Title

Ca2+-dependent changes in cyclic GMP levels are not correlated with opening and closing of the light-dependent permeability of toad photoreceptors.

Journal

The Journal of General Physiology, 80(4)

ISSN

0022-1295

Authors

Woodruff, ML
Fain, GL

Publication Date

1982-10-01

DOI

10.1085/jgp.80.4.537

Peer reviewed

Ca^{2+}-dependent Changes in Cyclic GMP Levels Are Not Correlated with Opening and Closing of the Light-dependent Permeability of Toad Photoreceptors

MICHAEL L. WOODRUFF and GORDON L. FAIN

From The Jules Stein Eye Institute, University of California School of Medicine, Los Angeles, California 90024

ABSTRACT We have measured the levels of 3′,5′-guanosine monophosphate (cyclic GMP) in isolated retinas from toad to investigate their correlation to the opening and closing of the light-dependent permeability of photoreceptors. When Ca^{2+}-induced changes in cyclic GMP concentration are compared with the Ca^{2+}-induced changes in the permeability of photoreceptor light-dependent channel, four quantitative dissimilarities are noted. First, when extracellular Ca^{2+} ($[Ca^{2+}]_o$) is reduced from normal physiological levels to between 10^{-6} and 10^{-7} M, the light-dependent permeability is increased, but cyclic GMP levels are not significantly changed. Second, when $[Ca^{2+}]_o$ is increased from 1.8 to 20 mM, the light-dependent permeability is suppressed, but cyclic GMP levels are decreased by only 10–15%, about one-quarter the decrease that can be obtained with bright illumination. Third, when $[Ca^{2+}]_o$ is increased from 10^{-8} M to 20 mM, the light-dependent permeability is closed rapidly, but the cyclic GMP decrease is slow. Fourth, when $[Ca^{2+}]_o$ is lowered to 10^{-8} M, the sensitivity of the light-dependent permeability to steady illumination is decreased by three to four orders of magnitude, but the sensitivity of the light-dependent decrease in cyclic GMP is not significantly affected. These observations indicate that there is no simple correlation between cyclic GMP levels and the permeability of the light-dependent channels and that Ca^{2+} can affect the conductance in the absence of changes in cyclic GMP content.

INTRODUCTION

Illuminating vertebrate photoreceptors leads to a rapid, large decrease in intracellular 3′,5′-guanosine monophosphate (cyclic GMP) concentration, and it has been proposed that this light-induced change might be the internal stimulus that decreases the ionic permeability of the photoreceptor membrane

Address reprint requests to Dr. Gordon L. Fain, Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, CA 90024. Dr. Woodruff's present address is Graduate Department of Biochemistry, Brandeis University, Waltham, MA 02254.

J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/82/10/0537/19 $1.00 537
Volume 80 October 1982 537–555

during visual transduction (Woodruff and Bownds, 1979). Several observations are consistent with this notion. Increasing internal cyclic GMP levels in isolated frog rod outer segments, either with low extracellular Ca^{2+} ($[Ca^{2+}]_o$) or phosphodiesterase inhibitors, increases the permeability of the light-dependent conductance. Decreasing cyclic GMP levels in outer segments by adding β,γ-methylene adenosine triphosphate decreases the light-dependent permeability (Woodruff et al., 1977). When intracellular cyclic GMP levels are increased by directly injecting cyclic GMP into photoreceptors, the membrane depolarizes and the latency of the light-induced decrease in permeability is delayed (Miller and Nicol, 1979). The depolarization, which also is observed when the photoreceptors are exposed to low Ca^{2+} (Lipton et al., 1977*a*; Brown et al., 1977; Bastian and Fain, 1979) and phosphodiesterase inhibitors (Lipton et al., 1977*b*), is probably caused by an increase in the conductance of the light-dependent channels.

In this paper we compare light- and Ca^{2+}-induced changes in cyclic GMP levels with toad rod physiology measured by the radiotracer method of the preceding paper (Woodruff et al., 1982) and by intracellular recording (Bastian and Fain, 1979, 1982). From these experiments we conclude that there is no simple correlation between cyclic GMP levels and the state of the light-dependent permeability.

METHODS

Materials and Solutions

The principal solutions used in this study are listed in Table I. Solution A is normal toad Ringer's solution and solution B is a low-Cl^- Ringer's solution. These are identical to solutions A and B in the previous paper (Woodruff et al., 1982). Solutions C–H are low-Cl^- Ringer's solutions containing various levels of Ca^{2+} from 10^{-6} to 10^{-9} M. Low-Ca^{2+} solutions were prepared as in previous experiments (Bastian and Fain, 1979, 1982). We calculated the free-Ca^{2+} concentrations in these solutions using the constants of Caldwell (1970). Note that because of the uncertainty of the dissociation constant for Ca^{2+}-EGTA (we have seen 10 different values published), the Ca^{2+} activity in these solutions may be different from the values shown by as much as 0.5 log units. The Cl^- was substituted with $CH_3SO_3^-$ to prevent photoreceptor swelling in low-Ca^{2+} solutions (Bastian and Fain, 1982). Ouabain, IBMX (isobutylmethylxanthine), Na aspartate, and the components of the Ringer's solutions listed in Table I were purchased from Sigma Chemical Co., St. Louis, MO. Components of the radioimmunoassay for cyclic GMP were purchased from Collaborative Research, Waltham, MA.

Light Stimulus

In the experiments testing the effect of light on cyclic GMP levels, retinas were illuminated with a full-field, 501-nm light whose unattenuated irradiance was 13.06 log quanta cm^{-2} s^{-1}. The light beam was heat filtered by 2 KG-3 infrared filters (Melles Griot, Irvine, CA). Assuming an effective collecting area of 29.5 μm for a rod photoreceptor (Fain, 1976), we calculate that this unattenuated light bleached 3.9×10^6 rhodopsin molecules per receptor each second. The irradiance was adjusted to this level before each experiment using a silicon photodiode. The light was attenuated by

interposing calibrated neutral density filters (Fish-Schurman Corp., New Rochelle, NY).

Preparation

We dissected dark-adapted retinas from the toad, *Bufo marinus*, and placed them receptor side up onto Millipore filters (HAWP 0013, 0.45 μm pore size), as described in the previous paper. We then cut each retina with its filter into eight pie-shaped sections with a razor blade and placed these into 10 ml normal toad Ringer's solution (solution A). We preincubated the sections in normal Ringer's solution for 8–10 min in darkness before using them in the various experimental procedures. The Ringer's solution was bubbled with oxygen during this incubation. To change solutions, we transferred the individual sections with forceps to a different small petri dish or spot plate well containing the new solution. The sections were briefly rinsed and then transferred to another dish or well containing the new solution for incubation. The retinas were kept well oxygenated by moving them to freshly oxygenated solution every 2–3 min during an experiment. After incubating the sections, we dropped them into 200 or 300 μl of 9% perchloric acid to quench any enzymatic reactions. We mixed the section in the acid quickly by vortexing.

TABLE I

COMPOSITION OF SOLUTIONS

Solution	NaCl	KCl	$CaCl_2$	$MgCl_2$	$NaHCO_3$	Na_2SO_4	$MgSO_4$	$NaCH_3SO_3$	KCH_3SO_3	EGTA	Free $[Ca^{2+}]_o$
					mM						*M*
A	106	2.5	1.8	1.2	0.13	1.8	—	—	—	—	1.8×10^{-3}
B	—	—	1.8	—	0.13	1.8	1.2	106	2.5	—	1.8×10^{-3}
C	—	—	1.8	—	0.13	1.8	1.2	104	2.5	1.81	1.0×10^{-6}
D	—	—	1.8	—	0.13	1.8	1.2	101	2.5	1.96	1.0×10^{-7}
E	—	—	1.8	—	0.13	1.8	1.2	100.6	2.5	2.30	3.0×10^{-8}
F	—	—	1.8	—	0.13	1.8	1.2	99	2.5	3.18	1.0×10^{-8}
G	—	—	12.8	—	0.13	1.8	1.2	97.5	2.5	5.85	3.0×10^{-9}
H	—	—	1.8	—	0.13	1.8	1.2	86.5	2.5	12.67	1.0×10^{-9}
I	106	2.5	20	—	—	—	—	—	—	—	2.0×10^{-2}

All solutions contained 3 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid) and 5.6 mM glucose and were buffered to pH 7.8 by adding NaOH. Solutions containing EGTA took relatively more NaOH to bring the pH to 7.8. We reduced the $NaCH_3SO_3$ concentration in these cases to maintain the Na^+ concentration at ~110 mM. Solution osmolarities were measured with a vapor pressure osmometer (Westcor, Inc., Logan, UT) to be between 210 and 230 mosmol. In the experiments of Figs. 3 and 4, which test the effect of illumination, 5 mM sodium aspartate was added to the Ringer's solutions.

Cyclic GMP Assay

We assayed cyclic GMP content in the acid-treated samples according to the procedure of Woodruff et al. (1977). Briefly, we centrifuged the acid treated samples (2,200 *g*, 30 min), took 110 μl of the supernatant for the cyclic GMP assay, and dissolved the pellet (retina, precipitated proteins, and Millipore filter) into 400 μl of 1 M NaOH for analysis of protein content according to Lowry et al. (1951). We neutralized 110 μl of the supernatant with KOH and used 10–70 μl of this for the cyclic GMP radioimmunoassay. We diluted the samples to 200 μl with 0.1 M imidazole buffer (pH 7.0 at 4°C), and then we added 100 μl of ^{125}I-succinyl cyclic GMP tyrosine methylester and 100 μl of cyclic GMP antiserum, bringing the total volume to 400 μl. We incubated the samples overnight at 4°C and then passed them through Millipore filters (type HA, 0.45 μm pore size), which retained the antibody complex. We counted the

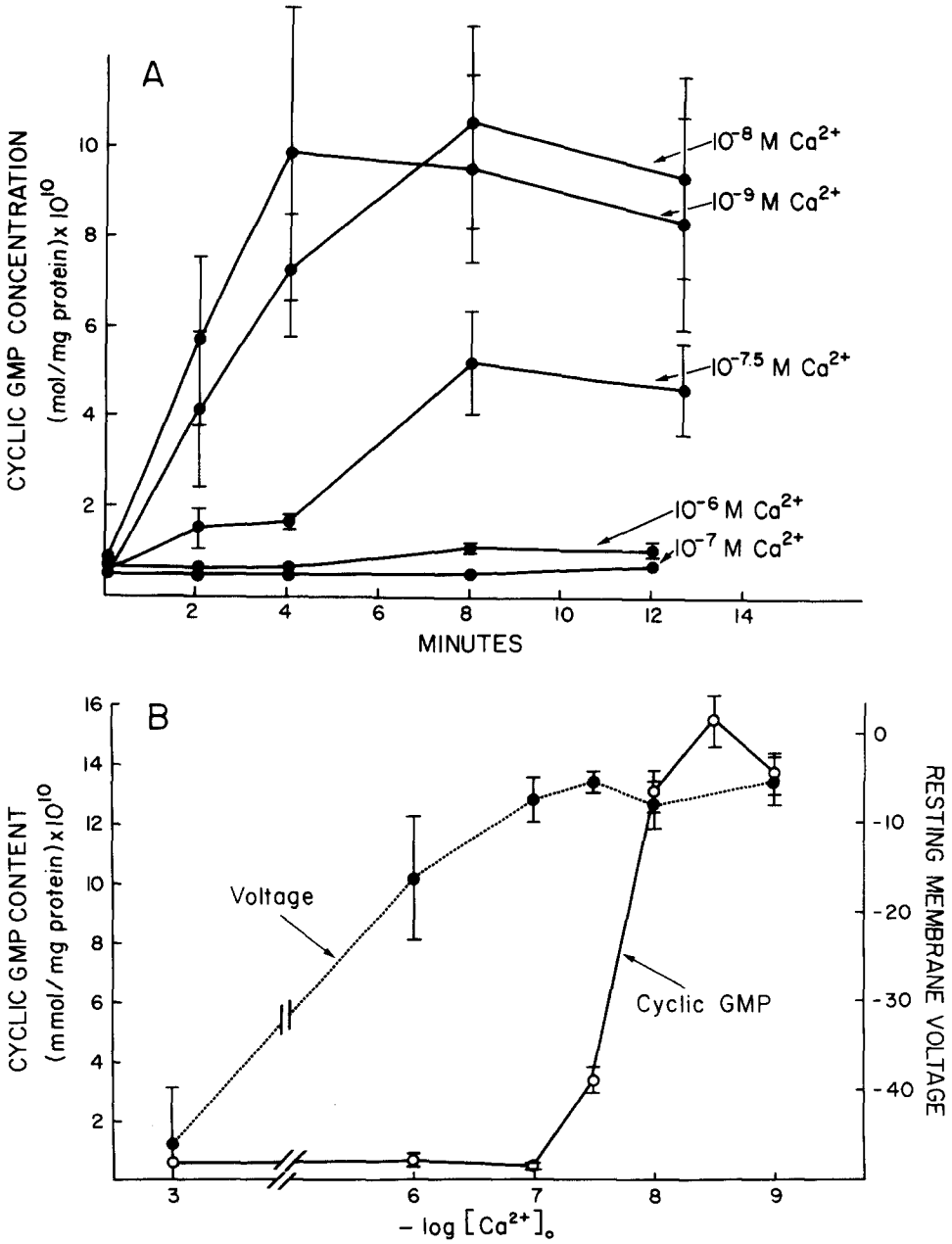


FIGURE 1. A. The time course of cyclic GMP increase in low $[Ca^{2+}]_o$. After 8–10 min incubation in normal toad Ringer's solution (solution A) retinas were transferred to low-Cl^- Ringer's solution (solution B) for 2–3 min and then into various Ringer's solutions with different free Ca^{2+} concentrations (solutions C–H). The data represent the mean (± SEM) of two or more determinations. B. The concentration dependence of low-$[Ca^{2+}]_o$-induced increase in cyclic GMP and the depolarization of the rod resting membrane voltage. For the cyclic

radioactivity on the filters with a Beckman 9000 gamma counter (Beckman Instruments, Inc., Fullerton, CA). Samples with known cyclic GMP levels were run in parallel with the experimental samples in order to generate a standard curve in each experiment.

RESULTS

After dissecting retinas from the toads and placing them in normal toad Ringer's solution (solution A), the level of cyclic GMP declined slightly over the first 10 min of incubation, from an average of 68.2 ± 10.2* ($N = 6$) pmol cGMP/mg protein to 56.3 ± 2.4 ($N = 42$) pmol cGMP/mg protein. In two determinations, retinas were left in normal Ringer's solution for 20 and 30 min, after which time they contained 59.0 and 56.5 pmol cGMP/mg protein. All the data in this report were obtained from retinas that were preincubated in darkness in normal Ringer's solution for 8–10 min before performing the different experimental manipulations. All data were obtained within 30 min of killing the toads.

When retinas were exposed to low $[Ca^{2+}]_o$, cyclic GMP levels increased dramatically. Fig. 1A shows the time course of the cyclic GMP increase after retinas are transferred to Ringer's solutions with various $[Ca^{2+}]_o$. Between 10^{-3} and 10^{-7} M Ca^{2+}, there was little or no change in cyclic GMP for the first 4 min of exposure. After 12 min of incubation a small increase in cyclic GMP levels could be observed at 10^{-6} and 10^{-7} M. When $[Ca^{2+}]_o$ was decreased to 3×10^{-8} M (labeled $10^{-7.5}$ M), cyclic GMP levels increased from 50–60 pmol cGMP/mg protein to ~500 pmol cGMP/mg protein at 8 min. Decreasing $[Ca^{2+}]_o$ further, to 10^{-8} and 10^{-9} M, resulted in more rapid and larger increases in cyclic GMP, to near 1,000 pmol cyclic GMP/mg protein.

The concentration dependence for the $[Ca^{2+}]_o$ effect is shown in Fig. 1B. It is clear that cyclic GMP content is a steep function of extracellular Ca^{2+} level, with most of the effect occurring between 10^{-7} and 10^{-8} M. Also shown in Fig. 1B (dashed line) is the effect of lowering $[Ca^{2+}]_o$ on the resting membrane potential of toad rods, taken from Bastian and Fain (1982). A depolarization of the cell from about −46 to −5 mV occurs when $[Ca^{2+}]_o$ is decreased from 10^{-3} to 10^{-7} M, a range of $[Ca^{2+}]_o$ where no noticeable increase in cyclic GMP can be measured. Large increases in dark current also occur over this range of Ca concentrations (see for example Yau et al., 1981; Greenblatt, 1982). The resting membrane potential is then stable as $[Ca^{2+}]_o$ is

* All errors given in this paper are standard errors of the mean, calculated with N − 1 weighing.

GMP data, the retinas were prepared and incubated in Ringer's solutions as in A. The data represent the amount of cyclic GMP found in the retinas after 8 min in the various low-Ca^{2+} solutions. The data for $pCa^{2+} = 6$, 7, and 7.5 includes the results shown in A. The data for $pCa^{2+} = 3$ was obtained by simply leaving the retinas in solution B for the 8-min incubation. The data represent the mean (± SEM) of five or more determinations. The voltage data are from Bastian and Fain (1982). The voltages reached the new depolarized values within 4 min of exposure to the new $[Ca^{2+}]_o$.

lowered from 10^{-8} to 10^{-9} M, although this is the region where the most dramatic change in cyclic GMP concentration occurred.

Effect of Ouabain on Cyclic GMP Levels

In our radioactive flux experiments (Woodruff et al., 1982), retinas were incubated in 0.5 mM ouabain in addition to low $[Ca^{2+}]_o$. To investigate the effects of ouabain exposure on cyclic GMP content, we added 0.5 mM ouabain to toad retinas after they had been incubated in normal toad Ringer's solution for 8–10 min. Cyclic GMP levels decreased by 33%, from an average of 62.5 ± 7.0 ($N = 6$) pmol cGMP/mg protein to 42.3 ± 2.9 ($N = 6$) pmol cGMP/mg proteins in 4 min. However, when ouabain-treated retinas were exposed to low Ca^{2+}, the time course and concentration dependence of the cyclic GMP increases were similar to those observed in retinas without ouabain. No increase in cyclic GMP was observed as $[Ca^{2+}]_o$ was lowered to 10^{-7} M, but a large and maximum increase was obtained with 10^{-8} M Ca^{2+}. After 8 min in 10^{-7} M Ca^{2+}, 0.5 mM ouabain, the cyclic GMP content was 35.9 ± 3.6 ($N = 3$) pmol cGMP/mg protein, a value not significantly different from the control level of 42.3 pmol/mg protein. In 10^{-8} M Ca^{2+}, 0.5 mM ouabain Ringer's solution, however, cyclic GMP levels increased to 1,084 ± 177 ($N = 5$) pmol cGMP/mg protein after 8 min.

Effect of Adding 5 mM IBMX to Ouabain-treated Retinas

In our tracer flux experiments we were able to observe a light-dependent accumulation of radioactivity in low $[Ca^{2+}]_o$ and in normal $[Ca^{2+}]_o$ if IBMX was added to the incubation medium. From the results presented thus far, it is clear that cyclic GMP levels are increased in low Ca^{2+}. We have found that cyclic GMP concentration also is increased in normal $[Ca^{2+}]_o$ (with ouabain) when we added 5 mM IBMX to the bath. In three retinas, cyclic GMP concentration in normal Ca^{2+}, 0.5 mM ouabain was 43.5 ± 6.9 ($N = 3$) pmol cGMP/mg protein. After a 4–5-min exposure to 5 mM IBMX, the incubation time used in our tracer experiments, the concentration increased to 151 ± 16 ($N = 3$) pmol/mg protein.

Effect of 20 mM Ca^{2+} on Cyclic GMP

In the previous report, we showed that the light-dependent permeability can be suppressed by exposing the retina either to a saturating illumination or to 20 mM Ca^{2+}. To investigate this phenomenon in more detail, we have compared the effects of bright light and high Ca^{2+} on cyclic GMP levels. Those results are shown in Fig. 2. In Fig. 2A, the open circles show the effect of increasing Ca^{2+} from 1.8 to 20 mM. The average cyclic GMP content decreased 10–15% within 20 s of changing to a 20 mM Ca^{2+} Ringer's solution, from 62.0 ± 4.0 ($N = 4$) pmol cGMP/mg protein to 52.2 ± 3.5 ($N = 4$) pmol cGMP/mg protein. When we added 20 mM Ca^{2+} to retinas bathed in 10^{-8} M Ca^{2+}, ouabain solution (Fig. 2B) cyclic GMP decreased 60% in 2 min, from 1,431 ± 309 ($N = 2$) to 582 ± 136 ($N = 2$) pmol cGMP/mg protein. The time course of cyclic GMP decrease when high $[Ca^{2+}]_o$ is added to retina bathed in 10^{-8} M Ca^{2+} is very similar to the time course of cyclic GMP

increase when Ca^{2+} is decreased (see Fig. 1A), which suggests that restoring Ca^{2+} simply reverses the effect of lowering Ca^{2+}. The dotted lines in Fig. 2 show the effects of a saturating illumination (3.4×10^6 rhodopsins bleached per receptor per second) on cyclic GMP levels. These data are taken from Fig.

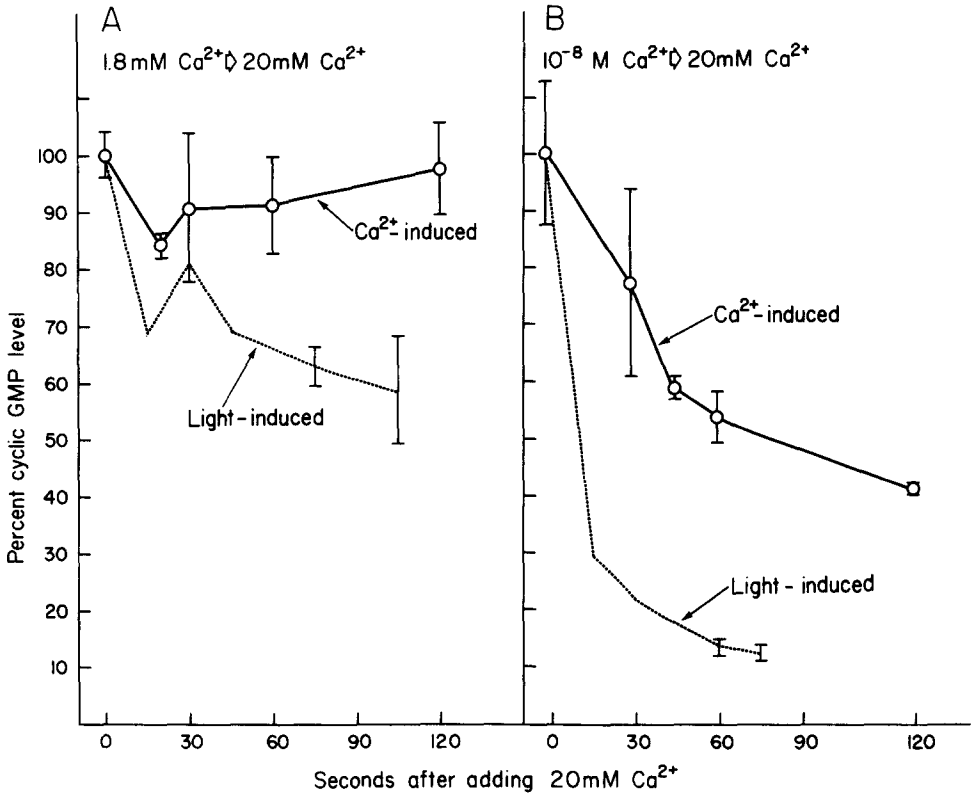


FIGURE 2. The effect of high extracellular Ca^{2+} on cyclic GMP levels in retinas incubated in normal Ringer's solution (A) or in low-Ca^{2+} Ringer's solution (B). Retinas were prepared and incubated in normal Ringer's solution as in Fig. 1, and then they were either left in normal Ringer's solution or incubated in low-Cl^-, low-Ca^{2+} Ringer's solution (solution F). At time zero on the abscissa they were exposed to solution I containing 20 mM Ca^{2+}. The initial concentrations of cyclic GMP were (in picomoles per milligram of protein): 1.8 mM Ca^{2+} = 62.0 (± 4.0, SEM, $N = 4$) and 10^{-8} M Ca^{2+} = 1,431 (±180, SEM, $N = 2$). The dotted lines in A and B indicate the decreases in cyclic GMP observed with light bleaching 3.4×10^6 rhodopsin molecules/receptor·s. The data are taken from Figs. 3*c* and *f*.

3. Notice that the decrease in cyclic GMP is much smaller in 20 mM Ca^{2+} than in bright light, even though the light-dependent permeability is completely suppressed in both cases (Woodruff et al., 1982). The decrease in cyclic GMP produced by 20 mM Ca^{2+} more closely resembles the effect produced by much dimmer light (e.g., 3.6×10^2 rhodopsin bleached per receptor per

second; Figs. 3*b* and *c*). We also have observed that 20 mM Ca^{2+} decreased cyclic GMP levels in IBMX-treated retinas from 151 pmol cGMP/mg protein (see above) to 74 ± 17 ($N = 3$) pmol cGMP/mg protein after 3 min. The time course of this effect was not investigated in detail.

Effect of Illumination on Cyclic GMP

The effects of light of various intensities on retinal cyclic GMP levels in both normal Ringer's solution and in low-Ca^{2+} Ringer's solution ($[Ca^{2+}]_o = 10^{-8}$

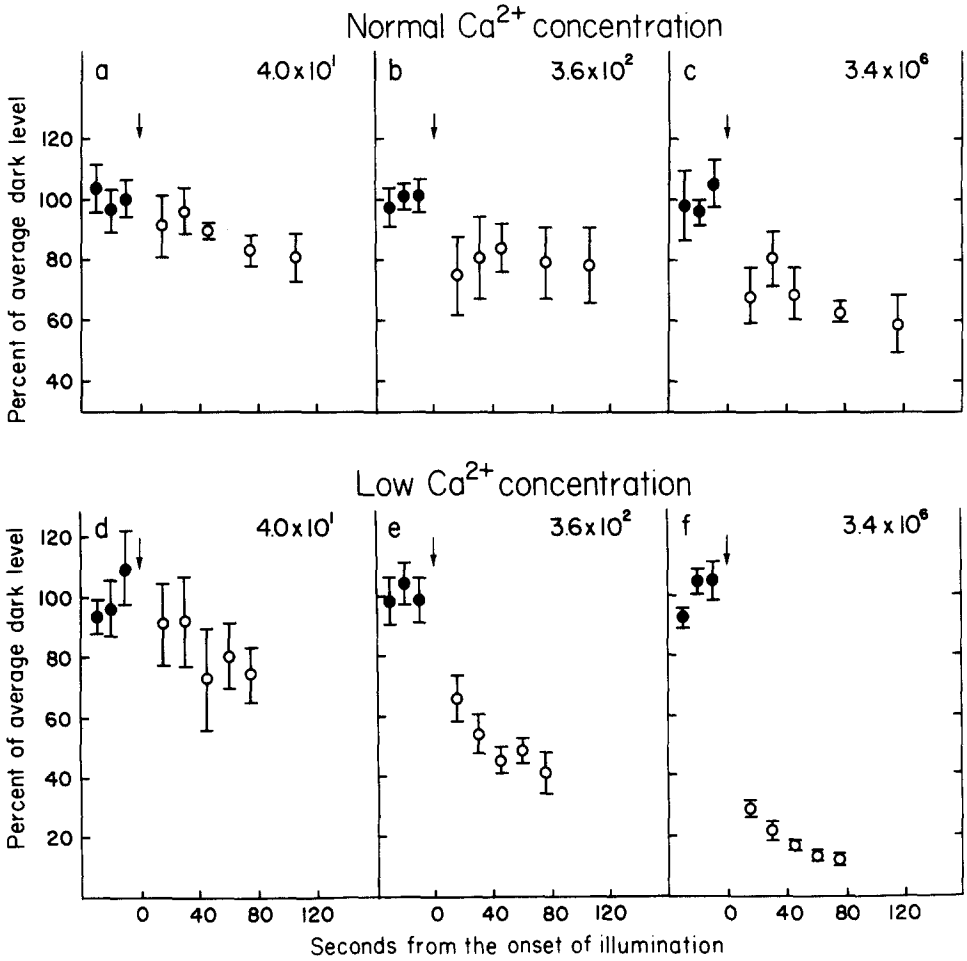


FIGURE 3. The effects of illumination on cyclic GMP concentration in retinas in normal Ringer's solution (*a–c*) and low-Ca^{2+} solution (*d–f*). All final incubation solutions contained 5 mM aspartate. The numbers in the top right-hand corner of each figure is the number of rhodopsin molecules bleached in each receptor per second. The arrows mark the onset of continuous illumination. The original concentrations of cyclic GMP were (in picomoles per milligram of protein): (*a*) 50.2, (*b*) 51.0, (*c*) 60.6, (*d*) 1,153, (*e*) 1,326, and (*f*) 1,164. The data represent the mean (± SEM) of four or more determinations.

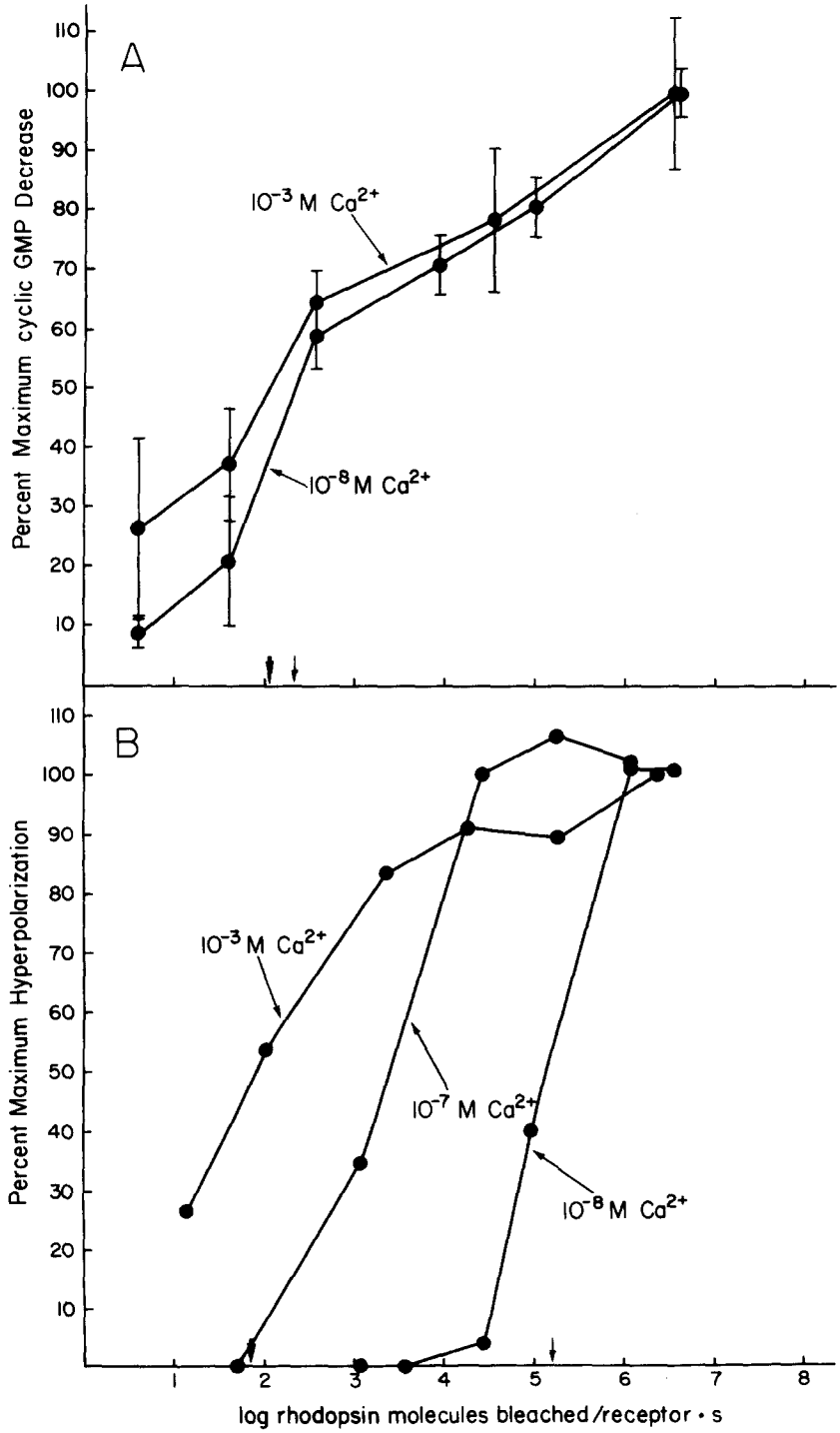
M) are shown in Fig. 3. In these experiments, 5 mM Na aspartate was included in the incubation solution. As mentioned in the previous paper (Woodruff et al., 1982), aspartate depolarizes horizontal and bipolar cells and eliminates the light-dependent activity in retinal cells proximal to the photoreceptors, so that the effect of illumination in these experiments most likely represents photoreceptor behavior.

At the lowest light intensity shown, which bleached 40 rhodopsin molecules/receptor·s, there was a small but significant decrease in cyclic GMP in normal Ringer's solutions (Fig. 3*a*). When we increased the light intensity 10-fold, the cyclic GMP decrease was larger, decreasing by 20% (Fig. 3*b*). Increasing the light intensity to 3.4×10^6 rhodopsin molecules bleached/receptor·s, a value that results in complete saturation in the photoreceptor voltage response (Fain, 1976) ("increment saturation") and eliminates the dark current (Baylor et al., 1979), decreased cyclic GMP in normal Ringer's solution from 60.6 ± 2.9 ($N = 4$) to 40.6 ± 1.7 ($N = 4$) pmol cGMP/mg protein, or by ~30–40% (Fig. 3*c*).

In low-Ca^{2+} Ringer's solution (10^{-8} M), cyclic GMP levels also were decreased by illumination. A light bleaching 40 rhodopsin molecules/receptor·s produced a small decrease in cyclic GMP levels amounting to ~10–15% at the most (Fig. 3*d*). A 10-fold-higher light intensity decreased cyclic GMP by ~55% (Fig. 3*c*). An even higher illumination bleaching 3.4×10^6 rhodopsin molecules/receptor·s, reduced cyclic GMP levels by 85%. In the latter case, this decrease amounted to a drop from 1,164 ± 47 ($N = 4$) to 168 ± 14 ($N = 4$) pmol cGMP/mg protein.

Fig. 4A shows the intensity-response functions for the light-dependent decrease in cyclic GMP in normal Ringer's and low-Ca^{2+} Ringer's solution. The two functions are normalized with respect to one another by expressing the decreases observed in normal and low Ca^{2+} as percentages of the decrease in the brightest illumination (see Figs. 3*c* and *f*). There is no significant difference in the light sensitivity of the cyclic GMP decrease when retinas are incubated in normal or low $[Ca^{2+}]_o$. In normal Ringer's solution, the half-maximal decrease in cyclic GMP was observed when light bleached ~100 rhodopsin molecules/receptor·s, and in 10^{-8} M Ca^{2+} this intensity increased somewhat to ~200 rhodopsin molecules/receptor·s. However, this difference is within our experimental error.

The large shift in light sensitivity of the toad-rod voltage response when $[Ca^{2+}]_o$ is lowered from 1.8×10^{-3} to 10^{-8} M is shown for comparison in Fig. 4B. The data are taken from Bastian and Fain (1982). The normalized peak response to steps of light (continuous illumination) is plotted as a function of light intensity. In normal Ringer's solution, half of the maximum hyperpolarization can be obtained with a light that bleaches ~85 rhodopsin molecules/receptor·s, a value very close to the intensity that reduced cyclic GMP by half. If retinas are incubated in 10^{-7} M Ca^{2+}, a half-maximal response is produced by bleaching 2.5×10^3 rhodopsin molecules/receptor·s; and in 10^{-8} M Ca^{2+}, by a light that bleaches 1.3×10^5 rhodopsin molecules/receptor·s. When $[Ca^{2+}]_o$ is lowered from 1.8×10^{-3} (normal Ringer's solution) to 10^{-8}


A
Percent Maximum cyclic GMP Decrease
10^{-3} M Ca^{2+}
10^{-8} M Ca^{2+}
B
Percent Maximum Hyperpolarization
10^{-3} M Ca^{2+}
10^{-7} M Ca^{2+}
10^{-8} M Ca^{2+}
log rhodopsin molecules bleached/receptor · s

M, the voltage response mechanism is desensitized by 1,600-fold, even though the sensitivity of the light-dependent decrease in cyclic GMP is not significantly changed.

DISCUSSION

Basal Level of Cyclic GMP

The level of cyclic GMP in dark-adapted toad retinas in normal Ringer's solution, 55–70 pmol/mg protein, is similar to the amount found by other investigators in other vertebrate retinas (see Table II). The differences in the values that are measured, which range from 25 (frog, *Rana esculenta*; Goridis et al., 1977) to 90 (mouse; Cohen et al., 1978) pmol/mg protein, may be caused by differences in species or to experimental protocol. In some of the published reports, the amount of rhodopsin in the experimental samples also is presented or can be calculated from the information given. We have normalized the cyclic GMP content to rhodopsin in these cases, and this is also shown in Table II. Most of the retinal cyclic GMP is probably in the photoreceptors. Orr et al. (1976) microdissected rabbit retinas and were able to show that ~90% of retinal cyclic GMP is found in the photoreceptors. This confirmed the earlier microdissection studies of Farber and Lolley (1974) and Lolley and Farber (1976) who found, respectively, 60% and 80% of mouse and rat retina

FIGURE 4. A. Intensity-response function for the light-dependent cyclic GMP decrease in normal and low-Ca^{2+} Ringer's solutions. Retinas were prepared and incubated in normal Ringer's solution (indicated as 10^{-3} M) or in low-Ca^{2+} Ringer's solution (solution F, indicated as 10^{-8} M) as in Figs. 2 and 3. They were then exposed to continuous illumination bleaching various amounts of pigment, as indicated on the abscissa. The maximum decrease was obtained in both normal and low-Ca^{2+} solutions with illumination bleaching 3.4×10^6 rhodopsin molecules/receptor·s (shown in Figs. 3*c* and *f*, respectively). The dark level of cyclic GMP was taken as the average of the three data points before the onset of illumination. The magnitude of the light effect was determined by comparing the dark-adapted level to the average level of cyclic GMP in the five retinal sections after the onset of illumination in each retina. The data are the mean (± SEM) of three or more determinations. The thick and thin arrows on the abscissa show the light intensity necessary for obtaining 50% of the maximum response in normal and low-Ca^{2+} Ringer's solutions, respectively. B. The intensity-response functions for the toad rod light-induced hyperpolarization. Retinas were incubated in normal toad Ringer's solution (solution A, indicated as 10^{-3} M) or in low-Ca^{2+} solutions containing 10^{-7} or 10^{-8} M Ca^{2+} (solutions D and F, respectively). The data were taken from Bastian and Fain (1982). Plotted are the normalized responses to steps of light (continuous illumination) at various light intensities. The peak of the hyperpolarizing responses was used for calculating the data. If the maintained voltage at each light intensity was used instead, that is, the "plateau" voltage (see Fain, 1976), the normalized intensity-response curves are not different from those shown here for the peak. The arrows on the abscissa are analogous to the arrows in A.

cyclic GMP in the photoreceptor layer. The data in Table II show that the variance in cyclic GMP concentration between retinas is smaller if cyclic GMP is normalized with respect to rhodopsin content instead of total retinal protein content, which is also consistent with the location of most of the cyclic GMP in the photoreceptors. Furthermore, the effect of light, low $[Ca^{2+}]_o$, and phosphodiesterase inhibitors on cyclic GMP content is the same for whole retinas and isolated rod outer segments (for example, compare Cohen et al., 1978 with Woodruff et al., 1977 and Woodruff and Bownds, 1979).

TABLE II

CYCLIC GMP CONTENT IN VARIOUS RETINAS

Investigation	Animal	cGMP	cGMP	Low-Ca^{2+}-induced increase	Maximum light-induced decrease
		pmol/mg protein	*mol/mol rhodopsin*		%
This study	Toad (*Bufo marinus*)	55–70	0.010–0.012	20×	35–40
Goridis et al. (1974)	Calf	64	—	—	70
	Frog (*Rana esculenta*)	28	0.014	—	67
Lolley and Farber (1976)	Rat	37	—	—	—
Goridis et al. (1977)	Frog (*R. esculenta*)	25.0	0.012–0.013	—	56
Farber and Lolley (1977)	Mouse	45–55	—	—	50
Cohen et al. (1978)	Mouse	68–90	—	20×	56
Woodruff and Bownds (1979)*	Frog (*R. catesbeiana*)	—	0.010	0.5×	40
Kilbride (1980)	Frog (*R. catesbeiana*)	41	0.007	10×	30

* Isolated rod outer segments.

Effect of Low Ca^{2+}

The 20-fold increase in cyclic GMP in toad retinas exposed to low $[Ca^{2+}]_o$ (Fig. 1) is similar to results for other retinas (Table II). Cohen et al. (1978) showed that incubation of mice retinas in 3 mM EGTA increases cyclic GMP levels by 20-fold. Kilbride (1980) obtained a 10-fold increase in cyclic GMP under similar conditions in frog (*R. catesbeiana*). When *R. catesbeiana* rod outer segments are first removed from the retina and then exposed to 3 mM EGTA, the increase in cyclic GMP is much more modest, amounting to only 50% over normal levels (Woodruff and Bownds, 1979). This same small increase was obtained by Polans et al. (1981). Although this might seem to indicate that a significant proportion of the low-$[Ca^{2+}]_o$-induced increase in cyclic GMP in whole retina is in cells other than the photoreceptors, we think that this is unlikely. Our reason for believing this is shown in Fig. 3*f*, where

>85% of the retinal cyclic GMP in low-Ca^{2+}-treated retinas is affected by illumination in the presence of 5 mM Na aspartate. In the presence of aspartate, only photoreceptors should show light-dependent changes, and this suggests that most of the retinal cyclic GMP must have been in the receptor cells, even in low-Ca^{2+} solutions. The low-$[Ca^{2+}]_o$-induced increase in rod outer segment cyclic GMP may be smaller because isolated outer segments have lost an important Ca^{2+}-regulating mechanism.

The kinetics of the low-Ca^{2+}-induced increase in cyclic GMP seems to be different for amphibian and mice retinas. We obtain a slow, steady increase that reaches a maximum after 4–7 min, depending on the concentration to which Ca^{2+} is reduced, followed by a fairly steady level after this increase. Kilbride (1980) obtained the same result in frog retinas. However, Cohen (1978) found that mouse cyclic GMP increased much more rapidly, reaching a maximum value (>1,000 pmol cGMP/mg protein) after only 2 min and then declining to a level only one-third of the maximum by 10 min.

The concentration dependence of the low-Ca^{2+}-induced increase in cyclic GMP was not determined by Cohen et al. (1978) or by Kilbride (1980). However, Polans et al. (1981) showed that cyclic GMP in frog isolated rod outer segment increased sharply between 10^{-8} and 10^{-9} M Ca^{2+}. They saw no increase in cyclic GMP above 10^{-8} M Ca^{2+}. As shown in Fig. 1B, toad cyclic GMP is also a sharp function of $[Ca^{2+}]_o$, but the effect is observed at higher levels of $[Ca^{2+}]_o$, most of the increase occurring between 10^{-7} and 10^{-8} M. We cannot say whether this 1-log-unit difference in Ca^{2+} sensitivity is caused by a difference in species (*B. marinus* vs. *R. catesbeiana*) or in the preparation (isolated rod outer segments vs. intact retinas).

For the $[Ca^{2+}]_o$-dependent cyclic GMP increase shown in Fig. 1B, we calculate a Hill coefficient of ~2.6. It has been shown that Ca^{2+} inhibits guanylate cyclase (Lolley and Rasc, 1981) and activates cyclic GMP phosphodiesterase (Robinson et al., 1980; Kawamura and Bownds, 1981), the enzymes that synthesize and degrade cyclic GMP, respectively. The steep relation between $[Ca^{2+}]_o$ and [cGMP] could result from the effect of Ca^{2+} on both these enzymes.

Light-dependent Decrease in Cyclic GMP

In toad retinas incubated in normal Ringer's solution we could decrease cyclic GMP levels by ~40% with a continuous light that bleached >3 × 10^6 rhodopsin molecules/receptor·s (Fig. 3*c*). Table II compares this with the light-dependent decreases observed in several other vertebrate retinas. The differences observed range from a 30% decrease in frog (*R. catesbeiana*) (Kilbride, 1980) to a 70% decrease in calf (Goridis et al., 1974). All the effects of illumination shown in this report were determined in retinas treated with 5 mM aspartate. Under these conditions it seems likely that most, if not all, of our light-induced decreases are caused by changes in photoreceptors. We find that aspartate, although it eliminates the response of cells proximal to the photoreceptors, has no effect on the basal level of cyclic GMP or the magnitude

of the low-$[Ca^{2+}]_o$-induced increase in cyclic GMP. Goridis et al. (1977) and Cohen et al. (1978) also found that the basal cyclic GMP levels and the light-induced changes in cyclic GMP levels were not affected by aspartate treatment.

Comparison between Cyclic GMP and Photoreceptor Light-dependent Permeability

Both Ca^{2+} and cyclic GMP have been proposed to be an internal messenger that regulates the conductance of the photoreceptor light-dependent permeability (see Hubbell and Bownds, 1979). Previous results have, in many cases, been difficult to interpret because changing Ca^{2+} clearly changes cyclic GMP levels and, as discussed in the previous paper, cyclic GMP may influence $[Ca^{2+}]_i$. However, our experiments show four situations in which Ca^{2+} has an effect on the photoreceptor light-dependent permeability that cannot be readily explained by Ca^{2+}-induced effects on cyclic GMP.

First, when $[Ca^{2+}]_o$ is reduced from 10^{-3} to 10^{-7} M, the membrane light-dependent permeability is increased, but there is no detectable change in cyclic GMP concentration. The change in resting potential of the photoreceptor shown in Fig. 1B occurs concomitantly with a decrease in the input resistance of the photoreceptor in turtle cones (Bertrand et al., 1978) and a large increase in the receptor "dark current" (Yoshikami and Hagins, 1971). It is therefore most likely caused by an increase in the light-dependent permeability in the receptor outer segments. Consistent with this, Wormington and Cone (1978) used osmotic swelling of isolated rod outer segments to demonstrate that a decrease in $[Ca^{2+}]_o$ from 10^{-5} to 10^{-7} M causes a significant increase in light-dependent sodium permeability. Recently, Yau et al. (1981) showed that replacing 1 mM Ca^{2+} with 10^{-5} M Ca^{2+} increased the rod outer segment light-sensitive current in toad from 20 to 170 pA, and similar results have been obtained by Greenblatt (1982).

It seems unlikely that cyclic GMP, which does not increase when $[Ca^{2+}]_o$ is lowered to 10^{-7} M, is involved in the increase in light-dependent permeability. It is possible that an increase in cyclic GMP in photoreceptors might be unnoticed in our experiments if it were balanced by a decrease in cyclic GMP in nonphotoreceptor cells in the retina. However, Polans et al. (1981) have shown that there is no cyclic GMP increase in rod outer segments isolated from frog retinas until Ca^{2+} is lowered below 10^{-8} M. Thus, it appears that lowering Ca^{2+} can increase the light-dependent permeability without affecting cyclic GMP levels.

Second, when retinas are exposed to 20 mM Ca^{2+}, the light-dependent permeability is closed. However, there is only a small decrease in cyclic GMP levels, less than half that observed with bright illumination (see Fig. 2). High $[Ca^{2+}]_o$ (20 mM) produces a rapid 30–40-mV hyperpolarization of the rod membrane potential and abolishes intracellularly recorded voltage responses, the dark current, and extracellular photocurrents (G. L. Fain and H. M. Gerschenfeld, unpublished observations; Yoshikami and Hagins, 1973). In 20 mM Ca^{2+} it seems likely that most of the light-sensitive channels are closed. However, 20 mM Ca^{2+} produces only a small change in the cyclic GMP levels,

a change comparable to that observed when the retina is exposed to light that bleaches a few hundred rhodopsin molecules in each receptor per second (see Fig. 3*b*). This is more than three orders of magnitude below the intensity necessary to saturate the voltage response ("increment saturation," see Fain, 1976) or to abolish the dark current (Yau et al., 1977).

Third, when 20 mM Ca^{2+} is added to retinas incubated in 10^{-8} M Ca^{2+} Ringer's solution, the light-dependent permeability closes (Woodruff et al., 1982) and cyclic GMP levels decrease; however, the cyclic GMP decrease occurs too slowly to be responsible for the permeability decrease. The permeability decrease produced by high $[Ca^{2+}]_o$ occurs nearly as rapidly as that induced by light (G. L. Fain and H. M. Gerschenfeld, unpublished observations; Yoshikami and Hagins, 1973, 1980). Increasing Ca^{2+} from 10^{-7} or 10^{-8} M to millimolar levels produces a rapid hyperpolarization of the membrane potential and reduction of the dark current, and both voltage responses and the light-sensitive photocurrent are suppressed (Bastian and Fain, 1979; Yau et al., 1981). Fig. 2B in this report shows that, in contrast, cyclic GMP levels are reduced much more slowly by high Ca^{2+}.

The cyclic GMP decrease probably occurs too slowly for it to be responsible for the high-$[Ca^{2+}]_o$-induced decrease in conductance. A comparison of Figs. 4A and B shows that, in 10^{-8} M Ca^{2+}, low and intermediate light intensities decrease cyclic GMP content, but those same lights have no effect on the membrane potential. Light levels that decrease cyclic GMP by 75% of the maximum decrease produce a just-detectable hyperpolarization of the membrane potential. This would represent a decrease in cyclic GMP from >1,000 pmol/mg protein to between 450 and 650 pmol/mg protein. Fig. 2B shows that cyclic GMP is reduced to a value <450–650 pmol/mg protein quickly by a saturating illumination, but a decrease in cyclic GMP to this level takes ~90 s when retinas are exposed to high $[Ca^{2+}]_o$. If the change in cyclic nucleotide concentration were directly responsible for closing the channels, the effects of high Ca^{2+} on cyclic GMP levels should occur much more quickly than this.

Fourth, exposing retinas to low $[Ca^{2+}]_o$ causes no significant change in the sensitivity of the light-induced cyclic GMP decrease (Fig. 4A), but causes a large decrease in sensitivity of the light-induced voltage response of the photoreceptor (Fig. 4B). Woodruff and Bownds (1979) demonstrated in rod outer segments that the sensitivity of the light-dependent cyclic GMP decrease was reduced ~10-fold by low $[Ca^{2+}]_o$, and recently Robinson et al. (1980) and Kawamura and Bownds (1981) showed that low $[Ca^{2+}]_o$ reduces the sensitivity of the light-activated cyclic GMP phosphodiesterase 10-fold. The data in Fig. 4A show that the sensitivity of the cyclic GMP decrease may be slightly reduced by exposure to low Ca^{2+} and are not inconsistent with the 10-fold change found in outer segments. However, the reduction in sensitivity of the voltage response at this Ca^{2+} level is three to four orders of magnitude.

The first three observations above indicate that the light-dependent permeability can be changed without a change in cyclic GMP. The data in Fig. 4 show the converse, that large changes in cyclic GMP can occur without corresponding decreases in permeability. This is easily observed in 10^{-8} M

Ca^{2+}, where a light intensity that bleaches 1,000 rhodopsin molecules/receptor·second gives 60% of the maximum decrease in cyclic GMP, but no voltage change. In 10^{-7} M Ca^{2+}, if we assume that the sensitivity of the light-induced cyclic GMP decrease is the same as it is at 10^{-3} M Ca^{2+} (and 10^{-8} M Ca^{2+}), a light bleaching 100 rhodopsin molecules/receptors·second would decrease cyclic GMP 20–30% of the maximum with no voltage change.

The data in Fig. 4 were normalized to show relatively decreases in cyclic GMP. If the absolute amount of cyclic GMP were the important factor controlling the light-dependent permeability then, in 10^{-8} M Ca^{2+}, when cyclic GMP levels are increased 10- to 20-fold, one might imagine that the photoreceptor voltage response might be "desensitized" because relatively more light would be required to bring the cyclic GMP levels back down into the physiologically relevant concentration range. However, this same argument cannot be used for retinas in 10^{-7} M Ca^{2+} where there is no significant increase in cyclic GMP but the voltage response is desensitized by 1.5 log units. In fact, we were unable to observe any simple correlation between the level of cyclic nucleotides and sensitivity. When Ca^{2+} is lowered from 10^{-8} to 10^{-9} M, the sensitivity of the voltage response decreases ~1.0 log unit (Bastian and Fain, 1982) but the cyclic GMP concentration is unchanged (see Fig. 1).

We draw two conclusions from these comparisons. First, there is no direct correlation between permeability of the light-dependent channel and the photoreceptor cyclic GMP levels; and second, Ca^{2+} is able to modulate the light-dependent permeability either by itself or through some mechanism not related to cyclic GMP. In drawing these conclusions, we have assumed that the major effects of changing extracellular [Ca^{2+}] are produced by some mechanism inside the rod, rather than outside on the external surface of the membrane. The evidence for this assumption is that exposure of rods to ionophores such as X537A or A23187, which increase the Ca^{2+} permeability of the plasma membrane, greatly alter the sensitivity of the rods to changes in external Ca^{2+} (Hagins and Yoshikami, 1974; Wormington and Cone, 1978; Bastian and Fain, 1979). These results are most easily interpreted if Ca^{2+} is assumed to be acting internally. We cannot discount the possibility that, under some circumstances, Ca^{2+} can affect the opening and closing of the light-dependent permeability from the external surface of the membrane (see for example, Yau et al., 1981). However, it is difficult to imagine how Ca^{2+} acting externally could produce large changes in the sensitivity of the light response (see Fig. 4B).

Our results are consistent with the proposal of Yoshikami and Hagins (1971) that Ca^{2+} directly regulates the permeability of the light-dependent channel; however, they are not inconsistent with the notion that changes in cyclic GMP indirectly influence the channel permeability. For example, cyclic GMP may regulate the permeability by modulating the intracellular Ca^{2+} concentration, with increases in cyclic GMP lowering $[Ca^{2+}]_i$ and decreases in cyclic GMP increasing $[Ca^{2+}]_i$. This would explain the observations that, first, injecting cyclic GMP into photoreceptors depolarizes the cell membrane potential and increases the latency of the light responses (Miller and Nicol,

1979); and second, that phosphodiesterase inhibitors applied externally depolarize the photoreceptor (Lipton et al., 1977*b*) and increase the light-dependent permeability (Brodie and Bownds, 1976; Woodruff et al., 1982). Changes in cyclic GMP may change the intracellular Ca^{2+} concentration, either by causing Ca^{2+} release from the disks or from other sites or by modulating Ca^{2+} uptake into internal stores. Since the decrease in cyclic GMP is rapid and occurs over the same range of light intensities as the photoreceptor light responses and the light-dependent increase in Ca^{2+} efflux from rods (compare our Fig. 4 with Fig. 5 of Gold and Korenbrot, 1980), it is conceivable that the decrease in cyclic GMP is responsible for triggering a release of Ca^{2+} during transduction. This hypothesis, though consistent with our experiments, must await confirmation from further experimentation.

We thank Susan Callery for excellent technical assistance.
This research was supported by National Institutes of Health EY 05359 (MLW), EY 01844 (GLF), EY 00331, and by a Bob Hope Award of Fight For Sight, Inc., New York.

Received for publication 4 November 1981 and in revised form 10 June 1982.

REFERENCES

BASTIAN, B. L. and G. L. FAIN. 1979. Light adaptation in toad rods: requirement for an internal messenger which is not calcium. *J. Physiol. (Lond.)*. **297**:493–520.

BASTIAN, B. L., and G. L. FAIN. 1982. The effects of low Ca^{2+} and background light on the sensitivity of toad rods. *J. Physiol (Lond.)*. In press.

BAYLOR, D. A., T. D. LAMB, and K.-W. YAU. 1979. The membrane current of style rod outer segments. *J. Physiol. (Lond.)*. **288**:589–611.

BERTRAND, D., M. G. F. FUORTES, and J. POCHOBRADSKY. 1978. Actions of EGTA and high calcium on the cones in the turtle retina. *J. Physiol. (Lond.)*. **275**:419–437

BRODIE, A. N., and D. BOWNDS. 1976. Biochemical correlates of adaptation processes in isolated frog photoreceptor membranes. *J. Gen. Physiol.* **68**:1–11.

BROWN, J. E., J. A. COLES, and L. H. PINTO. 1977. Effects of injections of calcium and EGTA into the outer segments of retinal rods of *Bufo marinus*. *J. Physiol. (Lond.)*. **269**:707–722.

CALDWELL, P. C. 1970. Calcium chelation and buffers. *In* Calcium and Cellular Function. A. W. Cuthbert, editor. St. Martins, London. 10–16.

COHEN, A. I., I. A. HALL, and J. A. FERRENDELLI. 1978. Calcium and cyclic nucleotide regulation in incubated mouse retinas. *J. Gen. Physiol.* **71**:595–612.

FAIN, G. L. 1976. Sensitivity of toad rods: dependence on wave-length and background illumination. *J. Physiol. (Lond.)*. **261**:71–101.

FARBER, D. B., and R. N. LOLLEY. 1974. Cyclic guanosine monophosphate: elevation in degenerating photoreceptor cells of the C3H mouse retina. *Science (Wash. D. C.)*. **186**:449–451.

FARBER, D. B., and R. N. LOLLEY. 1977. Light-induced reduction in cyclic GMP of retinal photoreceptor cells *in vivo*: abnormalities in the degenerative diseases of RCS rats and *rd* mice. *J. Neurochem.* **28**:1089–1095.

GOLD, G. H., and J. I. KORENBROT. 1980. Light-induced calcium release by intact retinal rods. *Proc. Natl. Acad. Sci. U. S. A.* **77**:5557–5561.

GORIDIS, C., P. F. URBAN, and P. MANDEL. 1977. The effect of flash illumination on the

endogenous cyclic GMP content of isolated frog retinae. *Exp. Eye Res.* **24:**171–177.

GORIDIS, C., N. VIRMAUX, H. L. CAILLA, and M. A. DELAAGE. 1974. Rapid, light-induced changes in retinal cyclic GMP levels. *FEBS Lett.* **49**(2):167–169.

GREENBLATT, R. E. 1982. Adapting lights and lowered extracellular free calcium desensitized toad photoreceptors by differing mechanisms. *J. Physiol. (Lond.)*. In press.

HAGINS, W. A., and S. YOSHIKAMI. 1974. A role for Ca^{2+} in excitation of retinal rods and cones. *Exp. Eye Res.* **18:**299–305.

HUBBELL, W. L., and M. D. BOWNDS. 1979. Visual transduction in vertebrate photoreceptors. *Annu. Rev. Neurosci.* **2:**17–34.

KAWAMURA, S., and M. D. BOWNDS. 1981. Light adaptation of the cyclic GMP phosphodiesterase of frog photoreceptor membranes mediated by ATP and calcium ions. *J. Gen. Physiol.* **77:**571–591.

KILBRIDE, P. 1980. Calcium effects on frog retinal cyclic quanosine 3′,5′-monophosphate levels and their light-initiated rate of decay. *J. Gen. Physiol.* **75:**457–465.

LIPTON, S. A., S. E. OSTROY, and J. E. DOWLING. 1977*a*. Electrical and adaptive properties of rod photoreceptors in *Bufo marinus*. I. Effects of altered extracellular Ca^{2+} levels. *J. Gen. Physiol.* **70:**747–770.

LIPTON, S. A., H. RASMUSSEN, and J. E. DOWLING. 1977*b*. Electrical and adaptive properties of rod photoreceptors in *Bufo marinus*. II. Effects of cyclic nucleotides and prostaglandins. *J. Gen. Physiol.* **70:**771–791.

LOLLEY, R. N., and D. B. FARBER. 1976. A proposed link between debris accumulation, guanosine 3′,5′ cyclic monophosphate changes and photoreceptor cell degeneration in retina of RCS rats. *Exp. Eye Res.* **22:**477–486.

LOLLEY, R. N., and E. RASC. 1981. Calcium regulation of receptor guanylate cyclase activity. *Invest. Ophthalmol. Vis. Sci.* (Suppl.) **20**(3):210.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193:**265–275.

MILLER, W. H., and G. D. NICOL. 1979. Evidence that cyclic GMP regulates membrane potential in rod photoreceptors. *Nature (Lond.)*. **280:**64–66.

ORR, H. T., O. H. LOWRY, A. I. COHEN, and J. A. FERRENDELLI. 1976. Distribution of 3′:5′-cyclic AMP and 3′:5′-cyclic GMP in rabbit retina *in vivo*: selective effects of dark and light adaptation and ischemia. *Proc. Natl. Acad. Sci. U. S. A.* **73:**4442–4445.

POLANS, A. S., S. KAWAMURA, and M. D. BOWNDS. 1981. Influence of calcium on guanosine 3′,5′-cyclic monophosphate levels in frog rod outer segments. *J. Gen. Physiol.* **77:**41–48.

ROBINSON, P. R., S. KAWAMURA, B. ABROMSON, and M. D. BOWNDS. 1980. Control of the cyclic GMP phosphodiesterase of frog photoreceptor membranes. *J. Gen. Physiol.* **76:**631–645.

WOODRUFF, M. L., and M. D. BOWNDS. 1979. Amplitude, kinetics, and reversibility of a light-induced decrease in guanosine 3′,5′-cyclic monophosphate in frog photoreceptor membranes. *J. Gen. Physiol.* **73:**629–653.

WOODRUFF, M. L., M. D. BOWNDS, S. H. GREEN, J. L. MORRISEY, and A. SHEDLOVSKY. 1977. Guanosine 3′,5′ cyclic monophosphate and the *in vitro* physiology of frog photoreceptor membranes. *J. Gen. Physiol.* **69:**667–679.

WOODRUFF, M. L., G. L., FAIN, and B. L. BASTIAN. 1982. Light-dependent ion influx into toad photoreceptors. *J. Gen. Physiol.* **80:**517–536.

WORMINGTON, C. M., and R. A. CONE. 1978. Ionic blockage of the light-regulated sodium channels in isolated rod outer segments. *J. Gen. Physiol.* **71:**657–681.

YAU, K.-W., T. D. LAMB, and D. A. BAYLOR. 1977. Light-induced fluctuations in membrane

current of single toad rod outer segments. *Nature* (*Lond.*). **269:**78–80.

YAU, K.-W., P. A. MCNAUGHTON, and A. L. HODGKIN. 1981. Effects of ions on the light-sensitive current in retinal rods. *Nature* (*Lond.*). **292:**502–505.

YOSHIKAMI, S., and W. A. HAGINS. 1971. Ionic basis of dark current and photocurrent of retinal rods. *Biophys. Soc. Annu. Meet. Abstr.* **10:**60*a*.

YOSHIKAMI, S., and W. A. HAGINS. 1973. Control of the dark current in vertebrate rods and cones. *In* Biochemistry and Physiology of Visual Pigments. H. Langer, editor. Springer-Verlag, New York. 245–255.

YOSHIKAMI, S, and W. A. HAGINS. 1980. Kinetics of control of the dark current of retinal rods by Ca^{++} and by light. *Fed. Proc.* **39:**1814. (Abstr.).