

In Situ cGMP Phosphodiesterase and Photoreceptor Potential in Gecko Retina

SATORU KAWAMURA and MOTOHIKO MURAKAMI

From the Department of Physiology, Keio University School of Medicine, Shinjuku-ku, Tokyo 160, Japan

ABSTRACT The possible involvement of phosphodiesterase (PDE) activation in phototransduction was investigated in gecko photoreceptors by comparing the in situ PDE activity with the photoreceptor potential. In the dark, intracellular injection of cGMP into a gecko photoreceptor caused a long-lasting depolarization. An intense light flash given during the depolarization phase repolarized the cell with a short latency comparable to that of the light-evoked hyperpolarizing response, which indicates that the activation of PDE in situ is rapid enough to generate the photoreceptor potential. PDE activity in situ was estimated quantitatively from the duration of the cGMP-induced depolarization, since it was expected that the higher the PDE activity, the shorter the duration. Under steady illumination, the enzyme exhibited a constant activity. On exposure to a light flash, PDE became activated, but recovered in the dark with a time course that was dependent on the intensity of the preceding stimulus. When PDE activity and photoreceptor sensitivity to light were measured in the same cell after a light flash, both recovery processes showed similar kinetics. Theoretical analysis showed that the parallelism in the recovery time courses could be explained if cGMP is the transduction messenger. These results suggest that PDE activation is involved not only in the generation but also in the adaptation mechanisms of the photoreceptor potential.

INTRODUCTION

Recent electrophysiological studies support the hypothesis that cGMP is the internal messenger in phototransduction in rods (Miller and Nicol, 1979; Kawamura and Murakami, 1983; MacLeish et al., 1984; Fesenko et al., 1985; Matthews et al., 1985). Since the hydrolytic enzyme of cGMP, cGMP phosphodiesterase (PDE), is activated on exposure to light (Miki et al., 1973), the level of PDE activation would be reflected in the level of cGMP in the light. Therefore, in order to elucidate the molecular mechanism of the light-evoked electrical response in rods, it is essential to know the behavior of PDE activity under various light conditions. However, PDE activity has been measured mainly in suspensions of purified disk membranes (Miki et al., 1973; Yee and Liebman,

Address reprint requests to Dr. Satoru Kawamura, Dept. of Physiology, Keio University School of Medicine, Shinjuku-ku, Tokyo 160, Japan.

1978). Since the plasma membrane was ruptured in these preparations, some essential soluble factor(s) may be lost and the PDE activity thus measured may not be relevant to that in situ.

In the present study, we tried to measure PDE activity in situ and then we compared its activity directly with the photoreceptor potential in gecko photoreceptors. In order to measure PDE activity in situ, cGMP was injected into the photoreceptor cell to induce a depolarization of the cell membrane (Miller and Nicol, 1979; Miller, 1982; Kawamura and Murakami, 1983). PDE activity was estimated from the duration of the depolarization, since the duration would be determined by the PDE activity, that is, the hydrolysis rate of the injected cGMP (Kawamura and Murakami, 1983).

The results of the present experiment suggested that cGMP is the transduction messenger and that PDE activation is involved not only in the mechanism of photoreceptor potential generation but also in the adaptation process of the gecko photoreceptors. A preliminary report on this work has been presented elsewhere (Kawamura and Murakami, 1985).

MATERIALS AND METHODS

Electrophysiology

In most of the electrophysiological experiments, geckos (*Gekko gekko*) were used. Geckos were dark-adapted for at least 3 h. The animals were decapitated and the eyes were enucleated. After the eye was hemisected, the posterior part was cut into several pieces and the retina was detached from the pigment epithelium under dim red light. The retina was placed with the photoreceptor side up on a filter paper moistened with Ringer solution (115 mM NaCl, 2.5 mM KCl, 2 mM MgCl₂, 1 mM CaCl₂, 10 mM HEPES, pH 7.8).

In most of the present studies, double-barreled microelectrodes were used (Kawamura and Murakami, 1983). One barrel, for cGMP injection, was filled with 25 mM cGMP dissolved in 500 mM potassium acetate (resistance, 400–600 M Ω), and the other barrel, for potential recording, was filled with 4 M potassium acetate (resistance, 200–400 M Ω). The typical input resistance was 100 M Ω (Kawamura and Murakami, 1983). The membrane potential was fed to a preamplifier (MEZ 8201, Nihon Koden, Tokyo, Japan) and the records were stored on FM tape for later analysis. Intracellular injection of cGMP was achieved by a negative current pulse supplied from a constant current source built into the preamplifier, but this method had some inconvenient aspects (see Results).

In some experiments comparing the intensity-response curves of the photoreceptor potential with PDE activation (Fig. 6), frogs (*Rana catesbeiana*) were used. The isolation of the frog retina and the measurement of the membrane potential were performed in a similar way as in gecko retina, except that the frog photoreceptor cell was penetrated with a single-barreled microelectrode filled with 4 M potassium acetate.

The light source was a 250-W tungsten lamp. In the experiment in gecko retina, we used an orange filter (>540 nm), and in frog retina, a yellow filter (>480 nm). Without attenuation, the light intensity was 2.4×10^6 rhodopsin molecules bleached per outer segment per second (rh/OS·s) in gecko retina and 2.5×10^6 rh/OS·s in frog retina.

Biochemical Measurement of PDE Activity

When necessary, we measured PDE activity biochemically using exogenous cGMP as the substrate (e.g., Fig. 6). In this measurement, rod outer segments were permeabilized so that the exogenous cGMP could have access to the PDE in the disk membrane. The

permeabilized outer segments were obtained by shaking a retina in a low-sodium solution that simulated the intracellular environment of the PDE. The composition of the solution was 10 mM NaCl, 110 mM KCl, 2 mM MgCl₂, 10 mM HEPES, pH 7.8. The calcium concentration was buffered to 10⁻⁹ M by adding 0.1 mM CaCl₂ and 2.78 mM EGTA (Polans et al., 1981).

PDE activity was measured with the pH assay method (Yee and Liebman, 1978; Kawamura and Bownds, 1981). This assay is based on the fact that hydrolysis of one cGMP molecule releases one proton and reduces the pH of the solution. Since the pH change is stoichiometrical in relation to the total number of protons released, it can be an indicator of PDE activity. Details of the manipulation are given elsewhere (Kawamura and Bownds, 1981; Kawamura, 1983). Briefly, however, 200 μ l of a suspension of permeabilized rod outer segments was made 0.5 mM in ATP and 0.5 mM in GTP so that the concentrations of the nucleotides in the suspension were similar to those in situ. cGMP was added to the suspension to make a final concentration of 4 mM. All of the above manipulations were carried out under infrared illumination with the aid of an infrared image converter (NVR 2015, NEC, Tokyo, Japan). When the rod outer segments were illuminated with a light flash, the pH of the suspension decreased. The pH change was monitored with a pH electrode (MI 410, Microelectrodes Inc., Londonderry, NH) and displayed on a pen chart recorder. PDE activity was calculated from a tangent of the pH trace.

cGMP (potassium salt) was purchased from PL Biochemicals (Milwaukee, WI) and the other chemicals were obtained from Nakarai (Kyoto, Japan).

RESULTS

Rapid Activation of PDE After a Light Flash

In the dark, an intracellular injection of cGMP produced a depolarization (record 1 in Fig. 1A), although the record during the current injection was not seen because of an imbalance in the recording system. The depolarization reached close to 0 mV (Kawamura and Murakami, 1983) and then slowly decayed, which indicates that the injected cGMP was slowly hydrolyzed and the internal cGMP concentration returned to the normal dark level. However, an intense light flash given during the decay phase shortened the duration and accelerated the repolarization kinetics (record 2), which suggests that the activated PDE in the light rapidly hydrolyzed the injected cGMP as well as the intrinsic cGMP. Record 3 in Fig. 1A shows the control photoreceptor potential evoked by a light flash of the same intensity and duration as those used in record 2. The subtraction of record 2 from record 1 gives the net "repolarizing effect" of the activated PDE. The result is shown by record 5 in Fig. 1B on an expanded time scale, together with the control photoreceptor potential (record 4). Fig. 1B shows that the latency of the repolarizing effect of PDE is almost the same (~100 ms) as that of the photoreceptor potential. In all of the eight cells tested, similar results were obtained. Therefore, the activation of PDE after a light stimulation occurred rapidly enough to meet the latency of the photoreceptor potential.

One might argue that the repolarizing effect was not caused by PDE activation but by the light-induced hyperpolarization in neighboring cells transmitted through electrical coupling between photoreceptors (see Gold, 1981, for a review). However, in the cells we used, this was not the main determinant of the repolarizing effect, for the following reason. Miller (1982) reported that, in toad

retina, when a light eliciting a nearly saturating photoreceptor potential was given during cGMP-induced depolarization, a very slight hyperpolarization with the normal latency of the control photoreceptor potential was superimposed. We made a similar observation in gecko retina (Fig. 1 in Kawamura and Murakami, 1983). These results suggest that the hyperpolarization in the neighboring cells was transmitted to the penetrated cell but that its effect was small (Miller, 1982). The contribution from neighboring cells under the intense light stimulation in Fig. 1 would not be appreciably different from that caused by the light eliciting

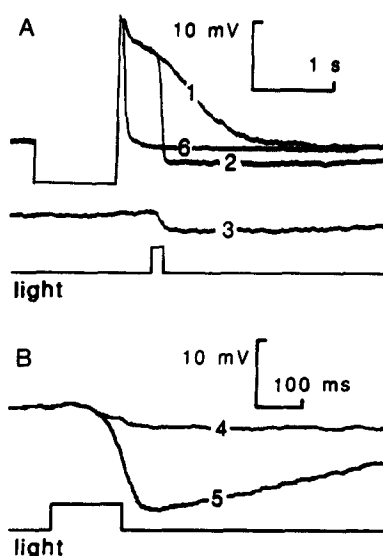


FIGURE 1. Rapid activation of PDE after a light flash. Record 1: depolarization in the dark induced by cGMP injection into a gecko photoreceptor with a negative current of 1.3 nA for 1 s. The membrane potential during the injection could not be observed because the recordings went off the scale of the chart recorder. Record 2: rapid repolarization induced by an intense light flash of 150 ms duration without attenuation by ND filters. Record 3: control photoreceptor potential elicited by the same light stimulation as in record 2. Record 4: photoreceptor potential of record 3 on an expanded time scale. Record 5: difference between records 1 and 2, indicating the net light-induced repolarization. Record 6: control response evoked by a negative current injection through the potassium acetate barrel of the electrode.

a nearly saturating photoreceptor potential, since the light-induced hyperpolarizations in the neighboring cells are similar in both cases.

The rapid repolarizing effect observed in Fig. 1 does not contradict the report by Miller and Nicol (1979) that, during the cGMP-induced depolarization, the latency of the repolarizing effect increases. The latency would depend on the level of PDE activation. A light of physiological intensity may activate PDE only slightly, and therefore it takes time to hydrolyze the injected cGMP. On the other hand, an intense light would cause enough activation of PDE to induce rapid hydrolysis. As will be shown below (Fig. 6), PDE activation becomes saturated by a very intense light.

It has been reported (Fain et al., 1980) that intracellular injection of a negative current activates some ion channel(s) of the frog photoreceptor membrane and causes a transient depolarization when the current is broken. This was also observed in some gecko photoreceptors, where it disturbed the estimation of the cGMP effect. Pressure injection was an alternative (Pinto and Brown, 1984) but was unsuccessful, since the electrode did not stay inside the cell long enough when pressure was repetitively applied. Therefore, before current injection of cGMP, we selected the cells that produced a minimum transient depolarization (record 6 in Fig. 1A), responding to a break of a negative current pulse injected through the potassium acetate barrel. In those cells, the resting potential was -30 to -15 mV and the amplitude of the photoreceptor potential was relatively small. However, cGMP injection was effective, and for this reason, we used this type of cell in the present experiment. Since the initial rapid repolarization in record 1 of Fig. 1A seemed to be the tail of the transient depolarization (record 6), we did not pay much attention to this repolarization.

PDE Activity under Continuous Illumination

To measure PDE activity during continuous illumination, cGMP was repetitively injected into a gecko photoreceptor and the time course of the change in the induced depolarization was observed. The induced depolarization in the dark is shown by record *a* in Fig. 2. The retina was then exposed to light, its intensity being increased stepwise, with a neutral density (ND) 6.0 filter (corresponding to 2.4 rh/OS·s) for 25 s, an ND 5.3 (12 rh/OS·s) for 22 s, an ND 5.0 (24 rh/OS·s) for 38 s, and finally an ND 4.3 (120 rh/OS·s) for 30 s. As the intensity and the duration of the illumination increased, the duration of the depolarization became shorter and the slope of the repolarization became steeper. Records *b* and *c* show the cGMP-induced depolarizations at the ends of the illuminations with the ND 5.0 filter and with the ND 4.3 filter, respectively. The peak of the cGMP-induced depolarization reached approximately the same level irrespective of the light intensity, which suggests that the amount of the injected cGMP was sufficient to depolarize the photoreceptor maximally in each injection.

The depolarization almost recovered to the control at ~ 50 s after termination of the illumination (record *d*). Although the current intensity of the repetitive cGMP injection was kept constant, the recovery of the cGMP-induced depolarization was sometimes incomplete. The data from those cells were discarded, since the incomplete recovery indicated that the amount of injected cGMP was not constant during the experiment.

From the above records, we tried to estimate PDE activity *in situ*. Although the intrinsic cGMP level is probably determined by the balance between the formation and degradation of cGMP, the lifetime of the injected cGMP would be determined mainly by the hydrolysis rate of PDE, that is, PDE activity. There would be several indices that correlate with PDE activity *in situ*, such as the time for decay of depolarization to its half-maximal value and the slope of the repolarization. However, we do not know how the membrane potential level correlates with the internal cGMP concentration, and therefore it would be difficult to estimate PDE activity quantitatively from the above indices.

Instead, in the present study, PDE activity was estimated from the time

required for complete repolarization (repolarization time), that is, the time necessary for full hydrolysis of the injected cGMP. As the amount of the injected cGMP was constant, the inverse of the repolarization time should be proportional to the number of hydrolyzed cGMP molecules in unit time, i.e., to PDE activity. However, the repolarization tapered off so gradually that the repolarization time was approximated at the point where the baseline intersected with an extrapolated straight line drawn along the repolarizing phase (see records *a-d* in Fig. 2).

The hydrolysis of the injected cGMP is a chemical reaction that probably occurs independently of the membrane potential. Therefore, the estimation of

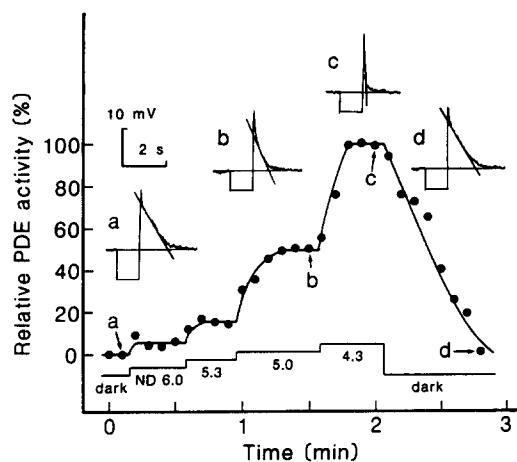


FIGURE 2. Constant PDE activity under continuous illumination. cGMP was injected with a negative current of 0.8 nA for 1 s in the dark as well as under attenuated light illuminations. The light intensity was increased stepwise as shown by the densities of the ND filters used. The light intensity with an ND 5.0 filter corresponded to 24 rh/OS·s. Records of cGMP-induced depolarizations at the times indicated by arrows are shown in records *a-d*. PDE activity was estimated from the inverse of the repolarization time, which was approximated at the intersection between an extrapolated line of a repolarizing phase (straight lines in records *a-d*) and the baseline (see text). The normalized values (solid circles) are plotted against time.

PDE activity from the repolarization time is also applicable, even when the membrane potential level is shifted by background illumination. In addition, the repolarization time was approximated at the time when the penetrated cell almost repolarized and therefore was nearly equipotential to the neighboring cells. Thus, the present estimation would also minimize the error caused by the electrical coupling between photoreceptors, although its contribution seemed to be small in the selected cells as described above.

From the records obtained in the experiment of Fig. 2, PDE activity was calculated as the inverse of the approximated repolarization time. Then the dark PDE activity was subtracted from each calculated PDE activity to obtain a net activity change caused by illumination. The results, normalized to the maximum

activity, are plotted in Fig. 2. PDE activity gradually increased at the beginning of each step of the intensity increase, stayed at a constant level as long as the intensity of the illumination was kept constant, and then gradually recovered to the dark level after termination of the illumination. Since cGMP was repetitively injected, stable measurement of the membrane potential was not possible.

PDE Activity Recovery as a Function of the Intensity of the Preceding Light Stimulus

In the dark, cGMP injection depolarized the cell, as shown by the top record in Fig. 3A. Then a light flash attenuated with an ND 1.3 filter (corresponding to 1.2×10^5 rh/OS·s) was delivered for 0.5 s and the recovery time course of the cGMP-induced depolarization was measured (the rest of the records in Fig. 3A). After recovery of the depolarization (top record in Fig. 3B), the experiment was repeated with a light flash attenuated with an ND 2.3 filter (1.2×10^4 rh/OS·s) (the rest of the records in Fig. 3B).

PDE activity was calculated in the same way as in Fig. 2, and the activity in the dark was subtracted from each value. The results obtained at two different levels of light intensity were plotted logarithmically against time after the flash (Fig. 3C). Since straight lines could be drawn in Fig. 3C, the recovery process of PDE activity could be approximated by first-order kinetics. The rate constant of the reaction was determined from the best-fit lines shown in Fig. 3C: 0.056 s^{-1} (time constant, 18 s) with the ND 1.3 filter and 0.11 s^{-1} (time constant, 9.1 s) with the ND 2.3 filter.

Therefore, the rate constant of PDE activity recovery was dependent on the intensity of the preceding light stimulus; that is, the lower the intensity, the higher the rate constant. Qualitatively similar results were obtained in all six cells examined, although the paired intensities were varied in each experiment.

The above results suggest that the mechanism of PDE activity recovery is complex: if activated PDE molecules simply decay, the rate constants should be equal irrespective of the intensity of the preceding light stimulus. As the PDE activity after a flash would be a net product of the activation and inactivation of PDE, the higher-intensity light may either facilitate the activation or inhibit the inactivation of PDE. Since PDE activation is triggered by metarhodopsin II (Fukada and Yoshizawa, 1981), one of the possibilities was that when a light of higher intensity was given, the lifetime of metarhodopsin II increased and PDE activation persisted longer.

The PDE activity recovery after a light stimulation is also light-intensity dependent in a suspension of permeabilized frog rod outer segment (Kawamura, 1983), and therefore this preparation could be used to test the above possibility. In this preparation, hydroxylamine (40 mM), which shortens the lifetime of metarhodopsin II (Bridges, 1962; Brin and Ripps, 1977), did not affect the PDE activity recovery appreciably (data not shown), which indicates that the lifetime of metarhodopsin II is not the determinant of the PDE activity recovery. As PDE activity is regulated by various factors in several steps of reactions (Stryer, 1983; Yamazaki et al., 1984), further experiments are necessary to elucidate the mechanism of the recovery process of PDE activity in detail.

Sensitivity Recovery of the Photoreceptor Potential

An example of the sensitivity recovery process of the gecko photoreceptor is shown in Fig. 4A. After a conditioning light was flashed at time 0, a test flash was given at various intervals, and four sample records were superimposed together with that of a control photoreceptor potential (the record before time

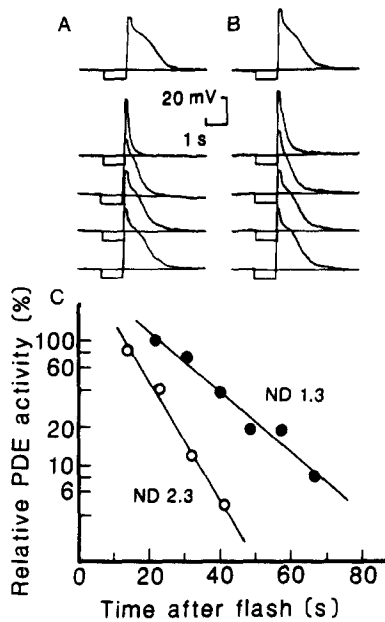


FIGURE 3. Recovery of PDE activity as a function of the preceding flash intensity. (A) cGMP was injected with a negative current of 1.3 nA for 1 s. After recording the cGMP-induced depolarization in the dark (top record), a light stimulus attenuated with an ND 1.3 filter (corresponding to 1.2×10^5 rh/OS·s) was given for 0.5 s. The record immediately after the cGMP injection went off the scale of the chart recorder. The time course of the recovery of the depolarization was measured every 9 s and the records at 22, 40, 58, and 76 s after the flash are shown (the rest of the records). (B) Same as in A except that the light intensity was attenuated with an ND 2.3 filter (1.2×10^4 rh/OS·s). The records at 14, 23, 32, and 41 s after the flash are shown. (C) PDE activity was estimated from the records in A and B in the same way as in Fig. 2. The net light effect on PDE activity was plotted on the logarithmic scale against time after the flash. Assuming first-order kinetics, best-fit lines were determined as shown by straight lines. The rate constants were 0.056 s^{-1} with an ND 1.3 filter and 0.11 s^{-1} with an ND 2.3 filter.

0). The peak amplitude of the photoreceptor potential elicited by a test flash became larger as the interval increased and fully recovered at the 60-s interval. Fig. 4B shows the superimposed records of the photoreceptor potentials to the test flashes in Fig. 4A on an expanded time scale. In the present study, we define the photoreceptor sensitivity as a fraction of the recovery of the peak amplitude elicited by a test flash given after a conditioning light flash. A semilogarithmic

plot of the differences in the peak amplitudes between the full response and the responses to the test flashes provides the time course of the sensitivity recovery of the photoreceptor potential.

In another cell, the same type of experiment was performed at two different intensities of conditioning light, and the sensitivity recovery is plotted in Fig. 4C. Assuming first-order kinetics, the best-fit lines were determined. The rate

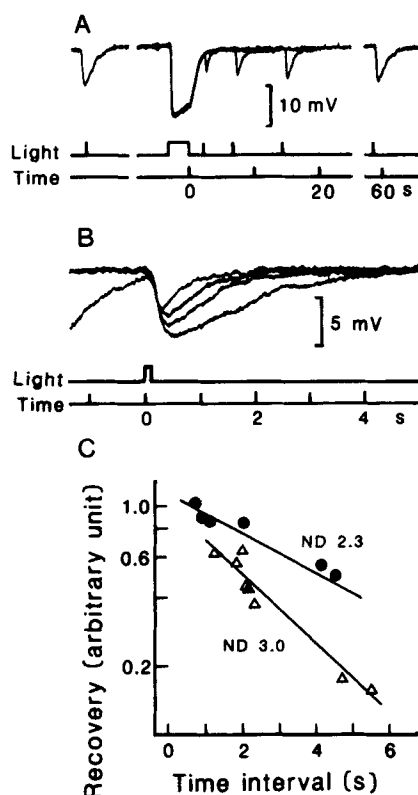


FIGURE 4. Photoreceptor sensitivity recovery as a function of preceding stimulus intensity. (A) Sensitivity recovery process of the photoreceptor potential after a light stimulus. A test flash was given at different time intervals after a constant conditioning light, and the records were superimposed. The record before time 0 is a control response elicited by a test flash in the dark-adapted state. (B) Recoveries of the sensitivity of the photoreceptor potential after a light flash. Four records elicited by the test flashes in A were superimposed and are shown on an expanded scale. To avoid crowdedness, the control response shown in A is not included. (C) Time course of the sensitivity recovery of the photoreceptor potential. The difference in amplitudes between the photoreceptor potentials evoked by a conditioning light flash and a test flash was logarithmically plotted against the time interval (see text). Assuming first-order kinetics, best-fit lines were determined and their rate constants were 0.23 and 0.35 s^{-1} for the conditioning light stimuli with ND 2.3 and 3.0 filters, respectively.

constant was 0.23 s^{-1} (time constant, 4.4 s) for the stimulus with an ND 2.3 filter ($1.2 \times 10^4 \text{ rh/OS}\cdot\text{s}$), and 0.35 s^{-1} (time constant, 2.9 s) with an ND 3.0 filter ($2.4 \times 10^3 \text{ rh/OS}\cdot\text{s}$); that is, the lower the light intensity, the higher the rate constant of the recovery. The results indicate that the sensitivity recovery of the photoreceptor is light-intensity dependent, and are consistent with the observation by Baylor and Hodgkin (1974) in turtle cones.

Since the rate constant of the recovery of the photoreceptor potential and that of PDE activity were both light-intensity dependent (Figs. 3 and 4), there seemed to be a close correlation between these two kinds of recoveries. Therefore, the two recovery processes were compared in the same cell by combining the methods used in Figs. 3 and 4. Because of the technical problem that the stable measurement of the photoreceptor potential throughout the experiment was difficult, double flashes of equal intensity were given, and the photoreceptor potential evoked by the first flash was taken as the control. The sensitivity, S , was defined by the equation of $S = (V_2 - V_i)/V_1$, where V_1 and V_2 are the peak amplitudes elicited by the first and the second flashes, respectively, and V_i is the expected membrane potential at the time when the potential change evoked by the second flash reached its peak (see inset records in Fig. 5). The sensitivity recovery, R , is defined by the equation $R = 1 - S$.

After the PDE activity recovery was measured twice (Fig. 5, circles), the photoreceptor sensitivity recovery was measured (triangles) with the same conditioning light as that used in the PDE activity recovery measurement. The two processes in Fig. 5 showed similar rate constants: 0.12 s^{-1} (time constant, 8.3 s) in the PDE activity recovery and 0.14 s^{-1} (time constant, 7.1 s) in the photoreceptor sensitivity recovery. The absolute value of the rate constant varied from experiment to experiment, depending on the light stimulus condition, but the ratio of the rate constants did not change so much and the average was close to unity (sensitivity recovery/PDE activity recovery; 1.2 ± 0.4 , $n = 5$). In order to fulfill the condition that was required in the theoretical analysis of the photoreceptor sensitivity (see Discussion), in Fig. 5, we used weak light flashes, which elicited a photoreceptor potential of $<4 \text{ mV}$.

Intensity-Response Curves of the Photoreceptor Potential and PDE Activation

Since there seemed to be a close correlation between PDE activity and the photoreceptor potential, as shown in Figs. 1–5, it was of interest to compare the intensity-response curve of the photoreceptor potential with that of PDE activation. For the measurement of the intensity-response curve of the in situ PDE activation, the PDE activity recovery should be measured at various light intensities. Practically, this was not possible because the electrode did not stay inside the cell long enough to complete the experiment, which forced us to use the biochemical method. In this respect, gecko retina was not adequate, and instead, frog retina was used for the following reason. In gecko retina, the isolation of the rod outer segments requires a purification process such as sucrose flotation, since the pigment epithelium firmly attaches to the outer segments. During this process, the plasma membrane is ruptured and all soluble components and some control factors of PDE are lost (Robinson et al., 1980). This might be a crucial

point in the physiological study of PDE activation. In frog retina, however, we can easily isolate rod outer segments without purification and therefore can prepare the permeabilized rod outer segments, which retain these control factors (Robinson et al., 1980). Since the same species should be used for a precise comparison between the intensity-response curves, frog retina was also used in the measurement of photoreceptor potential.

In Fig. 6, intensity-response curves of PDE activation (curve *a*) and the photoreceptor potential (curve *b*) are shown. It is evident that the response curve of PDE is located at an intensity range higher by two to three orders of magnitude

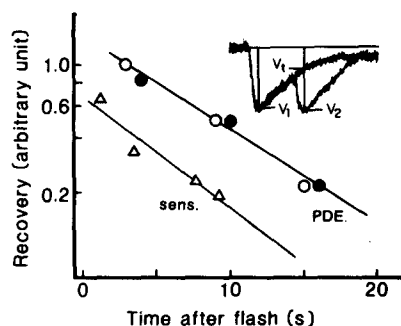


FIGURE 5. Recoveries of the photoreceptor sensitivity and PDE activity in the same cell. After a light stimulation of 50 ms duration attenuated with an ND 2.3 filter (1.2×10^4 rh/OS·s), the PDE activity recovery was analyzed as in Fig. 3. The measurement was repeated twice as shown by the circles. The maximum PDE activity was arbitrarily set to 1.00. In the same cell, with the conditioning light of the same intensity and duration as of the PDE activity measurement, the photoreceptor sensitivity recovery was analyzed using double flashes as shown by the inset records (for details, see text). The calculated value was plotted on the logarithmic scale against the time interval between the flashes. Assuming first-order kinetics, best-fit lines were determined (solid lines). The rate constants were 0.12 s^{-1} for the PDE activity recovery and 0.14 s^{-1} for the photoreceptor sensitivity recovery.

than that of the photoreceptor potential. The difference might be larger when a light flash of short duration was used, since the photoreceptor cell in this experiment was probably desensitized because of a relatively long illumination period. We used this stimulation because, for a precise comparison between the two intensity-response curves in Fig. 6, we applied the same stimulation condition in the measurement of the photoreceptor potential as was used in the biochemical measurement. In order to obtain a measurable pH change in the biochemical measurement of PDE activation, the duration of the light stimulation was necessarily >250 ms, since the intensity of the light source was limited. During this relatively long illumination period, the photoreceptor was probably desensitized. In fact, the number of rhodopsin molecules bleached per outer segment per flash necessary for eliciting a half-maximal photoreceptor potential was 6×10^2 and was ~ 10 times higher than the value in frog reported by Biernbaum and Bownds (1985). On the other hand, the half-maximal number for PDE activation was 1.5×10^5 , which is close to the value reported by Yee and Liebman (1978).

One of the possible sources of difference in the location of the intensity-response curves was considered in the following way. Although the permeabilized rod outer segments retained some control factors of PDE, other soluble cytoplasmic components might have leaked out from the outer segment. Since the rhodopsin concentration was $\sim 10 \mu\text{M}$ in the above *in vitro* study, and 2–3 mM in intact rod outer segments (Hárosi, 1975), the concentration of the cytoplasmic component(s) was usually diluted by >200 times in the biochemical experiment. This dilution might shift the intensity-response curve of PDE to a higher intensity range. To test this possibility, the rhodopsin concentration was varied from 2.8

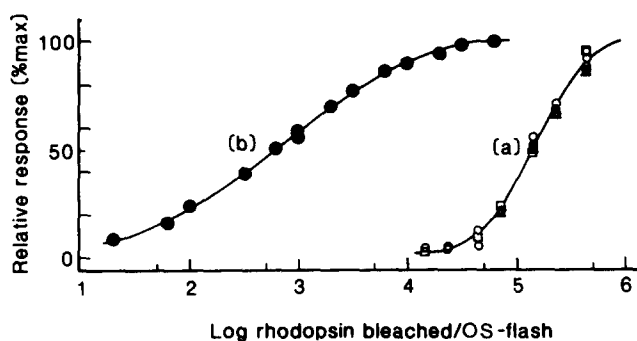


FIGURE 6. Intensity-response curves of PDE activation in isolated frog rod outer segments and the frog photoreceptor potential. (a) PDE activity: calibrated light stimulation of 250 ms duration was delivered to the frog rod outer segment suspension and PDE activity was measured as described in Materials and Methods. After normalization, PDE activity was plotted against the total amount of rhodopsin bleached per outer segment per flash. Experiments were carried out at three different rhodopsin concentrations: 2.8 (Δ), 8.3 (\circ), and 12 (\square) μM . (b) Photoreceptor potential: a frog rod photoreceptor was impaled with a single-barreled microelectrode and a calibrated light stimulation of 250 ms duration was given. The relative amplitude, normalized to the maximum response amplitude, was plotted.

to 12 μM , but no significant shift was observed, as shown by different symbols in curve *a*. When we increased the rhodopsin concentration further, the rate of cGMP hydrolysis became too high for reliable measurement in our present assay system. Since PDE can also hydrolyze cAMP but with a slower rate (Miki et al., 1973), we used cAMP as the substrate in the experiments performed at higher rhodopsin concentrations. Then the rhodopsin concentration was varied from 2.8 to 36.5 μM . Although the entire curve was shifted slightly (0.2 log units) toward a higher light intensity range when compared with that measured with cGMP as the substrate, the template of the curve did not change, despite the difference in the rhodopsin concentration (data not shown). This result is explained by the notion that PDE is activated through a diffusional process on the disk membrane (Liebman and Sitaramayya, 1984); i.e., it suggests that the light sensitivity of PDE *in situ* is the same as that *in vitro*.

Although the above experiment was performed in frogs, the result could be applied to geckos, since so far no large difference has been observed among various animal species in the light sensitivity of the rod photoreceptor (see

Korenbrodt, 1984, for a review), or in the PDE activation mechanism (see Stryer, 1983, and Yamazaki et al., 1984, for review).

From the above experiment, it seems that there is a large difference in the light intensity range of the intensity-response curves even in situ. However, this does not mean that PDE activation and the generation of the photoreceptor potential are not coupled. An interpretation will be given in the Discussion.

Effect of Background Light on the Recovery of PDE Activity

Since background illumination shortens the time to peak of the photoreceptor potential and facilitates the time course of the repolarization (Baylor and Hodgkin, 1974), it was of interest to examine whether the PDE activity recovery is accelerated in the presence of background illumination.

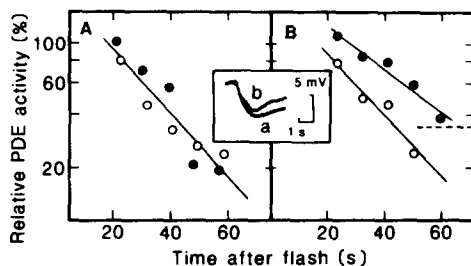


FIGURE 7. Effect of background illumination on the PDE activity recovery. cGMP was injected repetitively into a gecko photoreceptor with a negative current of 1.3 nA for 1 s. The PDE activity recovery after a light stimulation with an ND 2.3 filter (1.2×10^4 rh/OS·s) for 0.5 s was determined as in Fig. 3 (solid circles in A). The PDE activity recovery after a second flash in the presence of background illumination attenuated by an ND 5.0 filter was determined first without correction of the effect of the background illumination (solid circles in B), and then with subtraction of the constant activity caused by the background illumination (open circles in B). The dashed line in B indicates the steady level of PDE activation caused by the background illumination. After the background illumination was turned off, the PDE activity recovery was determined again (open circles in A). Assuming first-order kinetics, best-fit lines were determined and the rate constants were calculated. The rate constant of the recovery without the background illumination was 0.048 s^{-1} (circles in A) and 0.040 s^{-1} in the presence of the background illumination (open circles in B). The inset records show the photoreceptor potential with (record b) and without (record a) the background illumination.

The PDE activity recovery in situ after a light flash was first measured in the dark (solid circles in Fig. 7A) and the measured peak activity was arbitrarily set to 100%. Then background illumination was turned on. After the PDE activation became constant (34% of the peak activity; dashed line in Fig. 7B), we delivered a second light flash of the same duration and intensity as in Fig. 7A. The net recovery of PDE activity after the flash in the presence of background illumination was then calculated by subtracting the steady state PDE activity (34% of the peak activity), and the results are shown by the open circles in Fig. 7B. The solid circles in Fig. 7B show the PDE activity recovery without subtraction of the

steady activation of PDE under background illumination. After recovery of PDE activity to the level before the second flash, the background illumination was turned off and a third flash was given in order to measure the PDE activity recovery again (open circles in Fig. 7A).

In Fig. 7, A and B, assuming first-order kinetics, the best-fit lines were determined. The rate constant of the net recovery of PDE activity in the presence of background illumination (open circles in Fig. 7B) was 0.040 s^{-1} (time constant, 25 s), which is very similar to that in the absence of background illumination (rate constant, 0.042 s^{-1} ; time constant, 24 s). Although the time to peak of the photoreceptor potential was shortened and its repolarization process was facilitated under background illumination (record *b*) when compared with those without background illumination (record *a*), there was no facilitation in the PDE activity recovery, at least under the conditions used in the present experiment. Similar results were obtained in three separate experiments.

DISCUSSION

Validity of the PDE Activity Measurement In Situ

In the present experiment, PDE activity was estimated from the duration of the cGMP-induced depolarization. The estimation was not influenced appreciably either by the membrane potential level or electrical coupling between photoreceptors, as described in the Results.

Our estimation of PDE activity was based on the assumption that the hydrolysis of cGMP is the main pathway that restores the internal cGMP concentration to normal after the addition of exogenous cGMP. However, there are other possible pathways that can actively control the internal cGMP level, such as the binding of cGMP to PDE (Yamazaki et al., 1980) and the regulation of the cGMP formation by a feedback mechanism (Schwartz, 1985). However, the contribution of these factors to the present estimation seems to be negligible because the behavior of the in situ PDE activity (Figs. 1–3) was very similar to that of the PDE activity measured directly with biochemical methods, as summarized: (*a*) in a suspension of rod disk membranes, the latency of PDE activation is $<100 \text{ ms}$ (Yee and Liebman, 1978); (*b*) the level of PDE activation in rod disk membranes is determined by light intensity (Kawamura and Bownds, 1981); (*c*) PDE activity recovers with first-order kinetics, and the process is dependent on the intensity of the preceding light stimulus (Kawamura, 1983). These similarities would be an indication that the measurement of the in situ PDE activity in the present work was reliable.

Correlation Between the Photoreceptor Potential and In Situ PDE Activity

The present experiments revealed a close correlation between the in situ PDE activity and the photoreceptor potential, as follows. (*a*) The latency of PDE activation is short and is comparable to that of the photoreceptor potential (Fig. 1), which is consistent with the finding that PDE activation rapidly reduces the internal cGMP level (Woodruff and Bownds, 1979; Polans et al., 1981; Cote et al., 1984), and this reduction triggers the generation of the photoreceptor

potential (Fesenko et al., 1985). (b) Under continuous illumination, PDE activity is constant and its level is light-intensity dependent (Fig. 2). Although stable measurement of the membrane potential was not possible in Fig. 2, it appears that the photoreceptor potential behaves in the same way under continuous stimulation. (c) The kinetics of the recovery of PDE activity after a light flash are light-intensity dependent, as is the sensitivity recovery of the photoreceptor potential (Figs. 3 and 4). (d) The rate constant of the PDE activity recovery is similar to that of the photoreceptor sensitivity recovery (Fig. 5).

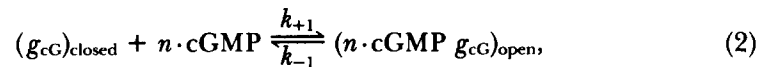
The physiological consequence of rapid activation and the constant activity during continuous illumination can be understood by the notion that cGMP acts as the internal messenger in gecko photoreceptors. Then the question is how the PDE activity recovery relates to the recovery of the photoreceptor sensitivity. In the next section, assuming that cGMP is the internal messenger, we show theoretically that the photoreceptor sensitivity recovery parallels the internal cGMP recovery. The results in Fig. 5 can be interpreted as an indication that internal cGMP recovers almost in parallel with PDE activity. On the basis of the conclusion of this analysis, we further consider whether we could explain the close correlation between the recovery time course of PDE activity and the recovery time course of cGMP after a light flash.

Analysis of Photoreceptor Sensitivity

Recent work by Fesenko et al. (1985) has revealed that cGMP directly regulates the membrane conductance. The dose-response relation between cGMP and the cGMP-sensitive conductance showed positive cooperativity. Therefore, the cGMP-sensitive conductance can be described by:

$$g_{cG} = g_{\max} \cdot \frac{S^n}{K_D'^n + S^n}, \quad (1)$$

where g_{cG} is the cGMP-dependent conductance, g_{\max} is the maximum conductance, S is the internal cGMP concentration, and n is a Hill coefficient in the following reaction:



where (g_{cG}) is the cGMP-sensitive ion channel and subscripts indicate the state of the channel. K_D' in Eq. 1 is determined by K_D , the dissociation constant of the reaction 2, which is

$$K_D'^n = K_D = k_{-1}/k_{+1}. \quad (3)$$

Using Eq. 1, we first obtained the expression of the cGMP-sensitive conductance change caused by the first flash. Then, taking the cGMP recovery into account, we obtained the expression of the cGMP-sensitive conductance at time t after the flash (for a detailed description, see the Appendix). Assuming that the second flash reduced internal cGMP to the same extent as that of the first flash, we then obtained the expression of the conductance change caused by the second flash. The assumption seems to be reasonable, since PDE activation under this condi-

tion appears to be linear and additive (see below). The conductance change would be linearly reflected in the voltage change under the conditions in Fig. 5, because the voltage change was small in that experiment (see Results and Appendix). Then the expressions of the cGMP-sensitive conductance changes could be incorporated into the equation of the sensitivity recovery, $R = 1 - (V_2 - V_i)/V_1$.

Adopting the values of $K_D = 30 \mu\text{M}$ (Fesenko et al., 1985), $S = 6 \mu\text{M}$ (Yau and Nakatani, 1985), and $n = 2$ (Fesenko et al., 1985; Yau and Nakatani, 1985), we simplified the equation. The equation obtained showed that the photoreceptor sensitivity recovery is a linear function of the cGMP recovery. Therefore, the data in Fig. 5 indicate that PDE activity recovers almost in parallel with the internal cGMP.

PDE Activity Recovery and cGMP Recovery

Since the internal cGMP level is determined by the balance of its formation and degradation, the next question is how the cGMP recovery parallels the PDE activity recovery. This was also considered theoretically.

The reaction scheme of the formation and the hydrolysis of cGMP is



In the above scheme, the rate constants α and β are functions of guanylate cyclase and PDE, respectively. The amount of cGMP, [cGMP], is determined by:

$$\frac{d[\text{cGMP}]}{dt} = \alpha[\text{GTP}] - \beta[\text{cGMP}]. \quad (4)$$

When a flash of light is given, PDE activity and thus β first increase and then decrease with first-order kinetics (for example, see Fig. 3). Then β can be expressed by:

$$\beta = p(qe^{-kt} + 1), \quad (5)$$

where p is the PDE dark activity, q is an activation ratio of PDE in the light, and k is the rate constant of the PDE activity recovery. To make the analysis simpler, we tentatively assume that the formation is constant irrespective of light conditions, that is, $\alpha[\text{GTP}]$ equals a constant α_0 , but this assumption will be reconsidered later on. Then the whole equation that gives the concentration change of cGMP is

$$\frac{d[\text{cGMP}]}{dt} = \alpha_0 - p(qe^{-kt} + 1)[\text{cGMP}]. \quad (6)$$

With a computer simulation using Eq. 6, we compared the time course of the recovery of cGMP after a light stimulation with that of PDE (Fig. 8). The result indicates that if the formation of cGMP is constant irrespective of light conditions, the cGMP concentration recovery does not precede the PDE activity recovery (for details of the simulation procedure, see the legend of Fig. 8). As shown in the experiment of Fig. 5, the sensitivity recovery and thus the cGMP recovery are almost equal to or slightly faster than the PDE activity recovery (the ratio of the rate constant of the sensitivity recovery to that of the PDE recovery is $1.2 \pm$

0.4). Therefore, the result in Fig. 5 is explained by assuming that cGMP is the transduction messenger and that cGMP formation is enhanced in the light as previously measured by Goldberg et al. (1983). Since we did not have detailed information about the kinetics of the formation of cGMP, no further analysis was conducted.

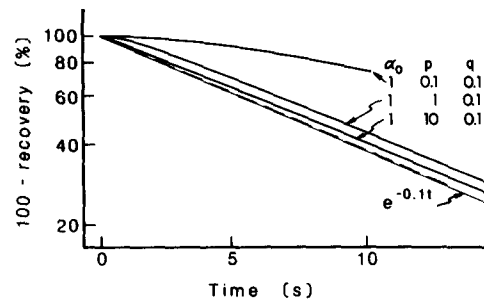


FIGURE 8. Computer simulation of the time course of the cGMP recovery after a light flash. The recovery time course of cGMP was simulated using Eq. 6 (see text). PDE activity was assumed to recover with first-order kinetics with a rate constant of 0.1 s^{-1} , a value close to the rate constant observed in the experiment of Fig. 5. For simulation, we varied the values of α_0 , p , and q independently from 0.1 to 10. (These values were arbitrarily chosen, since the actual values have not been measured.) With each group of α_0 , p , and q , the dark cGMP level was determined with Eq. 6 under the condition of $d[\text{cGMP}]/dt = 0$ at $t = \infty$. The initial cGMP level after a light flash was also calculated under the condition of $d[\text{cGMP}]/dt = 0$ but at $t = 0$. Each calculated value during the recovery phase was expressed as the percent of the total recovery and was subtracted from 100%. From the calculation, it was found that (a) α_0 is almost insensitive to the rate of the cGMP recovery, (b) the increase in the value of p is most sensitive to the cGMP recovery, and (c) the decrease in q is also sensitive, but less effective than p . In the figure, the calculated result at $\alpha_0 = 1$ and $q = 0.1$ is shown as a function of p . Although the PDE activity recovery (thin line) always preceded the cGMP recovery in any combination of the values shown above, both recoveries became similar when $p = 10$ and $q = 0.1$. Therefore, p was increased to 1,000 and q was reduced to 0.0001. Under this extreme condition (dashed line), the recovery time course of the internal cGMP became almost identical to that of PDE, but the cGMP recovery never preceded the PDE recovery. A further increase in p and a decrease in q did not affect the recovery time course of cGMP significantly.

Intensity-Response Curves of the Photoreceptor Potential and PDE Activation

As shown in Fig. 6, the intensity-response curve of PDE activation in permeabilized rod outer segments is located in an intensity range that is higher by two to three log units than that of the photoreceptor potential. This raises the possibility that PDE in permeabilized rod outer segment is desensitized. However, this possibility is ruled out by the fact that the increase in the rhodopsin concentration does not shift the intensity-response curve of PDE (see Results). In addition, the measurement of intrinsic cGMP shows that the cGMP decrease is remarkably large under very intense light, but it is small under a light of physiological

intensity (Woodruff and Bownds, 1979; Kilbride and Ebrey, 1979; Goldberg et al., 1983), which indicates that the activation of PDE in situ is not saturated by a light of physiological intensity.

Thus, the data in Fig. 6 provide a rationale of the assumption we made in the section *Analysis of the Photoreceptor Sensitivity*, where we assumed that the second flash changed the concentration of the messenger to the same extent as the first flash did. In Fig. 6, the intensity-response curve of PDE lies at a higher intensity than that of the photoreceptor potential. We could not detect PDE activity under low-intensity light in the present in vitro study, but other biochemical work indicates that the bleach of one rhodopsin molecule activates several hundreds of PDE molecules (Yee and Liebman, 1978). Then, when successive flashes of low light intensity are given, PDE activation is probably linear and additive, and therefore the change in the concentration of the messenger by each flash is constant.

A Possible Mechanism Underlying Light-induced Electrical Activities of Rod Photoreceptors

A possible mechanism deduced from the present work, together with the results of other work, is summarized as follows. Bleach of rhodopsin causes rapid activation of PDE (Fig. 1). A very slight activation of PDE is sufficient to evoke an electrical response of the rod photoreceptor (Fig. 6). The cGMP concentration in the outer segments is reduced immediately after a light flash (Woodruff and Bownds, 1979; Cote et al., 1984) by the activated PDE, and this reduction in cGMP probably hyperpolarizes the rod photoreceptors (Fesenko et al., 1985; Yau and Nakatani, 1985). When continuous illumination is given, PDE is activated at a constant level (Fig. 2), allowing the cGMP metabolism to be equilibrated at a constant level. After a light flash, the cGMP level in the outer segment is gradually recovered in the dark with a supplement of cGMP synthesized by the light-activated guanylate cyclase (Goldberg et al., 1983) in the inner segment, the most probable site for cGMP synthesis (Fleischman et al., 1980; de Azeredo et al., 1981; see Miller, 1983, for detailed discussion). In parallel with the restoration of the internal cGMP concentration, the photoreceptor sensitivity recovers (Fig. 5). Since the rate of the PDE activity recovery is a function of flash intensity (Fig. 3), this behavior is reflected in the recovery of internal cGMP and therefore in the recovery of the photoreceptor sensitivity (Fig. 4).

In our previous work (Kawamura and Murakami, 1983), it was shown that the recovery of the waveform of the light-induced hyperpolarization precedes the PDE activity recovery. In addition to this, the present experiments showed that the kinetics of the recovery of PDE activity were not altered by background illumination, although the time course of the waveform of the photoreceptor potential was accelerated (Fig. 7). This lack of correlation between the membrane potential and PDE activity may be explained by considering the depolarizing effect caused by an increase of the cGMP formation (Goldberg et al., 1983), changes of voltage-activated ion conductances (see Fain and Lisman, 1981, for a review), and electrogenic Na^+ - Ca^{2+} exchange (Yau and Nakatani, 1984).

The above explanation obviously lacks many quantitative descriptions, and further experiments will be necessary to reach a final conclusion. The electrophysiological measurement of PDE activity used in the present work is the only way currently available for real-time measurement of PDE activity in situ and can provide useful information in the study of the molecular mechanism of phototransduction and adaptation in rod photoreceptors.

APPENDIX

The dose-response relation between cGMP and the cGMP-sensitive conductance in rod outer segment can be described by:

$$g_{cG} = g_{\max} \cdot \frac{S^n}{K_D^n + S^n} \quad (1)$$

The definitions of g_{cG} , g_{\max} , S , n , and K_D are given in the Discussion. We define the cGMP concentration in the dark as S_d , and the concentration change of the cGMP induced by the first flash as ΔS . Then the conductance change due to the first flash (Δg_{cG}^1) is

$$\Delta g_{cG}^1 = g_{\max} \cdot \left[\frac{S_d^n}{K_D^n + S_d^n} - \frac{(S_d - \Delta S)^n}{K_D^n + (S_d - \Delta S)^n} \right] \quad (7)$$

We assume that, after a light flash, the concentration of cGMP recovers to the dark level according to a function $f(t)$. Since the photocurrent that reflects the concentration of the transduction messenger recovers more slowly than the membrane potential (Baylor et al., 1981), the recovery of cGMP after a flash would be slower than that of the photoreceptor potential.

At time t after a flash, the membrane conductance change determined by cGMP is still reduced to the level of Δg_{cG}^t , where

$$\Delta g_{cG}^t = g_{\max} \cdot \left\{ \frac{S_d^n}{K_D^n + S_d^n} - \frac{[S_d - f(t)]^n}{K_D^n + [S_d - f(t)]^n} \right\} \quad (8)$$

When a second flash is given at time t , the concentration change of cGMP caused by this flash is assumed to be the same as that caused by the first flash. This assumption seems reasonable since PDE activity is low at physiological light intensity (see text). The resultant concentration change of cGMP from the dark level is the sum of ΔS and $f(t)$, and the total conductance change (Δg_{cG}^2) is

$$\Delta g_{cG}^2 = g_{\max} \cdot \left\{ \frac{S_d^n}{K_D^n + S_d^n} - \frac{[S_d - f(t) - \Delta S]^n}{K_D^n + [S_d - f(t) - \Delta S]^n} \right\} \quad (9)$$

When the membrane voltage change is small, as in Fig. 5, the conductance change is linearly reflected in the photocurrent change. As can be calculated from the simultaneous recordings of the photocurrent and the membrane potential (Fig. 1 in Baylor et al., 1984), the peak photocurrent caused by a single flash is roughly linear to the peak amplitude of the voltage change. Since the current-voltage relation of the rod membrane is linear in the physiological range (Bader et al., 1979), the increment of the photocurrent caused by the second flash would be proportional to that of the photoreceptor potential. Therefore, we can assume the following relations:

$$\Delta g_{cG}^1 \propto V_1, \quad \Delta g_{cG}^2 - \Delta g_{cG}^1 \propto V_2 - V_1, \quad \text{and} \quad g_{\max} \propto V_{\max}, \quad (10)$$

where V_1 , V_2 , and V_i are the corresponding membrane potential changes (see inset records

in Fig. 5) and V_{\max} is the maximum voltage change. As described in the Results, we define the photoreceptor sensitivity as $(V_2 - V_1)/V_1$, and its recovery,

$$R = 1 - (V_2 - V_1)/V_1, \quad (11)$$

was plotted against the time interval between the two flashes.

Eqs. 7-10 are inserted into Eq. 11 and the following relation is obtained:

$$R = \left\{ \frac{S_d^n}{K_D'^n + S_d^n} - \frac{(S_d - \Delta S)^n}{K_D'^n + (S_d - \Delta S)^n} + \frac{[S_d - f(t) - \Delta S]^n}{K_D'^n + [S_d - f(t) - \Delta S]^n} - \frac{[S_d - f(t)]^n}{K_D'^n + [S_d - f(t)]^n} \right\} \times \text{constant}. \quad (12)$$

Eq. 12 can be simplified to:

$$R = \text{constant} \times \left\{ -\frac{1}{1 + \left(\frac{S_d}{K_D'}\right)^n} + \frac{1}{1 + \left(\frac{S_d - \Delta S}{K_D'}\right)^n} - \frac{1}{1 + \left[\frac{S_d - f(t) - \Delta S}{K_D'}\right]^n} + \frac{1}{1 + \left[\frac{S_d - f(t)}{K_D'}\right]^n} \right\}. \quad (13)$$

The dark cGMP concentration (S_d) has been estimated to be $6 \mu\text{M}$ (Yau and Nakatani, 1985) and the half-saturation of the conductance (K_D') is observed at $30 \mu\text{M}$ (Fesenko et al., 1985). On the basis of these values, S_d/K_D' was calculated to be 0.2, which indicates that $1 \gg (S_d/K_D')^n$. Therefore,

$$\frac{1}{1 + \left(\frac{S_d}{K_D'}\right)^n} \approx 1 - \left(\frac{S_d}{K_D'}\right)^n. \quad (14)$$

Since $S_d - f(t)$ and $S_d - f(t) - \Delta S$ are smaller than S_d , the same approximation can be made. Inserting these equations into Eq. 13, we obtain the following relation:

$$R = \frac{\text{constant}}{K_D'^n} \times \{S_d^n + [S_d - f(t) - \Delta S]^n - (S_d - \Delta S)^n - [S_d - f(t)]^n\}. \quad (15)$$

The value of n has been reported to be close to 2 (Fesenko et al., 1985; Yau and Nakatani, 1985). Rearranging Eq. 15 using the relation of $n = 2$, we obtain:

$$R = \text{constant} \times \frac{2\Delta S}{K_D'^n} \cdot f(t). \quad (16)$$

Eq. 16 indicates that the sensitivity recovery process is a linear function of the cGMP recovery.

This work was supported by a Grant-in-Aid (No. 59770103) from the Ministry of Education, Science and Culture of Japan to S.K.

Original version received 7 May 1985 and accepted version received 13 February 1986.

REFERENCES

- Bader, C. R., P. R. MacLeish, and E. A. Schwartz. 1979. A voltage-clamp study of the light response in solitary rods of the tiger salamander. *Journal of Physiology*. 296:1-26.

- Baylor, D. A., and A. L. Hodgkin. 1974. Changes in time scale and sensitivity in turtle photoreceptors. *Journal of Physiology*. 242:729-758.
- Baylor, D. A., G. Matthews, and B. J. Nunn. 1984. Location and function of voltage-sensitive conductance in retinal rods of the salamander, *Ambystoma tigrinum*. *Journal of Physiology*. 354:203-223.
- Biernbaum, M. S., and M. D. Bownds. 1985. Frog rod outer segments with attached inner segment ellipsoids as an in vitro model for photoreceptors on the retina. *Journal of General Physiology*. 85:83-105.
- Bridges, C. D. B. 1962. Studies on flash photolysis of visual pigments. IV. Dark reactions following the flash-irradiation of frog rhodopsin in suspensions of isolated photoreceptors. *Vision Research*. 2:215-232.
- Brin, K. P., and H. Ripps. 1977. Rhodopsin photoproducts and rod sensitivity in the skate retina. *Journal of General Physiology*. 69:97-120.
- Cote, R., M. S. Biernbaum, G. Nicol, and M. D. Bownds. 1984. Light-induced decreases in cGMP concentration precede changes in membrane permeability in frog rod photoreceptors. *Journal of Biological Chemistry*. 259:9635-9641.
- de Azeredo, F. A. M., W. D. Lust, and J. V. Passonneau. 1981. Light-induced changes in energy metabolites, guanine nucleotides, and guanylate cyclase within frog retinal layers. *Journal of Biological Chemistry*. 256:2731-2735.
- Fain, G., H. M. Gershenfeld, and F. N. Quandt. 1980. Calcium spikes in toad rods. *Journal of Physiology*. 303:495-513.
- Fain, G., and J. E. Lisman. 1981. Membrane conductances of photoreceptors. *Progress in Biophysics and Molecular Biology*. 37:91-147.
- Fesenko, E. E., S. S. Kolesnikov, and A. L. Lyubarsky. 1985. Induction by cyclic GMP of cationic conductance in plasma membrane of retinal rod outer segment. *Nature*. 313:310-313.
- Fleischman, D., M. Denisevich, D. Raveed, and R. G. Pannbecker. 1980. Association of guanylate cyclase with the axoneme of retinal rods. *Biochimica et Biophysica Acta*. 630:176-186.
- Fukada, Y., and T. Yoshizawa. 1981. Activation of phosphodiesterase in frog rod outer segment by an intermediate of rhodopsin photolysis II. *Biochimica et Biophysica Acta*. 675:195-200.
- Gold, G. H. 1981. Photoreceptor coupling: its mechanism and consequences. *Current Topics in Membranes and Transport*. 15:59-89.
- Goldberg, N. D., A. Ames III, J. E. Gander, and T. F. Walseth. 1983. Magnitude of increase in retinal cGMP metabolic flux determined by ¹⁸O incorporation into nucleotide α -phosphoryls corresponds with intensity of photic stimulation. *Journal of Biological Chemistry*. 258:9213-9219.
- Hárosi, F. I. 1975. Absorption spectra and linear dichroism of some amphibian photoreceptors. *Journal of General Physiology*. 66:357-382.
- Kawamura, S. 1983. Involvement of ATP in activation and inactivation sequence of phosphodiesterase in frog rod outer segments. *Biochimica et Biophysica Acta*. 732:276-281.
- Kawamura, S., and M. D. Bownds. 1981. Light adaptation of the cyclic GMP phosphodiesterase of frog photoreceptor membranes mediated by ATP and calcium ions. *Journal of General Physiology*. 77:571-591.
- Kawamura, S., and M. Murakami. 1983. Intracellular injection of cyclic-GMP increases sodium conductance in gecko photoreceptors. *Japanese Journal of Physiology*. 33:789-800.
- Kawamura, S., and M. Murakami. 1985. Functional role of phosphodiesterase in gecko photoreceptors. *Neuroscience Research*. Suppl. 2:S119-S125.

- Kilbride, P., and T. G. Ebrey. 1979. Light-initiated changes of cyclic guanosine monophosphate levels in frog retina measured with quick-freezing technique. *Journal of General Physiology*. 74:415-426.
- Korenbrod, J. I. 1984. Signal mechanisms of phototransduction in retinal rod. *CRC Critical Review of Biochemistry*. 17:223-256.
- Liebman, P. A., and A. Sitaramayya. 1984. Role of G-protein-receptor interaction in amplified phosphodiesterase activation of retinal rods. *Advances in Cyclic Nucleotide Research*. 17:215-225.
- MacLeish, P. R., E. A. Schwartz, and M. Tachibana. 1984. Control of the generator current in solitary rods of the *Ambystoma tigrinum* retina. *Journal of Physiology*. 348:645-664.
- Matthews, H. R., V. Torre, and T. D. Lamb. 1985. Effects on the photoresponse of calcium buffers and cyclic GMP incorporated into the cytoplasm of retinal rods. *Nature*. 313:582-585.
- Miki, N., J. J. Keirns, F. R. Marks, J. Freeman, and M. W. Bitensky. 1973. Regulation of cyclic nucleotide concentration in photoreceptors: an ATP-dependent stimulation of cyclic nucleotide phosphodiesterase by light. *Proceedings of the National Academy of Sciences*. 70:3820-3824.
- Miller, W. H. 1982. Physiological evidence that light-mediated decrease in cyclic GMP is an intermediary process in retinal rod transduction. *Journal of General Physiology*. 80:103-123.
- Miller, W. H. 1983. Physiological effects of cyclic GMP in the vertebrate retinal rod outer segment. *Advances in Cyclic Nucleotide Research*. 15:495-511.
- Miller, W. H., and G. D. Nicol. 1979. Evidence that cyclic GMP regulates membrane potential in rod photoreceptors. *Nature*. 280:64-66.
- Pinto, L. H., and J. E. Brown. 1984. Pressure injection of 3',5'-cyclic GMP into solitary rod photoreceptors of the tiger salamander. *Brain Research*. 304:197-200.
- 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. *Journal of General Physiology*. 77:41-48.
- Robinson, P. R., S. Kawamura, B. Abramson, and M. D. Bownds. 1980. Control of the cyclic GMP phosphodiesterase of frog photoreceptor membranes. *Journal of General Physiology*. 76:631-645.
- Schwartz, E. A. 1985. Phototransduction in vertebrate rods. *Annual Review of Neuroscience*. 8:339-367.
- Stryer, L. 1983. Transducin and the cyclic GMP phosphodiesterase: amplifier proteins in vision. *Cold Spring Harbor Symposium on Quantum Biology*. 48:841-852.
- 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. *Journal of General Physiology*. 73:629-653.
- Yamazaki, A., I. Sen, M. W. Bitensky, J. E. Casnellie, and P. Greengard. 1980. Cyclic GMP-specific, high affinity, noncatalytic binding sites on light-activated phosphodiesterase. *Journal of Biological Chemistry*. 255:11619-11624.
- Yamazaki, A., S. Uchida, P. J. Stein, G. L. Wheeler, and M. W. Bitensky. 1984. Enzyme regulation and GTP binding protein: an algorithm of control that includes physical displacement of an inhibitory protein. *Advances in Cyclic Nucleotide Research*. 16:381-392.
- Yau, K.-W., and K. Nakatani. 1984. Electrogenic Na-Ca exchange in retinal rod outer segment. *Nature*. 311:661-663.

- Yau, K.-W., and K. Nakatani. 1985. Light-suppressible, cyclic GMP-sensitive conductance in the plasma membrane of a truncated rod outer segment. *Nature*. 317:252-255.
- Yee, R., and P. A. Liebman. 1978. Light-activated phosphodiesterase of the rod outer segment. *Journal of Biological Chemistry*. 253:8902-8909.