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Coupling of photoexcited rhodopsin to inositol phospholipid hydrolysis in fly photoreceptors

(invertebrate phototransduction/guanine nucleotide-binding regulatory protein/phospholipase C/inositol trisphosphate/2,3-bisphosphoglycerate)

O. DEVARY*, O. HEICHAL*, A. BLUMENFELD*, D. CASSEL*†, E. SUSS‡, S. BARASH‡, C. T. RUBINSTEIN‡, B. MINKE‡, AND Z. SELINGER*§

Departments of *Biological Chemistry and ‡Physiology, The Hebrew University of Jerusalem, 91904 Israel

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ABSTRACT **Fly photoreceptor membranes were used to test the effect on defined biochemical reactions of light and of compounds causing photoreceptor excitation. Complementary electrophysiological studies examined whether putative second messengers excite the fly photoreceptor cells. This analysis revealed the following sequence of events: photoexcited rhodopsin activates a G protein by facilitating GTP binding. The G protein then activates a phospholipase C that generates inositol trisphosphate, which in turn acts as an internal messenger to bring about depolarization of the photoreceptor cell. Binding assays of GTP analogs and measurements of GTPase activity showed that there are 1.6 million copies of G protein per photoreceptor cell. The GTP binding component is a 41-kDa protein, and the light-activated GTPase is dependent on photoconversion of rhodopsin to metarhodopsin. Analysis of phospholipase C activity revealed that this enzyme is under stringent control of the G protein, that the major product formed is inositol trisphosphate, and that this product is rapidly hydrolyzed by a specific phosphomonoesterase. Introduction of inositol trisphosphate to the intact photoreceptor cell mimics the effect of light, and bisphosphoglycerate, which inhibits inositol trisphosphate hydrolysis, enhances the effects of inositol trisphosphate and of dim light. The interaction of photoexcited rhodopsin with a G protein is thus similar in both vertebrate and invertebrate photoreceptors. These G proteins, however, activate different photoreceptor enzymes: phospholipase C in invertebrates and cGMP phosphodiesterase in vertebrates.**

A central question in vision research is the identity of the biochemical pathway that underlies the phototransduction process. It is now well established that cGMP is the internal messenger of visual transduction in vertebrate rods and that a guanine nucleotide-binding regulatory protein (G protein, specifically transducin) couples the photoexcited rhodopsin to cGMP hydrolysis (reviewed in refs. 1 and 2). A G protein appears to be involved also in invertebrate phototransduction. Cephalopod rhodopsin has been found to interact with a G protein and to crossreact with vertebrate photoreceptor enzymes (3–5). In the fly a photopigment-dependent modulation of GTPase activity in eye membrane preparations was found to mimic photoreceptor excitation by colored lights, indicating that a G protein participates in phototransduction (6). The target enzyme for the invertebrate G protein, however, has not as yet been identified. Furthermore, the identification of the second messenger for invertebrate phototransduction is still under dispute as inositol trisphosphate ($InsP_3$) (7, 8) and cGMP (9) have been found to excite the *Limulus* ventral photoreceptors. Biochemical studies revealed a light-dependent increase in cGMP in squid retina (9, 10), whereas a light-induced increase in $InsP_3$ has been reported in both *Limulus* photoreceptors (8) and in squid retina (11). In none of these systems, however, has inositol phospholipid hydrolysis been studied in membrane preparations nor has the role of a G protein been analyzed.

We have initiated combined biochemical and electrophysiological studies of phototransduction in *Musca* and *Drosophila* flies, results of which can be used for detailed analysis of phototransduction employing photoreceptor-potential mutants of *Drosophila* (12).

MATERIALS AND METHODS

Preparation of Fly Photoreceptor Membranes. All experiments were performed on white-eyed *Musca domestica* or white-eyed Oregon R *Drosophila* flies. Five hundred eyes from *Musca* flies were dissected with a razor blade under white light and collected into 5 ml of ice-cold homogenization buffer [250 mM sucrose, 120 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 10 mM Mops (pH 7.0), 1 mM dithiothreitol, 10 μg of leupeptin per ml, and 1 μg of pepstatin A per ml]. All further operations were carried out at 4°C under dim, red light. The eyes were kept in the dark for 30 min and then homogenized in a tight Teflon/glass homogenizer. A crude membrane fraction was obtained by centrifugation of the homogenate for 15 min at 14,000 × *g*. The membrane pellet was suspended in homogenization buffer at a concentration of 200 eye equivalents per ml and kept in an aluminum foil-covered tube under liquid nitrogen. *Drosophila* photoreceptor membranes were prepared essentially as described above except that 2000 frozen *Drosophila* heads were used as starting material.

Measurement of Inositol Phospholipid Hydrolysis. *Musca* eyes or *Drosophila* heads were cut and halved with a razor blade. Equivalents of 100 *Musca* eyes or 200 *Drosophila* heads were incubated in the dark for 4 hr at 30°C in 0.5 ml of 150 mM NaCl, 10 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM Hepes (pH 7.4), 10 mM glucose, 5 mM succinate, and 20 μCi (1 Ci = 37 GBq) of [^{3}H]inositol per ml. Throughout the incubation the medium was equilibrated with 100% O_2 and gently shaken. At the end of incubation, free [^{3}H]inositol was removed by three replacements with a calcium-free medium interspaced by 5-min incubation periods. The crude membrane fraction was obtained by homogenization in Teflon/glass homogenizer under dim, red light using the homogenization medium described above, followed by centrifugation for 15 min at 14,000 × *g*. Membranes were suspended in

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Abbreviations: $InsP_3$, inositol trisphosphate; $InsP_2$, inositol bisphosphate; GTP[S], guanosine 5′-[γ-thio]triphosphate; GDP[S], guanosine 5′-[β-thio]diphosphate; G protein, guanine nucleotide-binding regulatory protein.

†Present address: Department of Biology, Technion, Haifa, Israel.
§To whom correspondence should be addressed.

homogenization buffer and kept under liquid nitrogen. Light-dependent inositol phospholipid hydrolysis was measured in 30 mM Mops (pH 6.7), 6 mM $MgCl_2$, 1 mM dithiothreitol, 0.01 mM GTP, 1 mM ATP, bovine serum albumin at 0.2 mg/ml, 5 mM creatine phosphate, and creatine kinase at 50 units/ml. The medium was adjusted with CaEGTA to give 50 nM free Ca^{2+}. The reaction was initiated by the addition of stock membranes to a reaction mixture at 30°C. At each incubation time a 0.1-ml aliquot was removed, the reaction stopped with an equivalent volume of 5% (wt/vol) trichloroacetic acid and centrifuged, and the supernatant was analyzed for inositol phosphates by chromatography on Dowex 1 columns (13).

Electrophysiological Measurements. Intracellular recordings were performed on intact *M. domestica* flies immobilized by wax; 2 M KCl-filled micropipettes of 100- to 150-MΩ resistance were used. Electrodes were lowered into the retina by way of a small hole in the cornea, covered by petroleum jelly. The indifferent electrode, a broken pipette filled with fly Ringer's solution (140 mM NaCl/2 mM $CaCl_2$/2 mM KCl/5 mM $MgCl_2$/10 mM Hepes, pH 7.0) was placed close to the recording pipette. A third pressure injection pipette was placed into the hole in the cornea. This pipette contained a Ringer's solution with part of the NaCl component replaced, by an equivalent concentration of the test compound. The electrical responses, amplified 100 times and filtered by a low-pass filter (26A2 Tektronix differential amplifier with 3dB point at 1 KHz), were sampled by an LSI 11/23 microcomputer (Digital Equipment, Maynard, MA) at 500 samples per sec, which is sufficient for the bandwidth of the signal (14). Increasing the sampling rate to 4000 samples per sec did not significantly change the power spectrum below 150 Hz. Power spectra were calculated by fast Fourier transform from blocks of 1024 points. The power spectra of 10 consecutive nonoverlapping blocks were averaged. The averaged spectra were further smoothed by a moving n point averaging with $n \leq 31$.

RESULTS

Quantitation and Identification of the G Protein. An advantage of the fly photoreceptors is that their photopigment is thermostable and photoreversible with a large spectral difference between rhodopsin (λ_{max}, 490 nm) and metarhodopsin (λ_{max}, 570 nm). By applying blue light (<490 nm), rhodopsin is converted to metarhodopsin (80%) and regenerated to rhodopsin (100%) by red light (>580 nm). Thus by the use of lights of different wavelength the content of metarhodopsin, the active form of the photopigment, can be manipulated (15). In the intact fly photoreceptor, conversion of a substantial amount of rhodopsin to metarhodopsin by blue light induces persistent excitation that far outlasts the light stimulus and is known as the prolonged depolarizing after potential (15, 16). A prolonged depolarizing after potential-like phenomenon is also observed in membrane preparations when light-dependent GTPase is monitored (6).

The number of G-protein molecules in the photoreceptors was determined by monitoring the time course of ^{35}S-labeled guanosine 5'-[γ-thio]triphosphate (GTP[S]) binding to eye membrane preparations, preilluminated with either blue or red light. The incremental binding of GTP[S] after blue illumination is relatively fast and saturates after 10 min, while GTP[S] binding to membranes preilluminated with red light is slow and progresses linearly over 10 min (Fig. 1). This background value was subtracted from the total binding after blue illumination. Binding assays to crude *Musca* eye membranes over a range of GTP[S] concentrations from 10^{-8} M to 10^{-7} M all saturated at 10 ± 2 pmol of GTP[S] bound per mg of membrane protein (n = 6), indicating that this value is the total number of binding sites in the membrane (data not shown). Calculations based on 1.8×10^4 peripheral retinula cells (17), an average yield of 5 μg of membrane protein per eye, and the assumption that one GTP[S] binds to one protein molecule, gives 1.6×10^6 molecules of G protein in a peripheral *Musca* photoreceptor cell. *Drosophila* membranes also demonstrated light-dependent GTP[S] binding but have not been studied as extensively as the *Musca* preparation.

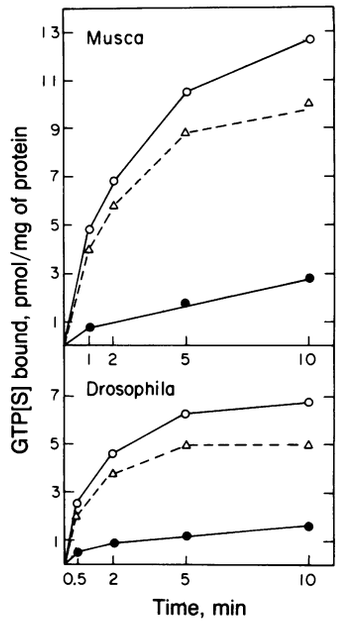

FIG. 1. Binding of GTP[^{35}S] to *Musca* and *Drosophila* eye membranes. Musca eye membranes or *Drosophila* head membranes were preilluminated at 4°C for 1 min with either red (●, Schott RG-610 edge filter) or blue (○, BG 28 filter, Schott; λ_{max}, 430 nm) saturating lights (6) followed by incubation at 25°C in the dark in 50 mM Mops (pH 6.7), 2 mM 2-mercaptoethanol, 5 mM $MgCl_2$, 0.2 mM ATP, 5 mM creatine phosphate, and creatine kinase at 50 units/ml. The final volume was 0.1 ml, and the specific activity of 20 nM GTP[^{35}S] was 3×10^4 cpm/pmol. The reaction was initiated by addition of 10 μg of membranes and terminated by the addition of 0.5 ml of cold rinsing solution (50 mM Mops/5 mM $MgCl_2$/2 mM 2-mercaptoethanol) followed by prompt filtration through 0.45-μm Oxoid membrane filters. Filters were rinsed twice with 2.5 ml of cold rinsing solution, and radioactivity was measured. Incremental binding due to the rhodopsin to metarhodopsin photoconversion (blue–red) is shown (△).

We employed an α-^{32}P-labeled azidoanilido analog of GTP (18) to identify the light-dependent G protein in the *Musca* eye membranes (6). Polyacrylamide gel electrophoresis and autoradiography revealed in the blue-illuminated membranes a labeled 41-kDa protein band (Fig. 2, lane 2) that was not seen in the systems illuminated with red light. Regardless of prior illumination one additional 39-kDa band was labeled. The role of this protein is unknown. Hypotonic washing, which abolished the light-dependent GTPase activity, largely reduced the amount of labeled protein in the 41-kDa band and somewhat reduced the amount of protein in the 39-kDa band (Fig. 2, lanes 3 and 4) suggesting that the 41-kDa protein is responsible for the light-dependent GTPase activity. In accord with this finding it has been reported (19) that a 41-kDa protein of octopus retinal membranes is ADP-ribosylated by pertussis toxin in the dark, and its ADP-ribosylation is inhibited by light. On the other hand in the *Calliphora* fly rhabdomeric membranes, a 41-kDa protein is ADP-ribosylated by cholera toxin in red- but not in blue-illuminated membranes (20).

Role of Inositol Phospholipid Hydrolysis in Phototransduction. To study how light activates the enzyme that generates the putative second messenger $InsP_3$ (7, 8), we have devel-

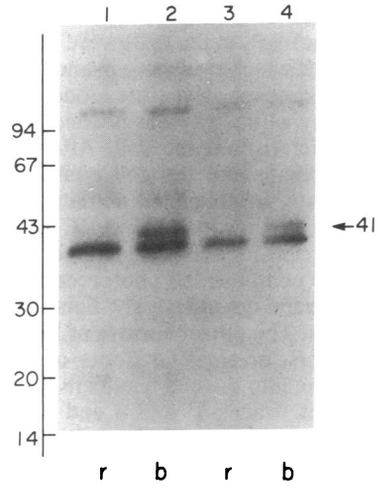

FIG. 2. Photoaffinity labeling of the light-activated G protein in *Musca* eye membranes. *Musca* eye membranes were preilluminated with either red (r) or blue (b) lights followed by incubation in binding solution for 20 min at 4°C in the dark as described in Fig. 1 except that the solution contained 10 nM α-^{32}P-labeled azidoanilido analog of GTP (18) (specific activity, 100 Ci/mmol; 1 Ci = 37 GBq). Separation of the free nucleotide was done by absorption of the membranes on nitrocellulose filter, washing the filter twice for 10 min with 20 ml of binding solution at 4°C, illumination for 30 sec with a mercury lamp (HBO; Zeiss) followed by insertion of the filters into the wells of a 10% polyacrylamide gel, electrophoresis, and autoradiography. Lanes: 1 and 2, from untreated membranes; 3 and 4, from membranes washed twice in hypotonic 5 mM Mops (pH 7.0), which resulted in a decrease of the light-dependent GTPase activity to 10% of the activity in the untreated membranes. Essentially the same results were obtained in five different experiments. Molecular masses in kDa are shown.

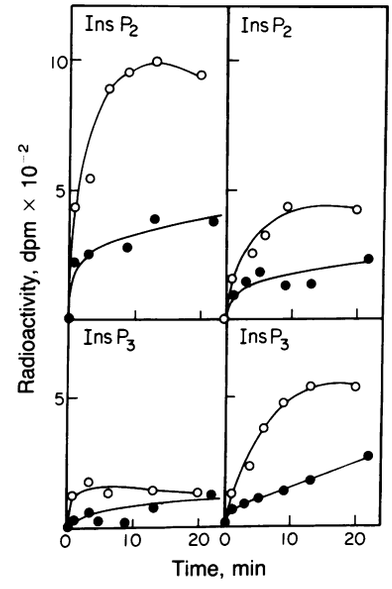

FIG. 3. Effect of 2,3-bisphosphoglycerate on light-induced inositol phospholipid hydrolysis in *Musca* eye membranes. (*Right*) Control without 2,3-bisphosphoglycerate. (*Left*) 2,3-Bisphosphoglycerate (10 mM) plus 10 mM $MgCl_2$ were added. Solid circles, systems incubated in the dark; open circles, systems illuminated with blue light.

oped a membrane preparation in which light-dependent inositol phospholipid hydrolysis can be studied under defined conditions and effects of activators and inhibitors can be analyzed. The *Musca* eye membrane preparation responded to blue illumination by a small increase in the accumulation of InsP_3 and a large increase in inositol bisphosphate (InsP_2), the respective products of inositol phospholipid hydrolysis by a phospholipase C type enzyme (Fig. 3 *Left*). The accumulation of inositol phosphate is characterized by a lag period indicating that this product may arise by subsequent stepwise enzymatic dephosphorylation of polyphosphorylated inositol (data not shown). The large accumulation of InsP_2 compared with that of InsP_3 in the *Musca* membranes raises the question of which of the two phosphatidylinositol phosphates is the major substrate of light-induced inositol phospholipid hydrolysis. To answer this we added 2,3-bisphosphoglycerate, a known inhibitor of InsP_3 phosphatase (21), and found a substantial decrease in the accumulation of InsP_2 and a concurrent large increase in the accumulation of InsP_3 (Fig. 3 *Right*). Furthermore, we have observed that under assay conditions, the concentration of phosphatidylinositol 4-phosphate in the fly membranes is five times higher than that of phosphatidylinositol 4,5-bisphosphate, indicating that the latter is a preferred substrate for the light-induced inositol phospholipid hydrolysis. It is also apparent that the fly eye membranes are endowed with the enzymatic system necessary to eliminate InsP_3 after it has been produced. Both the light-dependent preferential production of InsP_3 and a turn-off mechanism to stop its action are consistent with an internal messenger role for InsP_3 in fly phototransduction.

Two further criteria of an internal second messenger are: (*i*) introduction of the putative messenger into the cell should reproduce the physiological response; and (*ii*) agents that inhibit the inactivation of the messenger should augment the effect of the physiological stimulus. These criteria were tested for by light-induced introduction of InsP_3 and of 2,3-bisphosphoglycerate into the *Musca* photoreceptor cells. We used pressure injection of InsP_3 into the intact eye followed by a 1-min maximal-intensity white light to introduce the InsP_3 into the photoreceptors.

The introduction of hydrophilic molecules into fly photoreceptor cells was investigated in detail by Wilcox and Franceschini (22). They showed that colchicine enters the photoreceptors only upon illumination and then functionally interacts with microtubules disrupting pigment migration. Likewise Lucifer yellow shows light-dependent introduction that stains the whole photoreceptor cell up to its synaptic terminal (23). The integrity of the cells was tested by exclusion of trypan blue and monitoring normal pigment migration within the cell. Minke and Stephenson (14) showed that extracellular application of GTP[S] and guanosine 5'-[β,γ-imido]triphosphate had no effect on the membrane potential of *Musca* photoreceptors in the dark but induced noisy depolarization upon illumination in the presence of the nucleotides. They also found that the noisy depolarization, induced by consecutive light pulses in the presence of guanosine 5'-[β,γ-imido]triphosphate, was increased in a staircase fashion indicating that each light pulse introduced a certain amount of nucleotide into the cell. In the present experiments, white or red lights were used to introduce hydrophilic compounds into the photoreceptors and to stimulate the cells. These lights do not cause prolonged, postillumination excitation as was caused by a blue light (16).

The introduction of InsP_3 into the cell by 1-min maximal-intensity white light resulted in a large increase in baseline noise in the dark (compare the noise level before the stimuli in Fig. 4*A*). Subsequent application of an intense 1-sec white test light showed a response with an increased peak amplitude and an extended noisy depolarization that declined in the dark at a slower rate than the control (Fig. 4*A*). After InsP_3 plus 2,3-bisphosphoglycerate injection, one brief red flash was sufficient to create large voltage fluctuations in the dark (Fig. 4*B Lower*). One such flash in the presence of InsP_3 alone had no effect. The first maximal-intensity, 1-sec, white

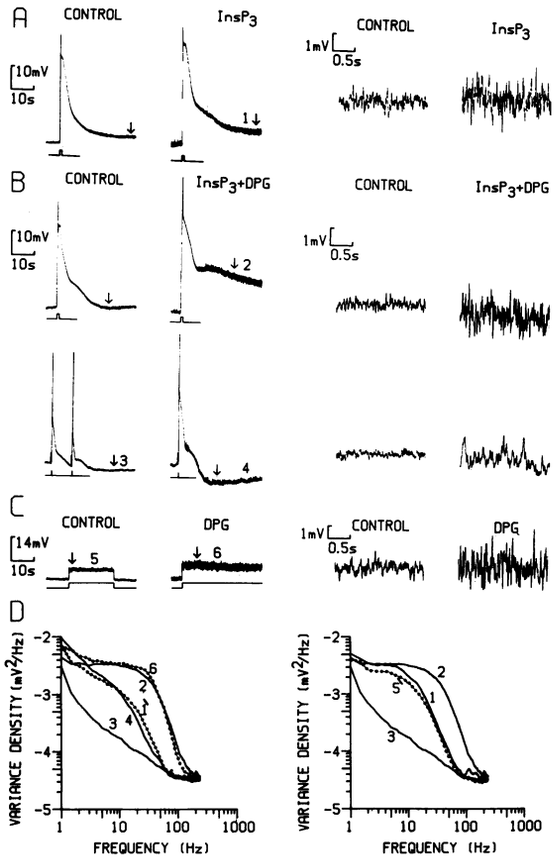

FIG. 4. Excitation of *Musca* photoreceptors by inositol-1,4,5-trisphosphate and by 2,3-bisphosphoglycerate. (*A*, *B*, and *C*) Records from different flies. The first column from the left shows the control responses before injection, and the second from the left shows responses to identical stimuli in the same cells after pressure injections of the indicated test compounds. The third and fourth columns from the left show enlarged segments of the regions of the first and second columns indicated by arrows. Segments from which power spectra were calculated are indicated by numbers. Test compounds were pressure injected in the dark into the retinal extracellular space resulting in a 1:10 dilution. The given concentrations of test compounds are those of solutions in the injecting pipette. (*A*) InsP_3 (1 mM) was introduced into the cell by maximal-intensity white illumination (100-W, 12-V halogen lamp; light energy at the level of the eye 20.5 mW·cm^{-2}) of a 1-min duration. (*B Upper*) Injection of a solution containing 1 mM InsP_3 and 50 mM 2,3,-bisphosphoglycerate (DPG) using a 1-sec white-light stimulation to introduce the compound into the cell. (*B Lower*) Responses to orange flashes, one short orange flash (150-J photographic flash in conjunction with Schott OG 570-nm edge filter) was sufficient to induce noise. The *Lower* responses preceded the *Upper* responses. (*C*) Injection of 50 mM DPG solution followed by a 1-min maximal-intensity white-light exposure. The responses shown in *C* are receptor potentials elicited by continuous, dim red light (Schott OG 590-nm edge filter with an intensity of 10.2 mW·cm^{-2} attenuated by 4 log units by a neutral-density filter). (*D*) Power spectra calculated from the noise of the various responses at the time segments indicated by numbers in *A*, *B*, and *C*. Spectra 1′, weak effect of InsP_3, and spectra 5′, control light response (dotted lines), were calculated from responses to InsP_3 and light, which are not presented in the figure. To compare power spectra of different experiments, a standard cell was selected, and power spectra of other cells were multiplied by the ratio of their dark noise level to that of the standard cell (trace 3). The ratios were 0.5, 0.39, 0.7, 0.51, for traces 1, 1′, 5′, and 6, respectively. Results similar to those presented in the figure were obtained in 11 different flies in which 4–7 cells were examined in each fly.

test pulse elicited a response with an increased peak amplitude and long (20 min), noisy depolarization (Fig. 4*B Upper*). Clearly combination of InsP_3 and 2,3-bisphosphoglycerate increased the effect of InsP_3 in that much lower light intensities (i.e., less InsP_3 in the cell) were needed to induce the excitatory effect, which was more pronounced and lasted longer. With 2,3-bisphosphoglycerate alone (Fig. 4*C*) 1 min of maximal-intensity white light was needed to introduce the 2,3-bisphosphoglycerate into the cell. After penetration of 2,3-bisphosphoglycerate into the cell continuous dim light gave a response with an enhanced amplitude and a higher noise level relative to the control (Fig. 4*C*).

The power spectrum reflects the shape of the elementary voltage events that compose the photoreceptor response. It was used to compare quantitatively light and chemically induced excitations. The power spectra of Fig. 4*D* show that introduction of InsP_3 or small amounts of InsP_3 plus 2,3-bisphosphoglycerate into the cell results in excitation similar to that of dim light (traces 1′ and 4 and traces 1 and 5′). However, large amounts of InsP_3 plus 2,3-bisphosphoglycerate result in a power spectrum that is different from those of any light or InsP_3 alone (trace 2). 2,3-Bisphosphoglycerate alone does not significantly increase the dark noise but does change the response to dim light so that its power spectrum resembles that of large amounts of InsP_3 plus 2,3-bisphosphoglycerate in the dark (traces 2 and 6).

These results indicate that InsP_3-induced unitary events are similar to those induced by dim light and that 2,3-bisphosphoglycerate enhances the effects of InsP_3 and of dim light, suggesting an endogenous production of InsP_3 during illumination.

Light-Dependent Inositol Phospholipid Hydrolysis Is Controlled by a G Protein. Several transduction mechanisms, including inositol phospholipid hydrolysis, are mediated by G proteins that are active in the GTP bound form and reverse to the inactive state upon hydrolysis of the bound GTP to GDP (24–26). We tested the role of a G protein in inositol phospholipid hydrolysis using GTP[S] and guanosine 5′-[β-thio]diphosphate (GDP[S]), hydrolysis-resistant analogs of GTP and GDP, respectively (27). In the control system incubated with GTP, illumination for 30 sec with blue light increased the rate of inositol phospholipid hydrolysis for ≈10 min. However, after illumination for 30 sec with red light, the rate of InsP_2 accumulation was not much different from that of a control system incubated in the dark. In contrast, with GTP[S], illumination for 30 sec with either blue or red light persistently activated the hydrolysis of inositol phospholipid (Fig. 5). Omission of GTP from the incubation medium only slightly decreased the light-induced increment in InsP_2 accumulation, apparently due to the endogenous guanine nucleotides (Fig. 6). Addition of 0.1 mM GDP[S] completely inhibited the light-induced hydrolysis of inositol phospholipid indicating that the phospholipase C activity is under stringent control of the G protein (Fig. 6). Consistent with these results is the finding that injection of GDP[S] to *Limulus* ventral photoreceptors inhibited the response to light but not to InsP_3 (28).

We have also observed an increased accumulation of InsP_2 and InsP_3 in *Musca* eye membranes incubated with fluoride ions (F^-) in the dark (data not shown), which resembles the ability of F^- to bypass the receptor and directly activate the adenylate cyclase and cGMP phosphodiesterase through its actions on the stimulatory G protein and tranducin, respectively (29, 30). In accord with these findings we have reported (14) that F^- excites the intact fly photoreceptors in the dark. This excitation can be accounted for by an increased inositol phospholipid hydrolysis elicited by F^-.

DISCUSSION

We have characterized a GTPase in fly photoreceptor membranes that is activated by photoconversion of rhodopsin to metarhodopsin (6). We now report that the G protein that gives rise to light-dependent GTPase activity is an abundant

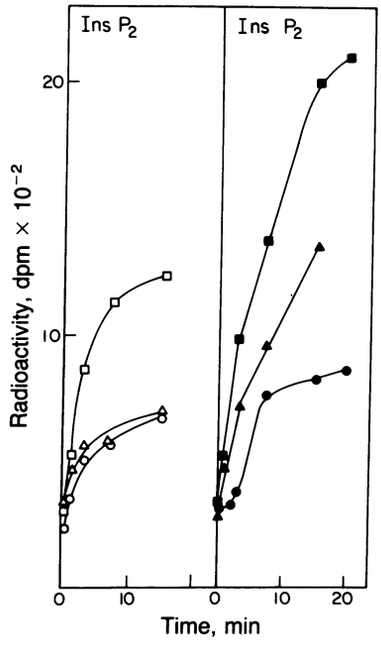

FIG. 5. Stimulation of light-induced InsP_2 production in *Musca* eye membranes by GTP[S]. (*Left*) Control with 0.1 mM GTP. (*Right*) GTP[S] at 10 μM without GTP. The reaction was initiated by illumination for 30 sec with red (△, ▲) or blue (□, ■) light as indicated. A control that remained in the dark (○, ●) was also included.

protein in the photoreceptor cell and that its GTP-binding component is a 41-kDa protein. Furthermore, we have identified the target enzyme that is activated by the fly photoreceptor G protein as a phospholipase C-type enzyme that catalyzes the hydrolysis of inositol polyphosphates. In its properties the fly photoreceptor G protein resembles other transducing G proteins (31). It is persistently activated by the hydrolysis-resistant analog GTP[S] but not by GTP. Light-dependent activation of phospholipase C is blocked by GDP[S], and F^- can bypass rhodopsin to activate the phospholipase C in the dark. Fluoride activation is apparently due to direct action on the G protein as inferred from our observation that fluoride activation of phospholipase C is greatly potentiated by aluminum and inhibited by prior incubation of the membranes with GDP[S] (data not shown).

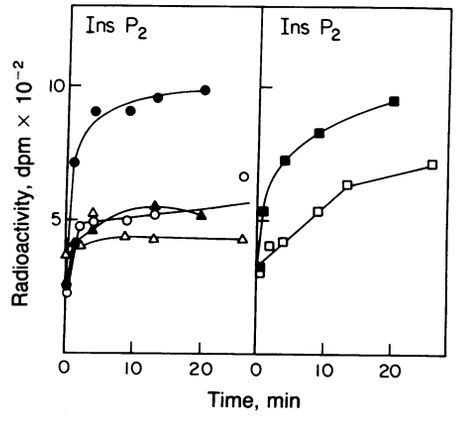

FIG. 6. Inhibition of light-induced InsP_2 production in *Musca* eye membranes by GDP[S]. GDP[S] (▲, △) was added to a final concentration of 0.1 mM. GTP was omitted from the systems containing GDP[S] and from the systems shown on the *Right* (■, □), but was present in control systems (●, ○). Solid symbols, blue light. Open symbols, dark.

Our biochemical experiments strongly suggest that the flow of information in the fly phototransduction cascade is from photo-excited rhodopsin to G protein and from the G protein to phospholipase C. We have also found that InsP_3 is the major inositol phosphate formed by the light-activated phospholipase C and that InsP_3 is rapidly hydrolyzed by specific phosphomonoesterase. Furthermore, introduction of InsP_3 into the cell excites the fly photoreceptor indicating that it may function as a second messenger in phototransduction. This has been further corroborated by demonstrating that when 2,3-bisphosphoglycerate, which inhibits InsP_3 hydrolysis, is introduced into the cell, it greatly potentiates the excitation by InsP_3 as well as augments the physiological excitation by dim light.

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