

# Light-induced Changes in GTP and ATP in Frog Rod Photoreceptors

## *Comparison with Recovery of Dark Current and Light Sensitivity During Dark Adaptation*

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**ABSTRACT** Light decreases GTP and ATP levels in purified suspensions of physiologically active frog rod outer segments still attached to their inner segment ellipsoids (OS-IS). (a) The GTP decrease is slower in OS-IS ( $t_{1/2} = 40$  s) than in isolated outer segments ( $t_{1/2} = 7$  s), which suggests there is more effective buffering in OS-IS. (b) The GTP decrease becomes detectable only at intensities greater than those required to saturate the photoresponse. As the intensity of a continuous light is increased over 4 log units, GTP levels decrease linearly with log intensity by as much as 60%. GTP is reduced to steady intermediate levels during extended illumination of intermediate intensity. (c) At levels of illumination bleaching  $>0.003\%$  of the rhodopsin, a decrease in ATP levels becomes detectable. (d) Following a flash, GTP levels fall and then rise with a recovery time dependent on the intensity of the flash. (e) After both 0.2 and 2% flash bleaches, the recovery of GTP levels parallels the recovery of light sensitivity, which is slower than the recovery of the dark current. This raises the possibility of a link between GTP levels and light sensitivity.

### INTRODUCTION

In this paper, we examine light-stimulated changes in the levels of ATP and GTP in purified suspensions of isolated frog rod outer segments still attached to the mitochondria-rich ellipsoid portion of their inner segments (OS-IS; cf. Biernbaum and Bownds, 1985). These structures, in contrast to isolated outer segments (OS), maintain dark currents and light sensitivities similar to those of photoreceptors on the living retina. Thus, it is possible to measure the time course of light-induced changes in the concentration of endogenous molecules,

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and to compare these changes directly with aspects of the electrophysiological response obtained concurrently from the same preparation.

Our previous studies using crude suspensions of intact OS have examined light-induced changes in cyclic GMP (Woodruff et al., 1977; Woodruff and Bownds, 1979; Polans et al., 1981) and GTP (Biernbaum and Bownds, 1979), and the nucleotide-dependent phosphorylations of rhodopsin and other proteins (Polans et al., 1979; Hermolin et al., 1982). From this work and from studies of light-sensitive enzymes assayed in more disrupted preparations (P. R. Robinson et al., 1980; Kawamura and Bownds, 1981), several roles for cyclic GMP and nucleoside triphosphates in amplification and desensitization of the photoreponse have been proposed (Bownds, 1980, 1981).

This paper examines GTP and ATP, both known to play a central role in photoreceptor function. GTP binds to the G-protein that links rhodopsin bleaching to activation of cyclic GMP phosphodiesterase (PDE), and GTP is then hydrolyzed during the inactivation sequence (Kuhn, 1980; Fung and Stryer, 1980; Fung et al., 1981; Kuhn et al., 1981; Hurley and Stryer, 1982; Fung, 1983; Yamazaki et al., 1983). GTP is also the substrate for guanylate cyclase, which de Azeredo et al. (1981) and Goldberg et al. (1983) have reported to be activated by light in frog photoreceptors. GTP measurements in isolated outer segments have raised the possibility that GTP plays a role in calcium transport (Biernbaum and Bownds, 1979).

ATP serves as substrate for protein phosphorylations (Hermolin, 1981), supports GTP synthesis by transphosphorylation (Berger et al., 1980; Schnetkamp and Daemen, 1981), and is used by the inner segment  $\text{Na}^+, \text{K}^+$ -ATPase for maintenance of the light-sensitive dark current. ATP also modulates phosphodiesterase activity (Liebman and Pugh, 1980; Kawamura and Bownds, 1981), possibly through phosphorylation of rhodopsin or some other protein (Hermolin et al., 1982; Sitaramayya and Liebman, 1983).

The nucleotide measurements presented in this paper demonstrate that illumination can decrease both ATP and GTP levels in OS-IS. The ATP decrease is not observed in isolated OS (Biernbaum and Bownds, 1979; W. E. Robinson and Hagins, 1979). The GTP changes show no correlation with light-induced current changes, but after bright light, GTP recovery parallels sensitivity recovery.

#### METHODS

##### *Preparation of Suspensions of Outer Segments with Attached Ellipsoids (OS-IS)*

All experiments were performed using purified suspensions of isolated frog rod outer segments attached to the ellipsoid portion of the inner segment (OS-IS), prepared under infrared illumination by suction treatment of the freshly dissected, dark-adapted retina and purified by discontinuous Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) gradient sedimentation (Biernbaum and Bownds, 1985). The Ringer's solution used in all experiments consisted of 105 mM NaCl, 2.5 mM KCl, 10 mM HEPES, 2 mM  $\text{MgCl}_2$ , 1 mM  $\text{CaCl}_2$ , 5 mM glucose, 5 mM  $\text{NaHCO}_3$ , pH 7.5–7.6. OS-IS were judged to be osmotically intact by their ability to exclude the fluorescent dye didansylcysteine (DDC) (Yoshikami et al., 1974) and by other criteria (Biernbaum and Bownds, 1985). In the experiments reported in this paper, only suspensions containing >50% OS-IS were used.

### *Measurement of Nucleoside Triphosphate Levels*

Levels of GTP and ATP in suspensions of OS-IS were measured by high-pressure liquid chromatography (HPLC) as described previously (Biernbaum and Bownds, 1979). The following protocol was used throughout. The required number of aliquots (100  $\mu$ l) were withdrawn from the purified suspension of OS-IS, which had been diluted with Ringer's medium containing Percoll. Initial dark control samples were quenched with 10% trichloroacetic acid (TCA; 200  $\mu$ l). Some of the remaining samples were then exposed to continuous or flash illumination as described previously (Brodie and Bownds, 1976; Biernbaum and Bownds, 1985) and quenched with TCA. Quenching of samples within 1 s of onset of illumination was performed using the rapid quench device described elsewhere (Cote et al., 1984). Dark control samples were sometimes quenched during the course of the experiment, and final dark control samples were quenched at the end of every experiment. Samples were chilled immediately after quenching and sedimented (15 min at 3,000 g) and the supernatants were analyzed by HPLC. Rhodopsin content was determined by difference spectroscopy (Bownds et al., 1971).

### *Measurement of Dark Current and Light Sensitivity*

OS-IS were assayed for dark current and light sensitivity using the suction electrode technique of Baylor et al. (1979), with the apparatus described by Biernbaum and Bownds (1985). All measurements were obtained on OS-IS from the same suspensions used for measurement of nucleotide levels or from identically prepared OS-IS suspensions. The dark current was determined by presenting a flash bleaching  $\sim$ 120 molecules of rhodopsin per OS-IS, an intensity sufficient to just saturate the photocurrent (Biernbaum and Bownds, 1985). (Flashes were focused as a 75-  $\times$  200- $\mu$ m rectangle centered at the tip of the electrode.) The flash intensity required to half-suppress the dark current was then determined. To examine the time course of dark current suppression and sensitivity changes, a flash bleaching 0.2 or 2% of the rhodopsin was presented. Recovery of dark current was recorded, and recovery of sensitivity was followed by measuring, every 15 s, the response to a test flash that had caused a half-maximal suppression of the dark current in the dark-adapted condition. The number of isomerizations was determined by bleaching a suspension of rods in the recording chamber with a diffuse beam from the unattenuated lamp, directly measuring the bleach by difference spectroscopy, and then attenuating the intensity with calibrated neutral density filters. When a photodiode was used, its response was calibrated against this.

OS-IS preparations were usually stable for several hours after isolation from the retina (Biernbaum and Bownds, 1985). Approximately 5% of the OS-IS tested showed deterioration sooner than this, with collapse of the dark current during the measurement of recovery of sensitivity. The incomplete data from such OS-IS, as well as from OS-IS that had dark currents of  $<8$  pA, were not included in the data averaging.

## RESULTS

### *Continuous Illumination Causes Sustained Decreases in GTP and ATP Levels*

Illumination of OS-IS causes levels of GTP and ATP to decrease. This is shown in Fig. 1, a composite of three experiments in which purified suspensions of OS-IS (Biernbaum and Bownds, 1985) were exposed to light bleaching 1% of the rhodopsin present per minute. GTP levels decrease for 1.5–2 min, with  $t_{1/2} = 40$  s, and remain low as long as the light is on. This change in GTP is slower than that occurring in crude suspensions of OS (lower dashed line; data from Biernbaum and Bownds, 1979). In crude OS suspensions, the GTP decrease has a

$t_{1/2} = 7$  s, and is complete within 20–30 s. This same time course was observed in the present work using Percoll-purified OS. The slower time in OS-IS is probably the result of more effective buffering of GTP levels (Biernbaum and Bownds, 1985; see also Fig. 4). While a slightly larger percentage decrease occurs in isolated OS, GTP concentrations are fivefold higher in OS-IS than in OS (Biernbaum and Bownds, 1985), and thus the decrease in OS-IS represents a greater utilization of nucleotide than in OS. The fall in GTP is accompanied by

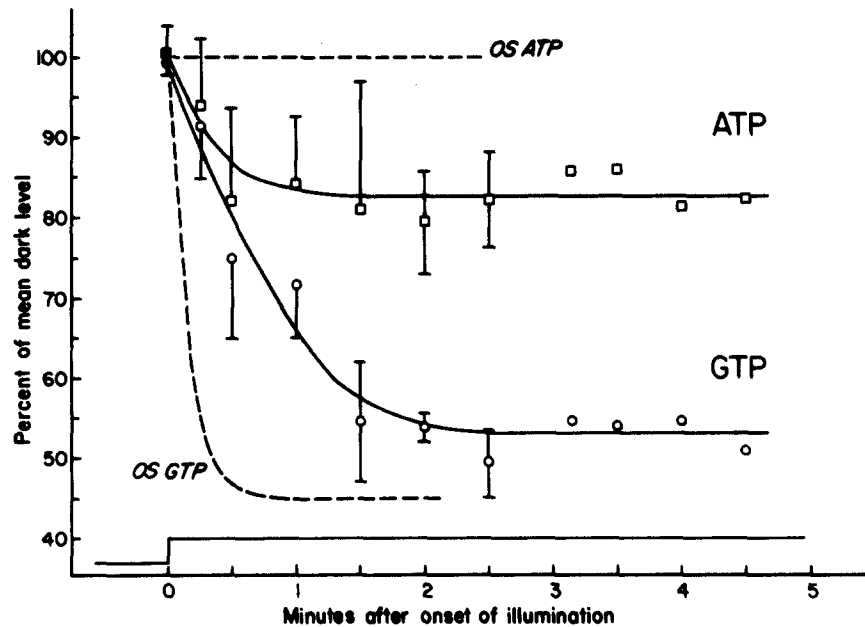


FIGURE 1. Bright continuous illumination causes sustained decreases in GTP and ATP levels in Percoll-purified OS-IS. Levels of GTP (open circles) and ATP (open squares) in OS-IS are plotted as a function of time (minutes) after the onset of illumination bleaching  $5 \times 10^5$  rhodopsin molecules per OS-IS·s. The figure is a composite of 36 determinations for each nucleotide from three separate preparations containing 70–80% OS-IS. Approximately 0.3 mol of GTP and of ATP is present per mole of rhodopsin. Illuminated levels, shown as means  $\pm$  SEM ( $n \geq 3$ ), except mean  $\pm$  range ( $n = 2$ ) at 2.5 min and single points from one experiment thereafter, are expressed as percent of mean dark control levels (closed symbols), obtained over the same time course and shown  $\pm$  SEM ( $n = 8$ ). The effect of illumination on GTP and ATP levels in crude isolated OS are indicated by lower and upper dashed lines, respectively (Biernbaum and Bownds, 1979).

an equimolar rise in GDP (not shown), which suggests that GTPase activity accounts for the decrease in GTP concentration. GDP levels in crude suspensions of OS rise by an amount equal to 50% of the light-induced GTP decrease (Biernbaum and Bownds, 1979).

Fig. 1 also shows that bright light causes levels of ATP in OS-IS to decrease by 15%, and ATP levels remain suppressed while the light is on. This change becomes maximal after 30 s, while GTP levels continue to fall. Measured over

14 separate experiments, illumination bleaching  $>0.3\%$  of the rhodopsin caused ATP levels to fall  $22.0 \pm 4.2\%$  (SEM). ATP levels in crude suspensions of isolated OS (upper dashed line) are unchanged by illumination (Biernbaum and Bownds, 1979), a result confirmed in the present work using Percoll-purified suspensions of OS. The data in Fig. 1 also demonstrate an observation we made throughout the study: the magnitude of the light-induced decrease in ATP averages 40–50% of the size of the GTP decrease.

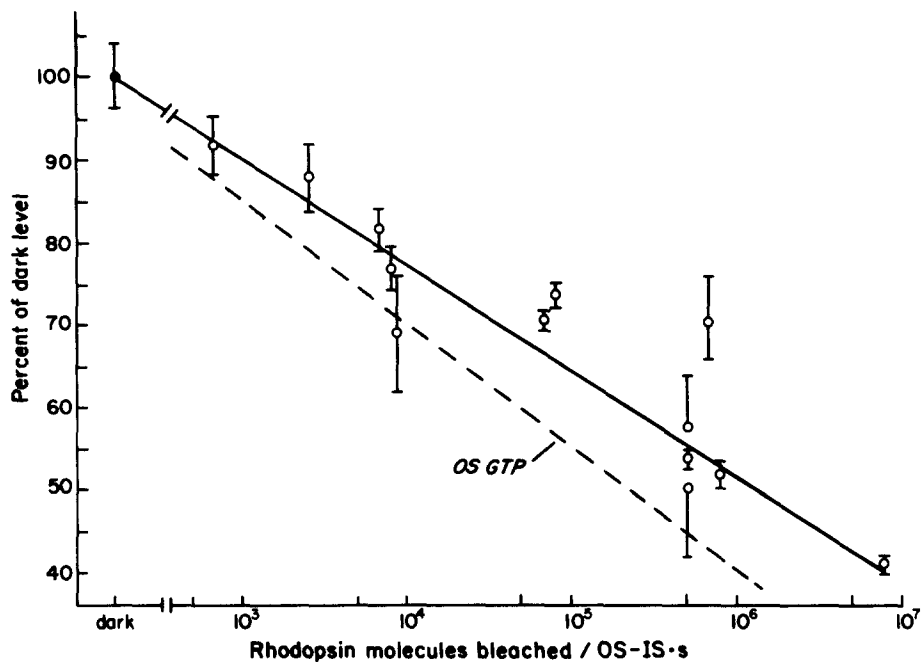


FIGURE 2. The light-induced decrease in GTP levels in OS-IS spans 4 log units of continuous light intensity. Levels of GTP in illuminated OS-IS (open circles), measured for maximal decreases after 2 min illumination, are expressed as percent of mean dark level (closed circle). GTP levels are plotted as a function of the incident light intensity, expressed as the number of rhodopsin molecules bleached per OS-IS · s. The figure is a composite of data from two separate preparations containing 50–75% OS-IS, with two to three determinations at each intensity in both experiments, plus data from the continuous light experiments in Figs. 1 and 3. Means are shown  $\pm$  SEM for  $n \geq 3$  and  $\pm$  range for  $n = 2$ , and dark GTP levels ranged from 0.1 to 0.3 mol per mole of rhodopsin. The line is hand-drawn. A reduction of 420 molecules of GTP per molecule of rhodopsin bleached is calculated at the lowest intensity shown. The GTP response in crude isolated OS is shown by the dashed line (Biernbaum and Bownds, 1979).

#### *Saturation of Photocurrent Response Precedes Measurable GTP Chemistry*

Maximum suppression of the dark current in OS-IS requires bleaching fewer than 200 rhodopsin molecules (Fain, 1976; Baylor et al., 1979; Bader et al., 1979; Lamb et al., 1981; Liebman et al., 1984; Biernbaum and Bownds, 1985);

much brighter illumination must be used to observe GTP (and ATP) decreases. The light sensitivity of the GTP pool in OS-IS is shown in Fig. 2. Levels of GTP, measured after 2 min of continuous illumination, are plotted as a function of the incident continuous light intensity (log scale).

The first detectable decrease in GTP shown in Fig. 2 occurs after  $8 \times 10^4$  molecules of rhodopsin per OS-IS have been bleached (by a light bleaching 670 rhodopsin molecules per OS-IS·s). A similar value ( $2 \times 10^4$  rhodopsins bleached per OS-IS) was obtained by Biernbaum and Bownds (1985), using dim flicker illumination bleaching 115 rhodopsin molecules per OS-IS per flash, and the same value is obtained from the flash response shown below in Fig. 4. Utilization of GTP is presumably occurring at lower light levels, but is detectable only after bleaching this number of rhodopsin molecules, independent of the timing of the illumination. At the limit of detection in Fig. 2, a decrease of at least 420 GTP molecules per rhodopsin molecule bleached is calculated. Because GTP is being replenished, the actual hydrolysis of GTP is undoubtedly much larger. The magnitude of the GTP response increases with the logarithm of the light intensity over four decades, from light bleaching 670 rhodopsin molecules per OS-IS·s to light bleaching  $8 \times 10^6$  rhodopsin molecules per OS-IS·s. The GTP response in isolated OS (dashed line, taken from Biernbaum and Bownds, 1979) spans this same range of intensities. In the experiments of Fig. 2, decreases in ATP could be measured with light intensities bleaching at least  $8 \times 10^5$  rhodopsin molecules per OS-IS·s.

Fig. 3 shows data from two experiments monitoring the time course of GTP changes at an intermediate light intensity. It demonstrates that a steady state level of GTP is reached after ~2 min and is maintained while the light is on. OS-IS were exposed to continuous illumination bleaching  $8 \times 10^5$  rhodopsin molecules per OS-IS·s, an intensity less than that causing a half-maximal GTP decrease (cf. Fig. 2). After onset of illumination, GTP levels decrease ~25% below dark levels, with a time course similar to that in Fig. 1. It would appear that during continuous illumination, a light adaptation occurs that sets a new equilibrium between GTP synthesis and decay, and thus a new steady level of GTP is established. This is consistent with evidence for GTP (and ATP) synthesis in OS-IS (Biernbaum and Bownds, 1985), and the presence of nucleoside diphosphokinase and other buffering enzymes in the inner and outer segment (Berger et al., 1980; Schnetkamp and Daemen, 1981).

#### *Flash Illumination Causes Transient Decreases in GTP and ATP Levels*

Following a flash, GTP first decreases by an amount graded with the intensity of the flash and then recovers more slowly to dark-adapted levels. This is shown in Fig. 4, where levels of GTP (upper panel) and ATP (lower panel) are plotted following flashes of increasing intensity. Illuminated levels of GTP and ATP (open symbols) are expressed as a percent of dark levels (closed symbols) in the minutes following the flash.

Small decreases in GTP levels (average 17%) are observed after a flash bleaching  $9 \times 10^4$  rhodopsin molecules per OS-IS, equal to 0.003% of the rhodopsin (triangles). In these experiments, the decrease corresponds to a re-

duction of at least 450–1,600 molecules of GTP per molecule of rhodopsin bleached. The lower limit of this range is the same value obtained in Fig. 2 with continuous illumination.

With brighter flashes, larger GTP decreases develop, the time to peak is longer, and recovery time is longer. Thus, after a 0.2% bleach (squares), GTP levels fall 25% below dark levels and remain suppressed for ~30 s. Recovery of GTP levels is underway by 45 s after the flash, and by 90 s after the flash, GTP levels are substantially restored. Full restoration to dark-adapted levels occurs by 3 min. Following a 2% bleach (circles), GTP levels fall over a 1.5-min period

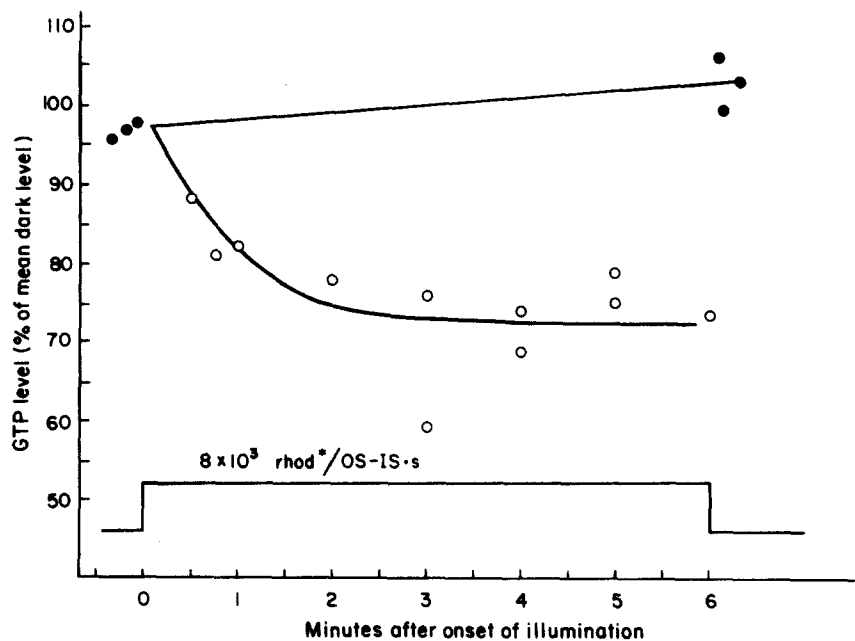


FIGURE 3. GTP levels are maintained at intermediate levels by continuous illumination of intermediate intensity. Levels of GTP, expressed as moles of GTP per mole of rhodopsin, are plotted as a function of time (minutes) after onset of illumination bleaching  $8 \times 10^3$  rhodopsin molecules per OS-IS·s. Dark and illuminated levels (closed and open circles, respectively) in two separate preparations containing 50–95% OS-IS are indicated.

( $t_{1/2} = 40$  s) and then begin a slow recovery. Complete restoration to dark levels occurs ~9 min after the flash.

The complete recovery of GTP levels in OS-IS contrasts with the partial recovery observed in isolated OS (Biernbaum and Bownds, 1979), which suggests greater synthetic capacity in OS-IS (cf. Biernbaum and Bownds, 1985). GTP levels recover at approximately the same rate after each of the flashes, by an amount equal to 10% of the dark level per minute. In the experiments of Fig. 4, this is equal to 0.03 mol of GTP per mole of rhodopsin per minute. This may set a lower limit on the GTP consumption that must be triggered by light to

cause measurable decreases. Finally, a comparison of Fig. 4 with Fig. 2 shows that flash illumination causes the same decrease in GTP as continuous illumination bleaching the same amount of rhodopsin within 2 min (i.e., before new equilibrium levels are established in the light).

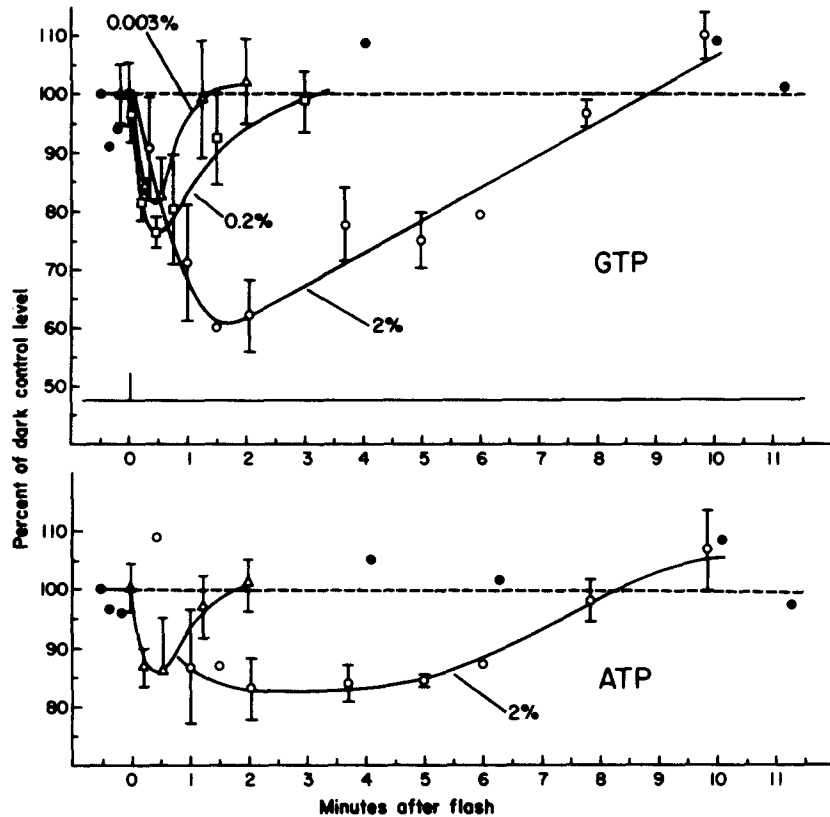


FIGURE 4. Flash illumination causes transient decreases in GTP and ATP levels that are graded with intensity. Levels of GTP (upper panel) and ATP (lower panel) in the light (open symbols) are plotted as a function of time (minutes) after flashes of increasing intensity: 0.003% bleach (triangles, two experiments), 0.2% bleach (squares, five experiments), and 2% bleach (circles, two experiments). Preparations contained 70–90% OS-1S. GTP and ATP levels are expressed as percent of dark control levels (closed symbols, mean value 0.3 mol of GTP per mole of rhodopsin). Means are shown  $\pm$  SEM with  $n \geq 3$ , except  $\pm$  SEM for dark ( $n = 19$ ) and subsecond ( $n = 18$ ) values after 0.2% bleach, and  $\pm$  range of two values after 2% bleach. All curves are hand-drawn.

The lower panel of Fig. 4 shows that flashes can also cause transient decreases in ATP levels. A small transient decrease is measured after a flash bleaching 0.003% of the rhodopsin present (triangles). A reduction of at least 500–1,300 molecules of ATP per molecule of rhodopsin bleached is calculated. Bleaching 2% of the rhodopsin present causes a prolonged decrease in ATP levels (circles).



As in Fig. 1, the time course of the ATP response diverges from that of GTP. Here, the start of recovery of ATP after the flash lags that of GTP: ATP levels remain suppressed during the period 1.5–6 min after the flash, while over this same period, the GTP decrease has reversed by >50%. This delayed recovery of ATP might reflect continued utilization of ATP for synthesis of GTP. Alternatively, the ATP might be utilized by other activities such as the ATP-dependent inactivation of PDE (Liebman and Pugh, 1980; Kawamura and Bownds, 1981) or protein phosphorylation reactions (Polans et al., 1979).

#### *Comparison of GTP and Sensitivity Recovery During Dark Adaptation*

To determine whether any correspondence exists between light-stimulated GTP and ATP changes and electrophysiological responses of the cell, membrane current recordings were obtained from OS-IS using the suction electrode technique of Baylor et al. (1979). Percoll-purified OS-IS maintain dark currents and light sensitivities similar to those of rods still attached to the living retina (Biernbaum and Bownds, 1985). In the present work, the recovery of both the dark current and sensitivity to light after a bright flash was monitored in many OS-IS preparations concurrently with nucleotide measurements. (Sensitivity is measured here not as current suppression per photon absorbed, but in a more approximate way—as response amplitude to a test flash during dark adaptation, with the test flash having the intensity required to half-suppress the current of a dark-adapted cell.) The data were obtained from OS-IS in the same Percoll-purified suspensions whose nucleotide responses are plotted in Fig. 4 or in identically prepared suspensions. In these OS-IS, the recovery of dark current precedes restoration of sensitivity. A similar separation between recovery of current (or membrane potential) and sensitivity has been reported by Baylor and Lamb (1982), Conner (1982), Kleinschmidt and Dowling (1975), and Toyoda et al. (1970).

Fig. 5 shows recovery toward dark-adapted levels of dark current (closed circles) and sensitivity to light (open circles) following flashes at two different intensities. The upper panel shows the response to a 0.2% bleach. For several seconds, the dark current is maximally suppressed, but within 30 s, 50% of the dark current is restored. No response to the test flash is observed over this period. The first response to the test flash is detected  $38 \pm 7$  s after the flash. Recovery of sensitivity has a half-time of 1–2 min and is complete in 2.5 min. Under these conditions, GTP recovery is also half-complete in 1–2 min (dashed line, taken from Fig. 4).

The lower panel of Fig. 5 shows recovery of current and light sensitivity following a 2% bleach. At this higher flash intensity, the dark current is suppressed longer and recovery is slower. The current remains maximally suppressed for 30 s, and 50% recovery of current requires 2 min. As before, the first response to the test flash is observed at this point, in this case  $2.1 \pm 0.2$  min after the flash, and the sensitivity recovery has a half-time of 5–6 min. GTP recovery (dashed line, taken from Fig. 4) also is half-complete in 5–6 min.

The data of Figs. 4 and 5 clearly demonstrate that GTP recovery from flash illumination is slower than recovery of dark current, but is approximately the

same as the recovery of light sensitivity. Note, however, that it is the relative recovery of GTP that correlates with the recovery of sensitivity, not absolute levels of GTP.

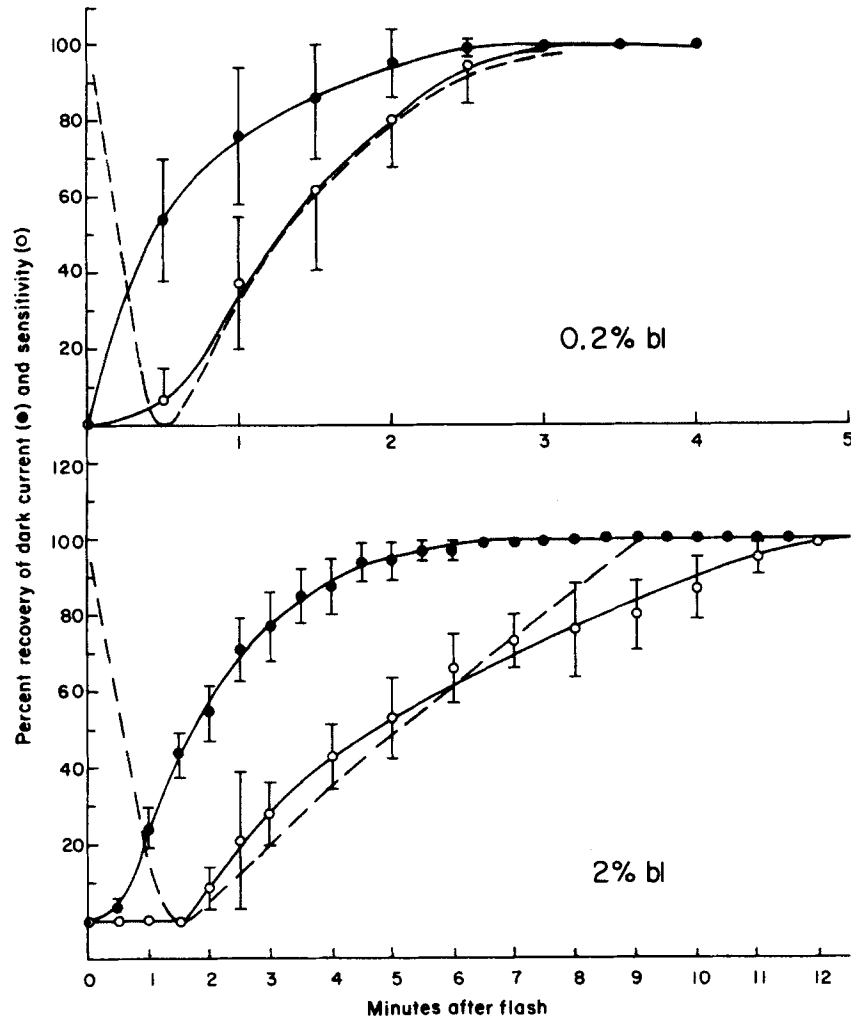


FIGURE 5. Restoration of dark current in OS-IS precedes recovery of light sensitivity after a flash. Recovery of dark current (closed circles) and of light sensitivity (open circles) is plotted as a function of time (minutes) after flashes bleaching 0.2% (upper panel, 7 cells, 2 experiments) and 2% (lower panel, 19 cells, 7 experiments) of the rhodopsin present. Recovery (mean  $\pm$  SEM) is expressed as percent of dark-adapted levels. Current recordings were obtained by the suction electrode technique described by Baylor et al. (1979) using the same or comparable Percoll-purified OS-IS whose GTP levels are plotted in Fig. 3 and which are indicated here by the dashed lines. Dark currents were 8–26 pA. The response to a flash half-saturating the photoresponse, bleaching 60 rhodopsin molecules per OS-IS (Biernbaum and Bowns, 1985), was used to measure recovery of sensitivity.

## DISCUSSION

Experiments in this and in the preceding report (Biernbaum and Bownds, 1985) establish that levels of ATP and GTP maintained in OS-IS for 1–2 h after isolation from the retina provide energy reserves sufficient to support excitation and adaptation. ATP and GTP concentrations were 1.5 and 1.8 mM, respectively, calculated with respect to extradiskal volume, the same as those reported by Zuckerman et al. (1982) and W. E. Robinson and Hagins (1979) for OS determined immediately after isolation from the retina and similar to those obtained *in vivo* by direct freeze-sectioning of the retina (de Azeredo et al., 1981; Berger et al., 1980). Large decreases in GTP and ATP concentration occur with intermediate and bright levels of illumination, which is compatible with a link between the energy metabolism of the system and adaptation processes. These decreases may be distinctive to adaptation mechanisms occurring at >0.2% bleaches. ATP and GTP decreases probably do not play a role in excitation, the suppression of conductance caused by dim illumination, because they are slow and are observed only at light levels higher than those required to saturate the conductance. It is important to recognize that these changes in levels of GTP and ATP give no measure of the flux through the pathways synthesizing and utilizing these nucleotides. The data in this and the preceding paper (Biernbaum and Bownds, 1985) indicate that OS-IS are synthesizing ATP and GTP and that such synthesis replenishes the utilization stimulated by light. Therefore, the reductions in GTP and ATP derived from the changes in steady state nucleotide levels measured here set only weak lower limits to the actual turnover of GTP and ATP.

The observed decreases in ATP or GTP would appear to be due to their increased utilization rather than to inhibition of synthesis; inhibition of synthesis would be expected to lower oxygen uptake, and illumination can have the opposite effect (Kimble et al., 1980). While the *in vivo* rates of synthesis are not known, several reactions that are directly or indirectly influenced by illumination are known to require nucleoside triphosphates: the  $\text{Na}^+, \text{K}^+$ -ATPase that maintains the ion gradients required for the dark current, the pathway that regulates cyclic GMP synthesis and degradation, and rhodopsin phosphorylation. The  $\text{Na}^+, \text{K}^+$ -ATPase can be excluded as a contributor to light-induced decreases because ATP hydrolysis by the pump will decrease after illumination.

Rhodopsin and enzymes of the cyclic GMP pathway, which constitute 90% of the protein in frog rod outer segments (Hamm, H. E., and M. D. Bownds, manuscript submitted for publication), seem likely to account for much of the ATP and GTP use. Concentration decreases in ATP are not observed until substantial rhodopsin bleaching has occurred, which suggests that it is only at this point that use of ATP to synthesize GTP (Berger et al., 1980; Schnetkamp and Daemen, 1981; Dontsov et al., 1978), phosphorylate rhodopsin (Kuhn and Dreyer, 1972; Bownds et al., 1972), and drive numerous other reactions overtakes the ability of the system to synthesize ATP. GTP is used by guanylate cyclase to synthesize cyclic GMP and by the GTPase (G-protein, transducin) that links rhodopsin bleaching to PDE activation.

The work of both de Azeredo et al. (1981) and Goldberg et al. (1983) has

shown that utilization of GTP for synthesis of cyclic GMP is stimulated by illumination. The observation by Goldberg et al. (1983) that illumination of rabbit retinas increases the turnover time of the cyclic GMP pool from seconds to milliseconds indicates that a substantial fraction of GTP utilization is occurring by this route. These findings, taken together with those of Cote et al. (1984) showing that decreases in the level of cyclic GMP in OS-IS are largely complete within 200–300 ms after illumination, indicate that activation of guanylate cyclase and utilization of GTP by guanylate cyclase is occurring long before decreases in GTP are measured. The changes in GTP measured here probably occur when the draw on GTP (by guanylate cyclase or G-protein) exceeds the ability of the system to synthesize GTP. A difficulty in evaluating this and other options for GTP (and ATP) use is that the turnover of these compounds may proceed at different rates in different parts of the outer segment. For example, an association has been shown between guanylate cyclase and the cilium that connects inner and outer segments (Fleischman et al., 1980), and Roof and Applebury (1984) have recently reported that the cilium extends along the length of the outer segment. It is possible that the rapid turnover of GTP associated with guanylate cyclase may occur in a functional compartment near the cilium and distinct from the remainder of the GTP pool in the cytoplasm.

It will be important to establish whether the correspondence between the recovery of GTP levels and the restoration of light sensitivity is observed as experimental conditions are varied. A question raised by this correspondence is whether GTP might serve a regulatory role. The enzymes involved in cyclic nucleotide metabolism and protein phosphorylation reactions contain many binding sites for ATP and GTP, some of which may serve not only in phosphate transfer reactions but as regulators of enzyme activity. Most of the known sites have binding constants of  $<100 \mu\text{M}$  (Pober and Bitensky, 1979), and the lowest ATP and GTP levels observed after bright illumination are  $\sim 400 \mu\text{M}$ . Thus, regulation by triphosphate concentration would require either regulatory sites with a higher binding constant, or that higher affinity sites be in a compartment in which the triphosphate change is larger than the average change.

The main point made by the observations in this paper is that ATP and GTP are well buffered in OS-IS preparations, and their concentrations are lowered only by illumination much greater than that required to produce a maximal current change. The light sensitivities of the ATP, GTP, and cyclic GMP decreases observed reflect their precursor-product relationship. ATP, as the precursor of GTP, begins to decrease when  $>10^5$  of the  $3 \times 10^9$  rhodopsin molecules in an outer segment have been bleached. GTP, the precursor of cyclic GMP, decreases after  $10^4$ – $10^5$  rhodopsins are bleached, and cyclic GMP decreases are observed with  $<10^5$  rhodopsins bleached (Cote et al., 1984). Regulatory roles for the GTP and ATP changes cannot be excluded, but it seems likely that their light-induced changes reflect their activity as precursors or substrates of compounds more directly involved in regulating sensitivity and conductance.

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