Cellular Mechanisms That Underlie Bleaching and Background Adaptation

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ABSTRACT Experiments were performed on rod photoreceptors isolated from the eye of the larval tiger salamander to determine if the same or different mechanisms underlie the desensitization produced by dim background light (background adaptation) and that which persists in the steady state in darkness after a significant fraction of the photopigment is bleached (bleaching adaptation). We have examined adaptational effects after light that bleached between ~50% and 95% of the photopigment under conditions which preclude pigment regeneration. The steady-state desensitization, far greater than that predicted by quantum-catch loss, is relieved upon regeneration of the visual pigment with 11-cis retinal. We measured the spread of desensitization along the long axis of the rod after a local bright bleach at one end by comparing responses to dim local test flashes elicited in different regions of the outer segment, before and after bleaching. The space constant for this spread was <2.5 μm. We have previously measured the space constant for the longitudinal spread of desensitization during a local dim background in Ambystoma rods to be 7 μm. This is similar to a space constant of 6 μm measured under similar conditions in Bufo rods by Lamb et al. (1981, J. Physiol. 319:463-496). If calcium carries the signal for background desensitization, this difference in space constant for background and bleaching adaptation precludes it as the messenger for the steady component of bleaching adaptation. Experiments with isobutylmethyl xanthine (IBMX) also indicate that Ca²⁺ as well as c-GMP are unlikely regulators of bleaching desensitization, since elevation of cytosolic levels of both of these internal messengers by IBMX has little effect on sensitivity in bleach-adapted cells. All of our findings are consistent with the notion that bleaching adaptation is not mediated by a freely diffusible cytoplasmic messenger.

INTRODUCTION

Adaptation in the visual system refers to changes in sensitivity which occur during or after exposure to light. It is this property that allows us to detect changes in light intensity over a range of ambient light intensity that exceeds a billionfold (see Lamb, 1986 for a review). Both physiological and biochemical experiments have established...
that a major component of the adaptation process occurs within the outer segments of retinal rods and cones.

Rushton (1965) was the first to describe two fundamentally different types of light adaptation. The first, called background or field adaptation, occurs in the presence of a steady light and is characterized by an elevation in threshold as well as improved temporal resolution. These adaptational effects occur at light intensities for which an insignificant fraction of the total pigment content of the outer segment is bleached. For example, in toad rods, threshold (the reciprocal of sensitivity) is raised twofold by a background light which bleaches only about four rhodopsin molecules per rod per second (Bastian and Fain, 1979). These workers concluded that since a toad rod contains over 2,000 rhodopsin-containing disks, this implies that absorption of a photon by a rhodopsin molecule in one disk affects the response to bleaching of other rhodopsin molecules in that disk and in other nearby disks. The spatial extent of spread of background adaptation from the site of photon absorption along the rod outer segment has been estimated to be between 5 and 20 μm (Hemila and Reuter, 1981; Lamb et al., 1981; Cornwall and Pan, 1985). Based on this as well as other experimental evidence it is generally accepted that for dim background lights, adaptation is mediated by a change in the concentration of a substance in the cytosol which is free to diffuse within the rod outer segment. Recent experiments on isolated rod and cone cells from the frog have suggested that background adaptation is mediated by a decrease in cytosolic free Ca^{2+} (C_{ai}^{2+}) (Cervetto et al., 1985; Matthews et al., 1988; Nakatani and Yau, 1988; Fain et al., 1989).

A second form of adaptation, termed bleaching or pigment adaptation occurs when the visual cells are exposed to higher intensities of light which bleach a significant fraction of the visual pigment (Rushton, 1965). Bleaching adaptation is greatly in excess of that expected due to the loss of quantum catch resulting from the reduction in rhodopsin concentration (Dowling, 1960; Rushton, 1961). Studies of bleaching adaptation in isolated retina or in solitary rods deprived of contact with the pigment epithelium, have shown that in darkness after the bleach, after an initial period of total insensitivity, partial recovery of sensitivity occurs over 30–60 min. The final steady-state level of desensitization that is achieved persists indefinitely in the absence of pigment regeneration (Weinstein et al., 1967; Grabowski and Pak, 1975; Brin and Ripps, 1977; Pepperberg et al., 1978; Leibovic et al., 1987). The total recovery of sensitivity requires the regeneration of rhodopsin through the application of exogenous 11-cis retinal (Pepperberg et al., 1978; Albani et al., 1980; Perlman et al., 1982). The steady-state component of bleaching adaptation has been shown to be spatially localized to the bleached region of the rod (Cornwall et al., 1983). However, it remains unclear whether the extent of the spread of background adaptation and the steady-state component of bleaching adaptation are similar or different.

The cellular mechanisms that are the basis of bleaching adaptation are poorly understood. It was first suggested by the work of Stiles and Crawford (1932) and Crawford (1937, 1947) that the adapting effects of bleaching light are equivalent to those produced by a steady background light at an intensity called the “equivalent background brightness.” Barlow (1956, 1964) subsequently proposed that bleaching
leads to increased photoreceptor noise similar to that produced by light. After the
demonstration by Baylor et al. (1979a) of discrete electrical responses in isolated
rods resulting from absorption of single photons, and the observation by Lamb
(1980) that discrete events similar to those elicited by light could be observed in
isolated rods in darkness after bright light, Lamb (1986) proposed a model which
asserts that the aftereffects of bright light are produced by photon-like events
identical to those produced by light but which "occur as a result of inactivated
photopigment molecules spontaneously reverting to active forms." It follows from
this hypothesis that bleaching adaptation should be mediated by the same diffusible
substance that mediates background adaptation.

The equivalent background hypothesis does not hold under all circumstances,
however, and this is especially true for temporal process in the retina (see Barlow,
1972). There also appear to be differences in the way in which background
adaptation (Hemila and Reuter, 1981; Lamb et al., 1981; Cornwall and Pan, 1985)
and bleaching adaptation (Cornwall et al., 1983) spread along the rod. Based on the
latter observations we have suggested that bleaching and background adaptation are
not equivalent, and that bleaching adaptation is not mediated by a freely diffusible
cytoplasmic messenger as background adaptation is presumed to be (Cornwall et al.,
1983). The studies reported here were undertaken to further examine bleaching and
background adaptation and their spread in isolated rod photoreceptors and to see if
underlying mechanisms are the same or different.

In this paper we describe three critical tests of the hypothesis that the steady-state
desensitizing effects of bleaching and background light are equivalent in solitary
rods. The first test is based on the observation that both bleaching and background
light reduce the maximum amplitude of the light response and produce shifts of the
response-intensity relation to higher intensity. The equivalence hypothesis predicts
that one should be able to find intensities of background and bleaching light which
produce equivalent steady-state changes in these parameters. The second experiment
uses focal light stimuli and in the steady-state compares the spatial spread of
bleaching and background adaptation along the longitudinal axis of a single rod
outer segment to see if they are equivalent. If the steady-state desensitizing effect of
bleaching and background illumination both result from a change in the cytosolic
concentration of the same substance, these effects should spread similarly along the
outer segments. The results of both of these tests suggest that the mechanisms that
underlie bleaching and background adaptation must be different. Third, we tested to
see if changes in $\text{Ca}^{2+}$ alone are sufficient to explain changes in sensitivity after
bleaching as has been suggested for background adaptation. These studies were
done by examining the effects on bleach-adapted cells of treatment with the
phosphodiesterase inhibitor isobutylmethyl xanthine (IBMX), an agent that not only
elevates the c-GMP level of rods (Cohen et al., 1978; Woodruff and Fain, 1982), but
also elevates $\text{Ca}^{2+}$ (McNaughton et al., 1986). In the latter two tests we failed to find
any evidence that changes in $\text{Ca}^{2+}$ underlie bleaching desensitization. All our
findings are consistent with our original suggestion that bleaching adaptation is not
mediated by a freely diffusible cytoplasmic messenger.
METHODS

Preparation and Solutions
All experiments were performed on the larval tiger salamander, *Ambystoma tigrinum* obtained from a commercial supplier (Lowrance Waterdog Farms, Tulsa, OK). Animals were kept in aquarium tanks at 7–9°C to retard transition from the larval to the adult form. They were fed once a week with goldfish obtained from a local pet store. Between 24 and 48 h before use, animals were warmed to room temperature and dark adapted for 12 h in a light-tight oxygenated container. All subsequent operations were carried out under infrared illumination with the aid of infrared image converters (FJW Industries, Mt. Prospect, IL). At the beginning of the experiment, an animal was decapitated and pithed. Both eyes were then removed and hemisected. The retinas were removed and placed in a saline solution that contained 108 mM NaCl, 2.4 mM KCl, 1.6 mM CaCl₂, 1.2 mM MgCl₂, 0.5 mM NaHCO₃, 15 mM dextrose, and 5 mM HEPES buffer, pH 7.8.

Light Stimulation
After removal from the pigment epithelium, the retinal tissue was chopped into small pieces with fine scissors and triturated by repeated passage of the solution containing bits of retina in and out of a Pasteur pipette, the tip of which was fire-polished to a diameter of ~0.5 mm. A few drops of the resulting suspension of retinal fragments which contained intact isolated rods and cones was placed between a glass coverslip located on the stage of a Zeiss inverted microscope (Invertoscope D; Carl Zeiss, Oberkochen, FRG) and the glass cone of a Nikon 10× water immersion objective (N.A. = 0.22; Nikon Inc., Garden City, NY) mounted on the condenser assembly. The microscope was fitted with an infrared television monitoring system that allowed continuous visualization of cells during subsequent manipulations. Red rods which were identified by their characteristic morphology and sensitivity to green light were drawn, inner segment first, onto recording pipettes which had been fire-polished to an inner tip diameter slightly smaller than the maximum diameter of the inner segment of the cell. The micropipette was connected via a silver/silver-chloride junction to the input of a current-to-voltage converter similar to that described by Baylor et al. (1979a, b).

Light stimuli were provided by a dual beam optical stimulator. Light from a single 120-W tungsten-halogen source was directed through two separate optical paths, each composed of a condenser lens, shutter, calibrated neutral density filters, narrow band interference filters, and a field lens. The separate beams were then combined and projected through a 10× eyepiece and the 10× objective which had been placed in the condenser holder of the Zeiss inverted microscope. The transmission characteristics of the neutral density filters and the interference filters were determined between 300 and 700 nm using a recording spectrophotometer. In addition, since some of the sensitivity measurements required that certain of the neutral density filters be used in series, the total density of these filters in combination was determined in situ by calculating optical density from measurements of light transmission. Transmission measurements were made at each wavelength using a photomultiplier tube placed in the light beam at the output of the photostimulator. Light stimulation of discrete regions of the outer segments was achieved by focusing narrow slits of light (520 or 560 nm, bandwidth at half intensity = 10 nm) on different parts of the cells. These slits were produced by the demagnified image of narrow slit apertures placed adjacent to the field lens in the optical stimulator. The slits were constructed by gluing razor blades to a small frame so that the space between the edges formed a slit aperture which was demagnified to be nominally 0.75 μm wide (test beam) or 5 μm wide (bleaching beam) at the plane of the preparation. One slit was mounted in each of the beams of the optical stimulator on micropositioners which allowed
independent positioning of each local stimulus to within 1 μm resolution at the plane of the preparation. Tests of the spatial localization of these discrete stimuli are illustrated in Fig. 1, a and b. In these tests a photomultiplier tube was substituted for one of the oculars of the inverted microscope and a discrete bar of light was focused at the plane of the preparation. This is illustrated by the hatched bar in Fig. 1 a, the left edge of which is located at relative position 0 on the abscissa. Relative output of the photomultiplier tube was monitored as a

\[ Z = \frac{I}{I_0} \]

\[ R = \frac{1}{2} \ln \left( \frac{I}{I_0} \right) \]

**Figure 1.** Spatial resolution of focal light stimuli. (A and B) Results of two procedures to estimate the degree of stimulus spread associated with focal light stimuli. (A) Relative change in light intensity (ordinate) measured as photocurrent from a photomultiplier tube placed in place of one ocular of the inverted microscope as the edge of a razor blade was moved across the stimulus field at the plane of the preparation from a position 8 μm to the left of the focused bar (total occlusion) to a position 4 μm to the right (unoccluded). Relative intensity changed 26-fold over ~4 μm (B) Second method for evaluating spatial spread of the focused stimulus. A second aperture (1-μm wide) formed by the approach of the edges of two razor blades under micromanipulator control was placed at the plane of the preparation. Relative intensity of light measured by the photomultiplier tube changed 12-fold as the image of the focused slit was moved from right to left across the stimulus field. See text for additional details.

razor blade attached to a micromanipulator was moved from right to left in 0.5-μm intervals across the plane of the preparation. As this test illustrates, a 26-fold change in light intensity was measured as the edge of the razor blade was moved from a position 2 μm to the left of the edge of the light bar to 1 μm to the right. The stimulus spread of a narrow slit was estimated in a different way (Fig. 1 b). In this case, a 1-μm wide aperture was placed at the plane of the preparation by approaching two razor blade edges under micromanipulator control. The
position of this aperture is illustrated by the hatched rectangle at 0 on the abscissa. Then the image of the 0.7-μm-wide stimulus slit was also focused at the plane of the preparation and positioned at 0.5-μm intervals between 8 μm to the left and 8 μm to the right of the aperture. In this case, the measured light intensity declined about 12-fold as the focused slit was moved 3 μm from the nominal center of the 1-μm aperture. The stimulus quality was roughly similar to that used by Lamb et al. (1981) who measured their slit width at half the maximum intensity to be 2.3 μm.

Preparation of 11-cis Retinal-containing Vesicles

The 11-cis retinal used in these experiments was the generous gift of Dr. Peter Sorter, Hoffman-LaRoche (Nutley, NJ). The procedures used for preparation of lipid vesicles was similar to those described previously by Yoshikami and Noll (1978) and Perlman et al. (1982). All manipulations were carried out in dim red light or under infrared illumination with the aid of infrared image converters. Briefly, ~10 mg of crystalline 11-cis retinal was weighed into a small vial. An appropriate volume of absolute ethanol was added to make a solution containing 10 mg/ml. A 100-μl aliquot from this stock solution was then placed in a scintillation vial, evaporated to dryness, and stored tightly capped under dry nitrogen at −80°C until use. Just before the time of an experiment, 25 mg of 1-α-phosphatidylycholine dissolved in chloroform/methanol solution (type V-E; Sigma Chemical Co., St. Louis, MO) was added to a scintillation vial containing retinoid and the contents were evaporated to dryness under a gentle stream of dry nitrogen. To be sure that all traces of solvent were removed, the open vial was placed in a lyophylizer and further evaporated under vacuum for an additional 30 min. 15 ml of saline solution which had been bubbled for 30 min with water-saturated, oxygen-free nitrogen was then added to the vial containing the dried retinoid and the contents were subjected to intermittent sonication (30 s on, 30 s off) using a Vibracell sonicator (Sonics and Materials, Inc., Danbury, CT) equipped with a 1-cm tip. The temperature of the sonicate solution was maintained below 5°C, and a moist stream of nitrogen was blown over the surface of the vial throughout this procedure. After 10 min of sonication, the sample was removed and stored in a refrigerator until use (~2 h).

The concentration of retinoid in the bulk vesicle suspension solution was calculated to be 0.23 mM. This value was verified as follows. A 100-μl sample of the stock ethanolic retinoid solution was diluted 1,000 times and its absorbance was measured in a recording spectrophotometer. From the known concentration and measured peak optical density we calculated the molar extinction coefficient of 11-cis retinal at 375 nm to be 24,000 cm⁻¹ M⁻¹, close to the 25,000 cm⁻¹ M⁻¹ value published by Brown and Wald (1956). A 1-ml sample of the vesicle-containing solution was then diluted 10-fold with ethanol and its optical density (OD) was measured in the recording spectrophotometer. Retinoid samples from ethanolic stock solutions and vesicle suspension solutions of which the measured peak densities were not within 10% of one another were rejected and not used in the experiments described.

Estimates of Pigment Bleaching

Measurements of the magnitude of visual pigment bleaching (Fig. 2) were made using a microspectrophotometer (MSP) with a measuring beam in the same orientation (side-on) as that used in the physiological experiments (MacNichol, 1978; Cornwall et al., 1983). This method can only be used for substantial bleaches, since changes in density of <0.005 are not accurately measurable with the MSP. Dissociated cells were mounted in the measuring chamber of the MSP and an OD difference spectrum was calculated from spectra measured before and after bleaching light. In order to eliminate so far as possible the contamination of spectra from photoproducts, it was necessary to wait up to 30 min in darkness before
difference spectral measurements were made. The bleaching beam in these experiments was adjusted to have exactly the same intensity, duration, and wavelength as was used in the physiological experiments. In the example shown in this figure, 10 s of intense bleaching light (560 nm) resulted in removal of ~70% of the photopigment.

RESULTS

Effects of Background and Bleaching Light

The data shown in Fig. 3 illustrate the effects that dim background light and intense bleaching light have on response amplitude and the response-intensity relation of isolated cells. The family of superimposed current records shown in the upper left inset were elicited in darkness in response to 220-ms flashes of light at intensities that increased in steps of ~0.5 log units. In these records, the light-induced reduction in inward current measured from the outer segment is plotted in the upward direction. The filled squares in the leftmost graph plot the relation of the peak amplitude of these responses to the log of the incident light intensity. The smooth curve through the data points was fitted by least-squares minimization to the relation:

\[ R = R_{\text{max}} \left[ \frac{I^n}{I^n + \sigma^n} \right] \]

where \( R \) is the response amplitude to a flash of intensity \( I \), \( R_{\text{max}} \) is the maximum amplitude response that can be elicited by bright flashes, \( \sigma \) is the intensity of light that elicits a response which is half of maximum, and \( n \) is a constant (Naka and Rushton, 1966; Baylor et al. 1979b). This equation was used to empirically estimate the position of the response-intensity relation on the intensity axis. In this cell, which
had not been previously exposed to background or bleaching light, $R_{\text{max}}$ was 47 pA, $\sigma$ was 3.8 photons $\mu^{-2}$, and $n$ was 0.98. In this cell the outer segment was contained within the recording pipette. However, in experiments reported elsewhere in this paper, results were qualitatively similar to those shown in Fig. 3, but peak responses were $\sim$50% smaller in amplitude. We believe this simply to be due to a less tight seal between the cell membrane and the recording pipette. In spite of this disadvantage, the latter method was chosen for subsequent measurements to avoid the complicating optical effects that result from passing the light stimulus through the glass.

![Figure 3](image-url)

**Figure 3.** Effects of background and bleaching light on response waveform and the response-intensity relation. (A, inset) Superimposed responses to 220-ms, 520-nm light flashes. Flash intensities differ by $\sim$0.5 long units. The time course of the light stimulus is shown above these and other current traces shown in the figure. Closed squares in the adjacent graph plot membrane current measured from the outer segment vs. log light intensity under dark-adapted conditions. The dashed curve is drawn according to Eq. 1 for this and all other response-intensity relations shown in the figure. Fitted parameters are given in the text. Insets B and C and their associated response-intensity relations (open squares and open triangles, respectively) show changes produced by two intensities of background light which differ by $\sim$1 log unit. Total recovery of sensitivity in darkness after the background is shown by the response-intensity relation (filled circles) measured 11.5 min after the brightest of the two backgrounds was terminated. The inset response records in D and the response-intensity curve described by the open circles were measured after 37 min of darkness, which followed a 10-s bleach. See text for additional details.
recording pipette. Measurements made in this way from the inner segments of 36 cells from 32 different retinas gave average peak responses of $19.0 \pm 5.0$ pA (SD). The open symbols in Fig. 3 recorded during background illumination or after bleaching and the accompanying families of flash responses, demonstrate that no intensity of background light could be found that produced an amount of response compression (reduction in the maximum amplitude of the photoresponse to a bright flash) and shift in $\sigma$ which was equivalent to that produced by the bleaching light. The test was performed in the following way. First, the cell was exposed to a background light that reduced the maximum amplitude of the light response to 43% of the dark-adapted value from 47 to 20 pA and shifted the response-intensity function 0.97 log units to the right (Fig. 3, inset B, open squares). Exposure of the cell to a background light that was 10-fold more intense further reduced the maximum amplitude of the response to 4.9 pA and further shifted $\sigma$ to the right (inset C, open triangles). Flash responses elicited in the presence of background light showed acceleration of the response time course when compared with responses elicited in darkness (not shown). These results are qualitatively similar to those reported previously by Baylor et al., 1979b).

After 16.5 min of exposure to the more intense background and 11.5 min of subsequent darkness, a dark-adapted response-intensity relation (filled circles) was measured. This relation is virtually identical to that measured prior to background exposure (filled squares), demonstrating that recovery following the background illumination was complete. Finally, following 37 min of darkness after a brief exposure to an intense bleaching light (10 s, 560 nm, which was measured from a separate set of spectral measurements [experiment in Fig. 2] to have bleached 70% of the photopigment), the response-intensity relation was again measured (inset D, open circles). At this time the maximum light response was 20 pA and $\sigma$ was shifted to the right by 2.2 log units relative to that measured in darkness before bleaching (a 0.5 log unit shift in $\sigma$ is expected from the decrease in quantal absorption). What is clear from these data is that dim background light and bleaching light which produced equivalent amounts of response compression (compare inset current records of maximum responses in B and D) were associated with shifts along the intensity axis which differed by 1.24 log units. On the other hand, the background light and bleaching light which produced roughly similar shifts in $\sigma$ relative to that under dark-adapted conditions were associated with widely different amounts of response compression.

The nonequivalent effects that dim background and bleaching light have on the photoresponse of isolated photoreceptors are further illustrated in Fig. 4. This figure compares the time course of changes in $\sigma$ and response compression associated with adapting light which bleached little pigment (background adaptation) and that which bleached a significant fraction of the dark-adapted pigment content (bleaching adaptation). These results are taken from the same cell as that shown in Fig. 3. Fig. 4 A shows a series of responses to flashes of different intensities taken after 11 min of exposure to the dimmer of the two backgrounds illustrated in Fig. 3. The response-intensity curve constructed from these data is illustrated by the open triangles in Fig. 4 D. It can be seen that the smooth curve fitted to these responses also provides a good fit for the response-intensity data which were obtained 4 (open circles) and 8 min (open squares) after the onset of the adapting light. Together, these
data indicate that a steady-state desensitization is achieved in <4 min from the onset of the adapting light. Similarly, when the cell was exposed to a 10-fold brighter background intensity (responses in Fig. 4 b), the response-intensity relation measured 2 (open inverted triangles) and 12 min (open diamonds) are all fitted by the same smooth curve, suggesting that even at this higher intensity background, which initially saturated the photocurrent response, the time it took for the steady state desensitization to be achieved was between 2 and 3 min. These data are in contrast to those shown in Fig. 4, C and E for the case of the same bleaching light as that shown in
Fig. 3. The responses to flashes shown in Fig. 4 C (top) were measured 11 min after the 10-s bleaching light, while those in Fig. 4 C (bottom) were elicited 37 min later. The same current response to the bleaching light is shown at the left in these two records for comparison. Initially, after termination of the bleaching light the photocurrent was saturated. Slowly as the dark current recovered, the light-response amplitude increased and the cell regained some of its responsiveness. Data that illustrate the time course of recovery to a steady-state level of desensitization after bleaching light are shown in Fig. 4 E. Response-intensity curves were measured 13 (open inverted triangles), 20 (open upright triangles), 32 (open circles) and 38 min (open squares) after the 10-s bleaching light. These data illustrate that in contrast to the results measured during background light, a steady new level of sensitivity following bleaching light was only achieved after ~32 min. We have demonstrated previously (Cornwall et al., 1983) that this recovery does not involve significant photopigment regeneration in darkness. As a comparison of data in Fig. 4, D and E shows, the rate of attaining steady-state desensitization after bleaching adaptation is more than 10 times slower than that measured during background light which reduced response amplitude by an equivalent amount.

Time Course of Effects of Bleaching and Background Light

Data in Figs. 3 and 4 suggest that bright light, which bleaches a significant fraction of the photopigment, has a permanent desensitizing effect on isolated rod cells. The time course with which steady-state desensitization is achieved after termination of the bleaching light is illustrated in Fig. 5. Both the effect on threshold response (closed squares, 10% of the maximum response) and \( \sigma \) (closed circles, light necessary to elicit a response that is half-maximal) are shown. Threshold and \( \sigma \) were determined before a bright 30-s 520-nm light which was measured in an independent MSP
experiment to bleach >85% of the visual pigment in the outer segment. No response could be elicited from the cell in darkness for ~30 min after the bleach. After this time, measurements of threshold and $\sigma$ were made until 70 min after the bleach. By this time, threshold had stabilized at a level 2.3 log units higher than before the bleach. Though some small recovery of sensitivity due to pigment regeneration cannot be ruled out, the large and sustained desensitization observed for periods as long as 6 h in darkness after bleaching suggest that photopigment regeneration under these conditions is minimal. The effects illustrated in Fig. 5 were demonstrated in four other cells.

**Regeneration of Sensitivity with 11-cis Retinal**

That the desensitization shown in Fig. 5 is due to pigment bleaching and not to deterioration of the preparation is illustrated in Fig. 6, which shows results from a
cell that was bleached by a 15-s, 520-nM light and allowed to achieve a postbleach steady desensitization as in the experiment illustrated in Fig. 5. After remaining in darkness for 55 min after the bleach, the cell was superfused for 23 minutes with normal saline solution to which lipid vesicles containing 11-cis retinal had been added. Rapid recovery of sensitivity commenced within 2 min of the addition of the retinoid to the bath. Both \( \sigma \) (closed circles) and threshold (closed squares) stabilized at new levels that were significantly different than those measured 60 min previously. At the lower right of Fig. 6 is shown a semilogarithmic plot of the decline in threshold and \( \sigma \) vs. time during treatment with 11-cis retinal. As illustrated in this plot, threshold and \( \sigma \) recovered as a simple exponential function of time with a time constant for recovery of ~11 min.

The time course of representative current responses measured in darkness from this cell before bleaching (a), 50 min after bleach (b), and 77 min after treatment with 11-cis retinal (c) are compared in the upper right of the figure. A reduction in the maximum amplitude of the flash response that could be elicited after the bleaching light was apparent from a comparison of the largest dark-adapted responses measured before bleaching (18 pA) with those measured 50 min after bleaching (8 pA, data not shown). Maximum amplitude responses elicited after treatment with 11-cis retinal showed that light-suppressible current in this cell recovered to 115% of that measured in darkness before bleaching.

**Adaptation Produced by Local Background and Bleaching Light**

If the mechanisms that underlie background and bleaching adaptation are the same, we would expect the extent of the spatial spread of desensitization along the longitudinal axis of the rod to be similar in the two situations. Alternatively, if the spatial spread of desensitization under these two conditions is different, then there must be some differences in the mechanisms that give rise to these two phenomena.

For evaluating the longitudinal spread of background adaptation along the long axis of a rod which was locally illuminated with a slit of dim background light we and others have used the relation:

\[
T = \left( \frac{S_F}{S_B} \right) - 1 = \frac{I_B}{I_o}
\]

(Lamb et al., 1981; Cornwall and Pan, 1985) where \( S_F \) is the flash sensitivity produced by a background light of intensity \( I_B \), \( S_D \) is the flash sensitivity in darkness, and \( I_o \) is a constant equal to the background intensity which halves the sensitivity. Similarly, for bleaching adaptation, we evaluate a desensitization parameter,

\[
T = \left( \frac{S_F^D}{S_B^D} \right) - 1
\]

along the rod at different positions from and at different times after local bleach, \( S_B^D \) is the local flash sensitivity measured in different positions along the rod outer segment with a dim test slit after a local bleach at one end of the outer segment.

The experiment to measure the longitudinal spatial profile after local bleaching light is illustrated in the inset of Fig. 7, which shows a cartoon of an isolated rod drawn inner segment first into a fire-polished recording pipette. The narrow transverse bars represent local test stimuli positioned at different distances from the area stimulated with the bleaching light. The broader stippled transverse bar
illustrates the position of the local bleaching stimulus at the distal tip of the rod outer segment. The three current traces above the diagram show the responses to three dim flashes applied locally at different positions along the rod outer segment at (from left to right) 0, 3, and 7 μm from the proximal edge (relative to the inner segment) of the locally bleached area at the distal tip of the rod. The responses to all these flashes were confined to the linear range of the response-intensity relation. The similar set of current traces shown below the diagram illustrates responses elicited at the same locations 30 min after a local bleach (same intensity and wavelength as used in Fig. 5) confined to the distal tip of the cell indicated by the stippled area.

The plots in Fig. 7 show the relationship between log T and distance from the site of the local bleach at different times after bleaching at the distal tip of the rod. The

profile of sensitivity was measured at 3, 20, and 45 min after bleaching. As indicated by the arrows at the top of the plot, ~3 min after the bleach, desensitization was maximal (no response could be elicited) at the site of illumination, whereas responses could be elicited in regions >4 μm distant from the bleached region. Sensitivity recovered slowly along the outer segment until a stable sensitivity profile was achieved after ~45 min.

Analysis of similar results from four other cells is illustrated in Fig. 8, A and B. The data points in Fig. 8 A were derived from the same measurements shown in Fig. 7, inset. From the straight line fitted to the semilogarithmic plot, the space constant for desensitization was estimated to be 2.2 μm in this cell. This relationship suggests that desensitization after the local bleach declines as a simple exponential function of

FIGURE 7. Measurement of the longitudinal spread of desensitization produced by a bleaching light localized to the distal tip of the rod. The inset shows records of responses elicited in the linear range of the response-intensity relation in darkness (top series of records) and after bleaching light confined approximately to the distal 4 μm of the rod tip (bottom series of records) at three different distances from the nominal edge of the focal bleaching light. The rod had been isolated and drawn into a recording pipette as shown in the cartoon. The larger of the two stippled areas indicates the location of the bleaching light; the smaller stippled rectangle is the test slit located in the position closest to the bleached area. The main figure plots the desensitization parameter (defined in the text) vs. distance from the nominal edge of the bleached area at 3, 20, and 45 min after bleaching. Data points were connected by eye. See text for additional details.
distance from the bleached site. The photographs in the inset are of the cell whose
data is illustrated in Fig. 8A. The picture on the right was taken from the television
monitor during the experiment. That on the left was taken after the experiment was
completed using only the bleaching and background slits for illumination and serve
to confirm the proper focus of these stimuli.

In Fig. 8B, the ratio of $T$ evaluated at different distances from the edge of the
bleached region to the maximum value measured at the center of the bleached
region is plotted vs. the distance from the bleached region. The continuous line
drawn through the data points was derived from the expression

$$
\frac{T}{T_{\text{max}}} = \exp\left(-\frac{x}{\lambda_{\text{bleach}}} \right)
$$

where $x$ is distance from the edge of the bleached region of the outer
segment and $\lambda_{\text{bleach}} = 2.5 \pm 0.76 \mu m$ (SD) is the space constant for the decline of the
desensitization parameter calculated from the average $\lambda_{\text{bleach}}$ determined in four cells
as shown in Fig. 8A.

The space constant for the spread of bleaching desensitization evaluated above can
be compared with previous measurements of the space constant for the spread of
background desensitization (Cornwall and Pan, 1985). In these experiments desensi-
Desensitization was evaluated at different distances from the locus of a region illuminated with a spatially restricted continuous background light from flash responses elicited before and during background illumination. In this study the average space constant for the decline of desensitization calculated from seven experiments was 7.1 μm.

The experiments illustrated in Fig. 8 together with this previous work (Cornwall and Pan, 1985) suggest that, on average, the space constants for the spread of desensitizing effects of spatially restricted bright bleaching and dim background light differ from one another by more than a factor of 2. Fig. 9 shows results from an experiment in which the extent of longitudinal spread of local bleaching and background adaptation were measured in a single cell. First, the space constant for dim background desensitization was evaluated (Fig. 9, inset, background). From the straight line which best fitted this data, a space constant of 7.3 μm was calculated. Then the cell was locally bleached in the same region and 18 min later the space constant for local bleaching was determined to be 3.2 μm (Fig. 9, inset, bleach). The main graph in Fig. 9 compares the relationships between normalized desensitization for the local bleaching and background stimuli in this cell. The dashed lines were drawn according to the simple exponential relations presented above where λ_{bleach} = 3.2 μm and λ_{background} = 7.3 μm. These data together with that of Cornwall and Pan (1985) demonstrate that the desensitizing effects of dim background and bright bleaching light spread differently along the rod outer segment.
Effects of IBMX on Bleaching Adaptation

A decrease in Ca\textsuperscript{2+} has been postulated to be the principal, if not the sole, regulator of sensitivity during background adaptation (Cervetto et al., 1985; Matthews et al., 1988; Nakatani and Yau, 1988; Fain et al., 1989). IBMX is a potent phosphodi-
esterase inhibitor known to increase cytosolic c-GMP levels (Cohen et al., 1978; Woodruff and Fain, 1982) as well as Ca\textsuperscript{2+} (Capovilla et al., 1983; McNaughton et al., 1986) in rods. Treatment of bleach-adapted rods with IBMX would be expected to elevate c-GMP levels and also to promote the return of Ca\textsuperscript{2+} toward normal dark-adapted levels (see Discussion) and thereby restore sensitivity. Fig. 10 reports an
experiment to test this possibility. The filled squares on the left of the figure show the response-intensity relation determined in darkness immediately before exposure to a 30-s, 520-nm bleaching light. \( \sigma \), measured before the bleaching light, was estimated to be 6 photons \( \mu \text{m}^{-2} \). Following 37 min of darkness after the bleach, the response-intensity relation illustrated by the open squares was obtained. At this time the maximum amplitude of the response was reduced by 59\% from 22 to 9 pA and \( \sigma \) was shifted by 1.75 log units. The superfusate was then changed to one containing 100 \( \mu \text{M} \) IBMX and the response-intensity relation was determined 5 (not shown) and 82 min (open circles) later. At the latter time, the largest response that could be elicited was close to the dark-adapted value (22 pA) and the responses to all

![](image)

**Figure 11.** Effects of IBMX on local bleaching desensitization. The response-intensity relation was evaluated at the proximal (closed circles) and distal (closed squares) ends of the outer segment before (A) and after (B) local bleach at the distal tip of the rod, and after treatment of the cell with 100 \( \mu \text{M} \) IBMX (C). A difference of sensitivity between bleached and unbleached regions of ~0.75 log units was preserved in the presence of IBMX in spite of a greater than fourfold increase in the amplitude of the current responses. See text for further details.

intensities of light exhibited marked slowing even though \( \sigma \) remained essentially unchanged at ~380 photons \( \mu \text{m}^{-2} \). The effect of IBMX on the amplitude and time course of the response was complete within 5 min of the start of superfusion with the test compound. Response-intensity relations obtained 5 and 82 min after the beginning of superfusion with IBMX were virtually identical, with \( \sigma \) differing by <0.2 log units. It can be seen that the time course of dim light responses after bleaching and in the presence of IBMX are considerably slower than those elicited in normal superfusate following IBMX. After 217 min of treatment with IBMX the superfusate was returned to normal and the response-intensity relation (open triangles) was obtained. The larger and slower flash responses were a transient effect requiring the
FIGURE 12. Recovery of light-suppressible current and dark-adapted response time course of bleached cells after treatment with 11-cis retinal–containing vesicles. (A, left) Responses to 220-ms flashes elicited 90 min after bleaching light but before application of 11-cis retinal. (A, right) Effect of 11-cis retinal on the response amplitude and the baseline circulating current after treatment with 11-cis retinal. 11-cis retinal was added to the bath 3 min before the beginning of the record. Maximum response amplitude recovered to 117% of that measured during the dark-adapted control period. The time course of the light stimuli is shown above the current traces. (B, insets) Records of response time course were taken before (center), in the steady state after bleaching adaptation (right) and after 11-cis retinal treatment (left). Associated response-intensity relations are plotted as the filled squares (dark adapted), open squares (bleach adapted), and filled circles (after 11-cis retinal). Smooth curves were fitted according to Eq. 1. (C) Time course of current responses elicited in the linear range of the response-intensity relation during control dark-adapted conditions (curve 1), bleaching adaptation (curve 2), and during steady-state recovery after treatment with 11-cis retinal (curve 3).
continued presence of IBMX and were quickly and completely reversed upon its
removal. These data clearly demonstrate that though there is an increase in
sensitivity (reduced photon flux necessary to produce a criterion photoreponse) of
the bleached rod after treatment with IBMX due to an increase in response
amplitude, \( \sigma \) was not significantly altered.

Fig. 11 demonstrates that the profile of desensitization produced by a local
bleaching light (see Figs. 7 and 8) is preserved during treatment with IBMX. In this
experiment, the low intensity portion of the response-intensity relation of the
proximal (closed circles) and distal (closed squares) ends of the outer segment were
measured in darkness when the inner segment was drawn into the recording pipette
(left panel). After local bleaching adaptation at the distal tip of the outer segment, the
response-intensity relation was again measured in these two regions. A difference of

sensitivity of \(-0.75\) log units was observed between these two regions 15 min after
termination of the bleaching light (Fig. 11 B). Fig. 11 C shows that after 33 min in
100 \( \mu M \) IBMX the differential of sensitivity between these two regions was essentially
unchanged even though the response amplitudes obtained were over fourfold
greater.

**Effects of 11-cis Retinal on Bleach-adapted Rods**

The data presented in Fig. 12 demonstrate that all the effects on the rod response
that occur when isolated cells are bleached and not allowed to regenerate their
photopigment are reversed after exposure of the cells to 11-cis retinal. Fig. 12 A
shows current records from a rod that demonstrate the recovery of light-suppressible
dark current which occurs after treatment with 11-cis retinal. Responses and
response-intensity curves from this cell are shown in Fig. 12 B. The effects of the bleaching light and subsequent application of 11-cis retinal on the time course of responses elicited in the linear range of the response-intensity relation are shown in Fig. 12 C. The time to peak of the dim-light response from the dark-adapted cell was estimated to be 750 ms. It is apparent from these responses that even after 90 min in darkness following the end of bleaching light, the time to peak of the dim-light response (500 ms) was significantly faster than that observed either in darkness before bleaching or after treatment with vesicles containing 11-cis retinal (780 ms).

Spectral Sensitivity of Dark-adapted and Pigment-regenerated Rods

Evidence that the recovery of sensitivity which occurs after treatment of bleach-adapted cells with vesicles containing 11-cis retinal is due to reconstitution of visual pigment is presented in Fig. 13. These data compare the spectral sensitivity of isolated rod cells that were bleached and subsequently resensitized using 11-cis retinal with spectral absorptance functions derived from microspectrophotometric recordings of either native visual pigment measured in dark-adapted cells or visual pigment regenerated after treatment with 11-cis retinal. It is apparent that the smooth curve describing the absorptance spectrum of regenerated rhodopsin provides a better fit to the spectral sensitivity data after bleaching and treatment with 11-cis retinal than does the native pigment absorptance spectrum. These data suggest that, after bleaching and resensitization in these rod cells, a substantial fraction of the native vitamin A₂-based pigment was replaced with a visual pigment whose absorption spectrum was shifted almost 20 nm to shorter wavelengths.

DISCUSSION

Bleaching Adaptation in Isolated Rods

The purpose of the work described in this paper was to determine if the same or different mechanisms underlie the desensitization produced by dim background light (background adaptation) and that produced when a significant fraction of the photopigment is bleached (bleaching adaptation). In these studies we chose to examine bleaching adaptation in solitary rod photoreceptors deprived of contact with the pigment epithelium in order to produce persistent levels of bleaching desensitization, which could be directly compared with the effects produced by dim background light.

After intense bleaching stimuli, the rods were at first completely insensitive to light stimulation (Figs. 5 and 7). Partial recovery to a steady-state level of desensitization was variable in time course depending on the extent of bleach but was complete for the largest bleaches (>90%) after 60–70 min of subsequent darkness. The steady-state desensitization persisted for as long as measurements were possible in these cells, but recovery of sensitivity could be accomplished by superfusion of the outer segment with solutions containing 11-cis retinal (discussed below). As demonstrated in Figs. 3, 4, and 12 A, the steady-state phase of desensitization arises from (a) a reduction in the magnitude of the light-suppressible current, and (b) a shift to higher intensities of the response-intensity relation. Though the degree of reduction of the light-suppressible current during the steady-state phase was somewhat variable, there
was a direct relation between the amount of reduction and the percent of pigment bleached. Surprisingly, however, even for light stimulation that bleached >90% of the pigment (Fig. 12), the reduction in light-suppressible current was no greater than 60%. This demonstrates that even under severely bleach-adapted conditions, bleached rods in the steady state are capable of responding to incremental changes in light intensity. The shift of the response-intensity relation along the intensity axis of between 2.5 and 3.0 log units after a >90% bleach roughly corresponds to results obtained by others (Grabowski and Pak, 1975, intracellular recordings from axolotyl; Frank, 1971, ammonia-isolated PIII response from frog; Ernst and Kemp, 1972, Ca**+-free–isolated PIII from rat; Hood et al., 1973, aspartate-isolated PIII response from frog; Cornwall et al., 1989, isolated skate photoreceptor), but varies significantly from the relation between the ERG B-wave and the percent of pigment bleached measured in rat (Weinstein et al., 1967), frog (Baumann, 1967), and that for the aspartate-isolated photoresponse from skate (Pepperberg et al., 1978). In the latter three cases, between 4 and 5 log units of desensitization were measured for roughly a 95% pigment bleach. The reason for this discrepancy is not known.

A variety of studies have demonstrated that the steady-state effects of bleaching adaptation which are observed in isolated retinas can be reversed by exposing the tissue to solutions containing 11-cis retinal (Pepperberg et al., 1978; Albani et al., 1980; Perlman et al., 1982). The experiments described in the present study demonstrate that these effects produced by exogenous application of retinoids result from events occurring within the rod outer segment and shed some light on the mechanisms regulating these changes. Our data (Figs. 6 and 12) clearly demonstrate that treatment of isolated cells with solutions containing 11-cis retinal results in complete recovery of sensitivity. That this resensitization results from synthesis of new photopigment is demonstrated by comparison of the spectral sensitivity relation after bleaching and treatment with 11-cis retinal with the absorptance spectra of the native and regenerated visual pigments (Fig. 13). Our measurements of the native visual pigment in the larval tiger salamander (dashed curve, Fig. 13) show a spectral peak at ~520 nm and a bandwidth at half-intensity of 4,930 cm⁻¹. These data are consistent with previous measurements of visual pigments in single cells of the larval tiger salamander (Harosi, 1975; Cornwall et al., 1984) and measurements of porphyropsin in solution (Bridges et al., 1967), and suggest that though these cells contain a mixture of rhodopsin and porphyropsin (Ernst et al., 1978), >90% of the pigment is porphyropsin. The absorptance spectrum of the regenerated visual pigment (small dots) and the spectral sensitivity after regeneration (larger filled symbols) in Fig. 13 are characteristic of rhodopsin (bandwidth at half-intensity of 4,200 cm⁻¹) and not porphyropsin.

The time required for recovery of sensitivity after application of chromophore in our experiments was somewhat variable and ranged between 10 and 20 min (for example see Fig. 6). This is somewhat faster than rates observed for rod responses in skate (~20 min for total recovery, see Pepperberg et al., 1978), bullfrog (~50 min, see Perlman et al., 1982), and rat (~40 min, see Albani et al., 1980), but different methods of application and different concentrations of 11-cis retinal were used in these studies. Though no systematic effort was made to examine the dose dependency of the resensitizing effects of 11-cis retinal, the rates of recovery of sensitivity
were lower when vesicles containing less retinoid were used. In a number of cases, the amplitude of the light-suppressible current measured after treatment with 11-cis retinal–containing vesicles was greater than that measured under control conditions prior to the bleach (see Figs. 6 and 12). We also often observed that the response-intensity relation after retinoid treatment was shifted slightly to lower intensity compared with control conditions, indicating that the cell was more sensitive than when the experiment began. It is possible that this resulted simply because a slight desensitization of the cell had occurred before these measurements during preparation and initiation of recording.

**Steady-state Desensitization Produced by Bleaching and Background Adaptation**

Experiments illustrated in Figs. 3 and 4 were designed to see if the equivalence of bleaching and background desensitization predicted by human psychophysical experiments could be observed in solitary rods under background-adapted conditions and bleached conditions. The data in these figures show that while it was possible to find some levels of bleaching and background light which produced equivalent changes in threshold (see Fig. 3, open circles, open triangles), the effects of bleaching and background light on σ and response compression were clearly not equivalent. It is apparent from comparison of the response-intensity relations in Fig. 3 (background, open squares; bleaching, open circles) measured when response compression was the same, that allowance for the loss of quantal absorption might bring these curves into closer register by shifting the bleaching curve to the left. Insufficient data were available, however, to test this point. This important question will be examined in a later study. No intensity or duration of bleaching light was ever found that permanently and totally depressed the response amplitude. Even for the brightest and largest bleaches, response amplitude was never depressed by \( >-60 \% \). In contrast, a bright background could produce complete suppression of the response to incremental light flashes.

**Longitudinal Spread of Bleaching and Background Adaptation**

We tested the equivalence of bleaching and background adaptation by comparing the extent of longitudinal spread along the long axis of outer segments (Figs. 7, 8, and 9). Just as with uniform bleaching of the rod, different phases of desensitization were observed after local bleaching (see Fig. 7). Initially, after the local bleaching, desensitization, though greatest in the illuminated region, extended over the entire length of the outer segment. This effect became progressively more restricted over the next 20–30 min to a narrow region of the rod corresponding closely to the nominal area bleached. At steady state, the effective longitudinal distance over which the e-fold decline of desensitization occurred was \(<3 \mu m\) (Fig. 8). This distance is \(<50\%\) of the average distance of 7 \(\mu m\) over which the effects of background desensitization was measured to decline (Cornwall and Pan, 1985 and Fig. 9). This latter estimate for the spread of background desensitization in *Ambystoma* rods is close to the mean value of 6 \(\mu m\) that was measured previously in toad rods by the same methods (Lamb et al., 1981) and of 5–20 \(\mu m\) estimated in isolated frog retina by a different technique (Hemila and Reuter, 1981). All of these data regarding the spread of the effects of background adaptation are consistent with the observations
of Bastian and Fain (1979) that substantial adaptation can be observed to occur over the entire outer segment at intensities of background light so low that only a very small fraction of discs within a rod are absorbing photons.

Our data demonstrate that the upper limit for the effective spread of bleaching adaptation in these cells is \( \sim 2.5-3 \, \mu m \). Two arguments, however, suggest that this apparent spread may be an artifact of our method of measurement, and we suggest, as have Baylor and Lamb (1982), that the effects of bleaching adaptation are more localized, perhaps even confined to the discs containing bleached photopigment. First, the longitudinal distance over which an \( e \)-fold decline of bleaching light intensity (stimulus spread) occurred (\( \sim 2 \, \mu m \), Fig. 1) is very similar to the space constant for the decline of the effects of bleaching desensitization. Secondly, data in Figs. 8, 9, and 11 B demonstrate that local gradients of sensitivity persist for periods in excess of 4 h (the longest duration of our experiments) after local bleaching. Over such extended periods, any local gradients of concentration of desensitizing substances in the cell should have disappeared. McNaughton et al. (1980) and Lamb et al. (1981) have estimated the effective diffusion coefficient for activation and background desensitization to be of the order of \( 10^{-7} \, cm^2 \, s^{-1} \) in toad rods. Similarly, we have confirmed that this is also true for background desensitization in Ambystoma rods (Cornwall and Pan, 1985). Furthermore, we calculate from a simple one-dimension diffusion model and a diffusion coefficient on the order of that cited above, that if a pulse of positive or negative transmitter occurred at one end of a rod outer segment roughly 20 \( \mu m \) long, its concentration would be effectively constant along the rod cytoplasm within 10 s (Cornwall and Pan, 1985). Third, Corson et al. (1989) have shown that a nontransducing visual pigment can be regenerated and sensitivity substantially restored in isolated bleached rods after superfusion with lipid vesicles containing an analogue of 11-cis retinal, which cannot isomerize about the 11-12 bond. These experiments suggest a central role for opsin unattached to retinoid in bleaching adaptation because sensitivity was observed to recover as the nonisomerizable chromophore was bound to opsin.

**The Effect of IBMX on Bleach-adapted Rods**

Recent evidence from experiments on salamander rods and cones has indicated that background adaptation is mediated largely, if not completely, through a decrease in \( Ca^{2+} \) resulting from a decrease in \( Ca^{2+} \) flux across the plasma membrane (Matthews et al., 1988; Nakatani and Yau, 1988; Fain et al., 1989). According to this idea, light leads to a decrease in \( Ca^{2+} \) influx through the plasma membrane conductance. Continued efflux through the \( Na^{+}-Ca^{2+} \) exchanger leads to a decrease in \( Ca^{2+} \). This results in an increased rate of c-GMP production via an increase in the activity of guanulate cyclase (Hodgkin and Nunn, 1988; Koch and Stryer, 1988) or to a decrease in the activity per molecule of activated phosphodiesterase (Torre et al., 1986). Our results suggest that the mechanisms that regulate sensitivity under bleach-adapted conditions are more complicated. The data in Fig. 10 demonstrate that treatment of a bleach-adapted rod with 100 \( \mu m \) IBMX results in an increase of the light-suppressible dark current to a level similar to that measured previously under dark-adapted conditions. If the level of \( Ca^{2+} \) is set by its influx through the
c-GMP-activated conductance in the plasma membrane and its efflux via the Na/Ca exchanger, then Ca\(^{2+}\) should have been increased by IBMX in the experiment of Fig. 10 to a level near that expected before bleaching. However, the results show that, whereas dark current during treatment with IBMX was nearly at its dark-adapted level, σ did not change, and only a very small increase in sensitivity resulted. Furthermore, the experiment of Fig. 11 shows that the profile of desensitization along the outer segment is preserved during treatment with IBMX. We conclude from the data in Figs. 10 and 11 that in addition to changes in cytosolic calcium and c-GMP, some other factor is important in regulating sensitivity under bleach-adapted conditions.

**Mechanisms Underlying Bleaching Adaptation**

We have examined adaptational effects after lights that bleach between ~50% and 95% or more of the pigment under conditions which preclude regeneration of the photopigment and in particular, we have followed the persistent effects of bleaching adaptation under true steady-state conditions for periods up to 4 h. These effects appear effectively confined to regions of the outer segment containing bleached photopigment and are relieved only upon regeneration of the visual pigment. We found more than a twofold difference in the spatial longitudinal spread of the effects of local bleaching and local background light (Figs. 7–9). In toad rods (Lamb et al., 1981), the space constant for the spread of background adaptation is ~6 μm, which is very close to the 7 μm for the spread of background adaptation in tiger salamander rods (Fig. 9 and Cornwall and Pan, 1985). If calcium carries the signal for background desensitization (Cervetto et al., 1985; Matthews et al., 1988; Nakatani and Yau, 1988; Fain et al., 1989), this difference in space constant would rule it out as the messenger for the steady component of bleaching adaptation. The experiments with IBMX also indicate that Ca\(^{2+}\) and c-GMP are unlikely regulators of bleaching desensitization, since elevation of cytosolic levels of both of these internal messengers by IBMX has no effect on σ in bleach-adapted cells.

All of the known photoproducts in the vertebrate bleaching cascade except two decay spontaneously (for a review, see Abrahamson and Weisenfeld, 1972). These substances are free opsin, and all-trans-retinol. All-trans-retinol is free to diffuse within the membranes of the outer segment (Liebman and Entine, 1968) but is thought to be transported from the retina to the pigment epithelium via a protein in the interphotoreceptor matrix (interphotoreceptor matrix retinoid binding protein) (Chader et al., 1983; Okajima et al., 1989) where an isomerase transforms retinol back to the cis form (Bernstein et al., 1987). Therefore, since free opsin in some form appears to be the only photoproduct whose concentration in the outer segment after bleaching is undiminished with time, we conclude that it or some substance intimately associated with it may be the mediator of the persistent desensitizing effects that we and others (Pepperberg et al., 1978; Perlman et al., 1982) have observed under these conditions.

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