some steady level of transducin activation will be achieved. If the rate of phosphodiesterase activation is determined by the product of active transducin and inactive phosphodiesterase concentrations, then the rate at which additional phosphodiesterase molecules will be activated might increase with increasing background illumination if the pool of activated transducin increases more rapidly than the depletion of phosphodiesterase that can be potentially activated by light. Thus the initial kinetics of the photoresponse elicited by a flash in the presence of background illumination might be enhanced as the background intensity increases, potentially accounting for the Ca^{2+} independence of the decreased time to peak with background lights.

A comparison of the results shown in Figs. 6 and 8 suggests that a critical level of Ca^{2+} may be required for acceleration of the response recovery by background illumination (see discussion below). These results, in combination with those presented

for the recovery rates of flashes in darkness (Fig. 10), indicate that in the low Ca^{2+} conditions the response recovery is faster even in the absence of background illumination. This implies that the pathway or feedback mechanism regulating the return of the membrane current to its baseline is sensitive to the level of intracellular Ca^{2+} and that under these low-Ca^{2+} conditions background illumination does not provide any further regulation or enhancement of this pathway.

Site of Ca^{2+} Action

The prevailing model for light adaptation is that a light-induced decrease in Ca^{2+} acts on the enzymes controlling cyclic GMP levels and therefore is involved in the regulation of sensitivity and response kinetics (Bownds, 1980; Yau and Nakatani, 1985; Torre et al., 1986). If, for example, the lowering of Ca^{2+} known to occur upon illumination causes either a stimulation of the guanylate cyclase (synthetic pathway) or an inhibition of the light-activated phosphodiesterase (hydrolytic pathway), this might desensitize the cell to subsequent illumination. This would also facilitate the return of dark cyclic GMP levels and consequently recovery of the conductance.

An important question is whether the effects of Ca^{2+} on the enzymes of the cyclic GMP cascade occur between 220 and 140 nM Ca^{2+}, the range over which Ca^{2+} is decreased by illumination (Ratto et al., 1988). In this narrow window, none of the published studies report significant effects of Ca^{2+} on the cyclic GMP content of retinal rods (Cohen et al., 1978; Woodruff and Fain, 1982), the guanylate cyclase (Lolley and Racz, 1982; Pepe et al., 1986), or the phosphodiesterase (Robinson et al., 1980; Kawamura and Bownds, 1981); however, Koch and Stryer (1988) have reported recently that the activity of guanylate cyclase from bovine ROS is greatly enhanced when the Ca^{2+} concentration is lowered from 200 to 50 nM, the window of physiological importance; the change was half-maximal at 90 nM. Whether this effect plays a role in adaptation is open to question since bovine rods, unlike amphibians rods, show little change in response kinetics with background illumination and exhibit saturation of the photoresponse without adaptation (Baylor et al., 1984).

Our results are consistent with the idea that a decrease in Ca^{2+} concentration may lead to an activation of guanylate cyclase, which accelerates the recovery of the flash response (cf. Torre et al. 1986; Koch and Stryer, 1988). If in the low-Ca^{2+} + A23187 conditions, the cyclase is already activated, one would not expect subsequent background illumination to cause any further reduction in the Ca^{2+} level or additional cyclase activity. Recovery of the flash response in darkness for 100 nM Ca^{2+} + A23187 thus should be faster than that in 1 mM Ca^{2+} or 300 nM Ca^{2+} + A23187, and this is what is observed. However, if acceleration of the response recovery during background illumination requires that a fall in Ca^{2+} activates guanylate cyclase, then why does background illumination accelerate the response recovery in 300 nM Ca^{2+}-Ringer's + A23187? Under these conditions the intracellular Ca^{2+} level should be slightly above the normal value of 220 nM in darkness (see Results). The presence of the ionophore should prevent or greatly diminish any light-induced changes in the intracellular Ca^{2+} level, the cyclase should remain at its reduced level of activity, and background illumination should not accelerate the

recovery kinetics of the flash response. Fig. 8 demonstrates, however, that background illumination does accelerate the recovery of the flash response under these conditions.

This observation has several potential explanations. One is that the 300 nM concentration of Ca^{2+} inhibits guanylate cyclase and thereby lowers the concentration of cyclic GMP and closes a significant fraction of the light-sensitive channels. Desensitization results, because the number of channels that can be closed by light is reduced. This is consistent with the decreased value of S_F observed in 300 nM Ca^{2+} + A23187, and also the decrease in the maximum amplitude of the photoresponse in 300 nM Ca^{2+} + A23187 (10.3 ± 1.9 pA, $n = 10$) as compared with 1 mM Ca^{2+}-Ringer's (17.8 ± 2.0 pA, $n = 5$; see Table I). Further reduction of cyclic GMP concentration by background illumination might have the effect of reducing the amount of time required for its replenishment after a flash, thus accelerating the recovery of the photoresponse. Such a model is difficult to evaluate because the respective roles of cyclase and phosphodiesterase in regulating the recovery from a flash are not known, and possible light activation of the cyclase (Pepe et al., 1986) has not been considered. A quantitative treatment of the role of increased cyclase activity on the waveform of the flash response is given in Bownds and Thomson (1988).

According to a scheme in which the activity of guanylate cyclase is regulated by intracellular Ca^{2+}, one might predict that a sufficiently high Ca^{2+} concentration would inhibit the cyclase enough to deplete cyclic GMP levels and abolish the light response. This is what is observed when A23187 is added to a suspension of OS-IS in 1 mM Ca^{2+}-Ringer's; the OS-IS is loaded with sufficient Ca^{2+} to suppress the light response. However, under these conditions of elevated intracellular Ca^{2+} the photoresponse can be restored, without lowering the intracellular Ca^{2+}, by the addition of the phosphodiesterase inhibitor IBMX (see Fig. 9 in Nicol et al., 1987). What then permits the recovery of the photoresponse? Perhaps the hydrolytic pathway is now sufficiently suppressed to allow some restoration of cyclic GMP levels. This would imply that the guanylate cyclase activity is only partially suppressed by elevated intracellular Ca^{2+} and that the relation between Ca^{2+} concentration and cyclase activity may not be simple. Recent results of Sather et al. (1988) also question the simple relation between Ca^{2+} concentration and guanylate cyclase activity as the control mechanism for recovery of the light-adapted flash response.

In summary, the results presented above suggest that the Ca^{2+} concentration may be an important regulator in setting the response sensitivity of the rod and that a critical level of Ca^{2+} may be required for the mechanisms of light adaptation to modulate the kinetics of the photoresponse. Background illumination does desensitize the photoresponse in rods depleted of Ca^{2+}; this is consistent with the notion that there may be parallel pathways that control the adaptation process in rod photoreceptors. The results presented in this study would seem to exclude the notion of a simple relation between the intracellular concentration of Ca^{2+} and the activity of guanylate cyclase to regulate the recovery of the membrane current in the presence of background illumination. Further biochemical studies will be required to assess the relative roles of Ca^{2+} and light in that they influence the activities of the guanylate cyclase and the phosphodiesterase.

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