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Stimulation of phospholipase A_2 activity in bovine rod outer segments by the $\beta\gamma$ subunits of transducin and its inhibition by the α subunit

(arachidonic acid/guanosine 5′-[γ-thio]triphosphate/light activation/pertussis toxin/retina)

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ABSTRACT **In the rod outer segments (ROS) of bovine retina, light activation of phospholipase A_2 has been shown to occur by a transducin-dependent mechanism. In this report, the transducin-mediated stimulation of phospholipase A_2 is shown to require dissociation of the $\alpha\beta\gamma$ heterotrimer. Addition of transducin to dark-adapted transducin-poor ROS stimulated phospholipase A_2 activity only with coincident exposure to white light or, in the dark, with addition of the hydrolysis-resistant GTP analog, guanosine 5′-[γ-thio]triphosphate (GTP[γ-S]). Both light and GTP[γ-S] induced dissociation of the transducin subunits and led to severalfold increases in the phospholipase A_2 activity of transducin-rich, but not transducin-poor, ROS. In contrast, pertussis toxin treatment of transducin, which stabilizes the associated state of this G protein, prevented the stimulation of phospholipase A_2 by exogenous transducin in the presence of light. Addition of purified transducin subunits to dark-adapted transducin-poor ROS revealed that phospholipase A_2 stimulation occurred by action of the $\beta\gamma$ subunits. This is in contrast to the transducin-mediated increase in cGMP phosphodiesterase activity, where activation occurs by action of the α subunit. The α subunit, which itself slightly stimulated phospholipase A_2 activity, inhibited the $\beta\gamma$-induced stimulation of phospholipase A_2. This inhibition appears to be the result of subunit reassociation since addition of GTP[γ-S] abolished the inhibitory effect of the α subunit on the $\beta\gamma$-induced increase in phospholipase A_2, while pertussis toxin treatment of the subunits further inhibited phospholipase A_2 activity. Modulation of phospholipase A_2 activity by the transducin subunits is, therefore, a mode of action for these subunits in signal transduction.**

Guanine nucleotide-binding regulatory proteins, G (or N) proteins, play a major role in receptor-mediated signal transduction (1–3). These G proteins are heterotrimers, which, upon ligand-induced stimulation, dissociate into α and $\beta\gamma$ subunits (1, 3). The α subunits of these G proteins serve as substrates for specific bacterial toxins (4–7), have high affinity GTP binding sites (1, 2), and an intrinsic GTPase activity that plays a role in cycling the G proteins through their active (dissociated) and inactive (associated) states (1–3). The dissociated GTP-bound forms of the G protein α subunits are the active effectors of intracellular regulatory proteins such as adenylate cyclase and cGMP phosphodiesterase (1–3). The $\beta\gamma$ subunits, in contrast, function primarily to facilitate the reassociation of the heterotrimeric complex, thereby deactivating the dissociated α subunits of those G proteins that share common $\beta\gamma$ subunits (1, 8). The $\beta\gamma$ complex also appears to be required for or to enhance the interaction of specific G proteins with activated receptors (9, 10).

In the rod outer segments (ROS) of the retina, the major G protein is transducin (2). The dissociated α subunit of this G protein couples the light activation of rhodopsin to stimulation of cGMP phosphodiesterase activity (2, 11, 12) and is a substrate for both pertussis and cholera toxin (6, 7). It has recently been shown that light also activates phospholipase A_2 in dark-adapted ROS of bovine retina by a transducin-dependent mechanism (13, 14). We now report that the $\beta\gamma$ subunits of transducin, dissociated by either light or guanosine 5′-[γ-thio]triphosphate (GTP[γ-S]), stimulate phospholipase A_2 activity when added to dark-adapted transducin-poor ROS. The α subunit of transducin, by facilitating reassociation of the heterotrimeric complex, blocks the increase in phospholipase A_2 activity observed with the $\beta\gamma$ subunits.

MATERIALS AND METHODS

Preparation of ROS and Isolation of Transducin. Dark-adapted transducin-poor and transducin-rich ROS membranes were prepared as described (14). Since transducin accounts for >95% of the G protein content of ROS membranes (14), the ROS transducin content could be estimated by the GTP[γ-S]-binding capacity of the ROS, as measured by the procedure of Sternweis and Robishaw (15). For isolation of transducin that was >95% pure, light-activated ROS were isolated, purified, and GTP-extracted as described (14). Excess GTP was removed from the transducin by DE-52 chromatography (16) prior to its use in phospholipase A_2 assays. The transducin was then stored at 4°C and used within 3 days since the ability to modulate phospholipase A_2 activity appeared to be lost upon freeze–thawing.

Isolation of Transducin Subunits. Transducin subunits were separated chromatographically using either heptyl Sepharose (15) (prepared by reaction of heptylamine with CL-4B Sepharose; Pharmacia) or CL-6B blue Sepharose (17) (Pharmacia). For subunit separation, transducin was activated by the procedure of Sternweis (8), diluted to a final concentration of 0.25 mg of protein per ml in 0.2% cholate/1.5 M NaCl/0.25 M Tris·HCl, pH 8.0/0.05 M $MgCl_2$/0.01 M EGTA (TME buffer), then added to the heptyl Sepharose column (20 ml of packed heptyl Sepharose per 6 mg of transducin). The α and $\beta\gamma$ subunits were eluted by using a 200-ml linear cholate/NaCl gradient (0.6% cholate/1.25 M NaCl to 1.4% cholate/0.5 M NaCl) in TME buffer after an initial 50-ml linear gradient of 0.2% cholate/1.5 M NaCl to 0.6% cholate/1.25 M NaCl.

For subunit separation using CL-6B blue Sepharose, activated transducin was diluted to 0.1 mg of protein per ml in 20 mM Tris·HCl, pH 7.5/0.5 mM $MgCl_2$/0.05 mM EGTA/1 mM

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Abbreviations: GTP[γ-S], guanosine 5′-[γ-thio]triphosphate; ROS, rod outer segments.

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dithiothreitol (TMED), then mixed with blue Sepharose (4 ml of packed CL-6B Sepharose per 5 mg of transducin) on an orbital shaker for 2 hr at 4°C. The Sepharose was batch-loaded onto a column and the flow-through was passed over the column a second time. The $\beta\gamma$ subunits were eluted with TMED (100 ml per 4-ml column) and the α subunits were subsequently eluted with TMED containing 1.5 M NaCl (40 ml per 4-ml column).

Fractions containing the isolated α and $\beta\gamma$ subunits were identified by gel electrophoresis of aliquots of the fractions on $NaDodSO_4$/polyacrylamide gels (18), using a 5% stacking gel and a 15% running gel. Samples were prepared by the procedure of Neer *et al.* (19), loaded onto the gels, and the proteins were separated by electrophoresis and then visualized by silver stain (Bio-Rad) (20). The fractions containing either α or $\beta\gamma$ subunits were pooled, concentrated by filtration (Amicon YM10), then dialyzed overnight with several changes of TMED to remove the cholate and/or NaCl as well as residual GTP, Al^{3+}, and F^- ions, all of which interfered either with the phospholipase A_2 assay *per se* or with experiments designed to determine the effect of subunit reassociation. The transducin subunits were stored at 4°C and used within 3 days of the initial extraction of the transducin.

Pertussis Toxin Treatment. Dark-adapted ROS membranes, isolated transducin, or transducin subunits were incubated with activated pertussis toxin (10 ng/ml) as described (14, 21). Controls included membranes or transducin subunits that had been incubated with the same buffers under toxin-free conditions.

Phospholipase A_2 Assays. *In vitro* assays of phospholipase A_2 were performed as described (14), with reactions stopped by addition of 50 μl of 1 M formic acid, and fatty acids were extracted by further addition of 200 μl of *n*-heptane. Phospholipase A_2 activity was assessed as described (14). To ensure that the arachidonate was released by action of phospholipase A_2 and not the result of phospholipase C acting in concert with diglyceride lipase, the lipid-rich lower phase was analyzed for the formation of lysophosphatidylcholine using phosphatidyl[^{14}C]choline (New England Nuclear) as the substrate. The presence of lysophosphatidyl[^{14}C]choline was demonstrated by two-dimensional thin-layer chromatography (22) and visualization of the radiolabeled lipids by autoradiography. In addition, the *in vitro* assays were performed as described above using either phosphatidyl[^{3}H]-inositol (New England Nuclear) or 1-stearoyl-2-[^{14}C]arachidonoyl glycerol (Amersham) as the substrate. Under the reaction conditions described (pH 8.8), only minimal levels of either radiolabeled arachidonate or inositol were obtained.

The α and $\beta\gamma$ preparations occasionally had significant amounts of "intrinsic" phospholipase A_2 activity. This was also noted in some G protein preparations from bovine brain (C.L.J. and R. Kahn, unpublished observations). Based on immunoblot analysis of the fractions with anti-phospholipase A_2 antibody graciously provided by T. Parks (Boehringer Ingelheim), this activity was found to be due to a phospholipase A_2 contaminant not detected in silver-stained gels that often accompanied the subunits through the isolation procedures. For the present studies, the α and $\beta\gamma$ subunits had minimal phospholipase A_2 activity.

Materials. 1-Palmitoyl-2-[1-^{14}C]arachidonoyl-*sn*-glycero-3-phosphocholine (54.5 mCi/mmol; 1 Ci = 37 GBq), [^{3}H]arachidonic acid (91.2 Ci/mmol), and GTP[γ-^{35}S] (1106 Ci/mmol) were from New England Nuclear. Pertussis toxin was from List Biological Laboratories (Campbell, CA).

RESULTS

Transducin-Mediated Light Activation of Phospholipase A_2 Requires Subunit Dissociation. The light activation of phospholipase A_2 in ROS membranes of bovine retina occurs by a transducin-dependent mechanism (13, 14). As shown in Fig. 1, purified exogenous transducin caused a modest (25%) increase in phospholipase A_2 when added to dark-adapted transducin-poor ROS membranes. Exposure to light, which dissociates transducin subunits via photoexcitation of rhodopsin (2), markedly enhanced the activation of phospholipase A_2 by exogenous transducin, leading to a 2.5-fold increase in phospholipase A_2 activity (Fig. 1). Similar results were observed upon addition of GTP[γ-S] together with exogenous transducin. This hydrolysis-resistant GTP analog has been shown to dissociate isolated transducin into its α and $\beta\gamma$ subunits, mimicking the action of light (2, 23). In the absence of exogenous transducin, exposure to light had only a minimal effect on the phospholipase A_2 activity of transducin-depleted dark-adapted ROS membranes (Fig. 2 *Right*), and addition of GTP[γ-S] to these membranes caused a slight but significant inhibition. This inhibition is proposed to result from activation of a G protein that mediates the inhibition of phospholipase A_2 (14). The loss of the light and GTP[γ-S]-induced phospholipase A_2 activity in the dark-adapted transducin-poor ROS coincided with the loss of $\approx$75% of the membrane transducin (14), as assessed by the GTP[γ-S]-binding capacity of the membranes. At the same time, there were no significant changes in either the

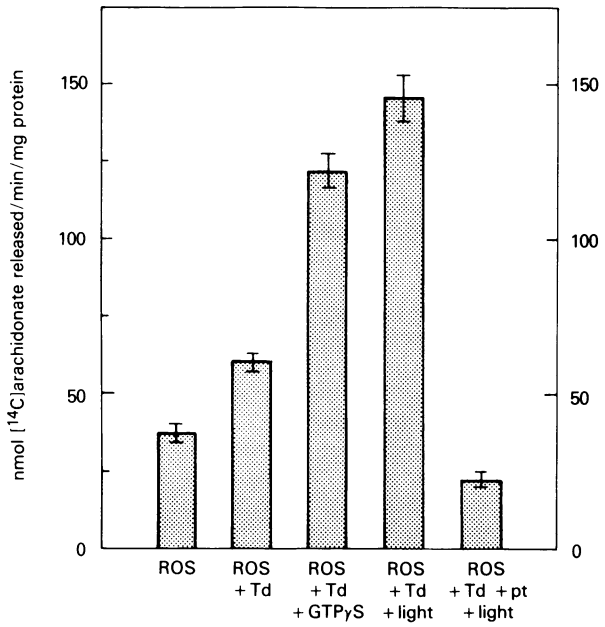

FIG. 1. Exogenous transducin stimulates phospholipase A_2 in dark-adapted transducin-depleted ROS membranes of bovine retina. The phospholipase A_2 activity of dark-adapted ROS membranes (20 μg) was measured as nmol of [^{14}C]arachidonate released per min per mg of protein after 10 min of incubation at 37°C under dim red light in the presence or absence of exogenous transducin (5 μg). Preparation of dark-adapted transducin-poor ROS, isolation of pure transducin, and the phospholipase A_2 assay conditions were as described (14). To determine whether dissociation of the transducin subunits was required for phospholipase A_2 activation, the effect of exogenous transducin (Td) on the phospholipase A_2 activity of dark-adapted transducin-poor ROS was assessed in the presence of GTP[γ-S] (100 μM) or upon exposure to white light (300 W). Alternatively, exogenous transducin was treated with pertussis toxin (pt, 10 ng/ml) prior to its addition to dark-adapted ROS, using the procedures described (14). The transducin content of the transducin-poor membranes, as assessed by the GTP[γ-^{35}S]-binding capacity of the membranes, was somewhat variable between preparations ($\pm$0.15 pmol per mg of membrane protein). To adjust for this variability, values from individual experiments were normalized to a transducin content of 0.8 pmol per mg of membrane protein, exclusive of the transducin that was added. The results are mean $\pm$ SEM in triplicate determinations from three separate experiments.

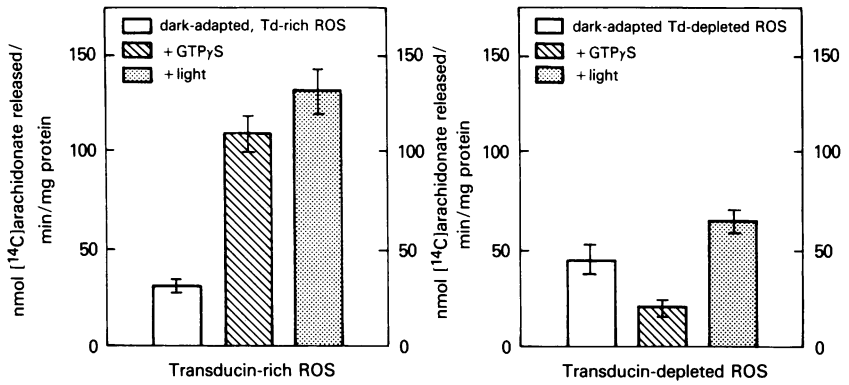

FIG. 2. Stimulation of phospholipase A_2 by light and GTP[γ-S] is decreased in dark-adapted ROS membranes depleted of transducin. Transducin-depleted membranes were prepared as described in *Materials and Methods*, while transducin-rich membranes were obtained by minimal washing (two washes with isotonic buffer) of the ROS membranes after the sucrose gradient. The dark-adapted ROS membranes were incubated (20 μg of membrane protein) in the presence of either GTP[γ-S] (100 μM) or white light (300 W). Phospholipase A_2 activity was assayed as described (Fig. 1). Values from separate experiments were normalized to a transducin content of 0.8 pmol per mg of protein for the transducin-depleted membranes and 3.2 pmol per mg of protein for the transducin-rich membranes. Values are mean ± SEM for three experiments performed in triplicate. (*Left*) Modulation of phospholipase A_2 activity in transducin-rich ROS. (*Right*) Modulation of phospholipase A_2 activity in transducin-poor ROS.

rhodopsin content of the membranes or in the amount of melittin-stimulated phospholipase A_2 activity (14).

Since neither light nor GTP[γ-S] enhanced the phospholipase A_2 activity of dark-adapted transducin-poor ROS in the absence of added transducin (Fig. 2 *Right*), the increased phospholipase A_2 activity observed when these agents were added together with transducin appears to be the result of a light or GTP[γ-S] effect on the exogenous transducin—i.e., dissociation of the transducin subunits. These results indicate a requirement for dissociation of the transducin subunits in the activation of phospholipase A_2. Additional evidence is provided by the observation that pertussis toxin treatment of exogenous transducin, which stabilizes this G protein in its associated state (7), prevented the activation of phospholipase A_2 obtained by addition of exogenous transducin in the presence of light (Fig. 1).

The net increase in phospholipase A_2 activity obtained with exogenous transducin could also be enhanced by decreasing the basal phospholipase A_2 activity of the membranes. Pertussis toxin treatment of dark-adapted transducin-depleted ROS reduced basal phospholipase A_2 activity (14). Under these conditions (data not presented), the increase in phospholipase A_2 activity achieved with exogenous transducin approached the 4- to 5-fold stimulation of phospholipase A_2 seen with transducin-rich ROS upon light exposure or addition of GTP[γ-S] (Fig. 2 *Left*). While statistically significant increases in phospholipase A_2 activity could be observed at transducin concentrations as low as 100 ng (data not presented), to obtain a 2-fold increase in phospholipase A_2 activity without prior reduction of basal activity, the exogenous transducin concentration added had to be $\geq$1 μg. This concentration may appear to be high but is actually only twice the amount of transducin remaining in 20 μg of dark-adapted transducin-poor ROS, based on the fact that transducin accounts for 10% of the ROS membrane protein (2) and the observation that transducin-poor ROS have lost 75% of the membrane transducin (14).

The $\beta\gamma$ Subunits of Transducin Stimulate Phospholipase A_2. To examine the role of the individual subunits of transducin in phospholipase A_2 activation, purified transducin was isolated from bovine ROS membranes, and the α and $\beta\gamma$ subunits were separated by either heptyl Sepharose or CL-6B blue Sepharose chromatography of activated transducin. When purified ROS that had been extensively washed were used as the source of transducin, the α and $\beta\gamma$ subunit preparations obtained by heptyl Sepharose chromatography were >95% pure, as assessed by densitometric analysis of polyacrylamide gels that had been previously developed by silver-staining. The α and $\beta\gamma$ subunits separated by blue Sepharose chromatography were of comparable purity, but the number of fractions containing $\beta\gamma$ subunits free of α were considerably fewer. The yield of pure $\beta\gamma$ subunits was, therefore, substantially lower. When added to dark-adapted ROS membranes depleted of transducin, the $\beta\gamma$ subunits (5 μg) caused a 2.5-fold increase in phospholipase A_2 activity. The α subunit, in contrast, showed a smaller increase ($\approx$50%). When added at equimolar concentrations, the stimulation observed with the $\beta\gamma$ subunits was consistently 3 to 5 times that observed with the α subunit over a wide range of concentrations (100 ng to 50 μg).

The α Subunit Inhibits $\beta\gamma$-Induced Stimulation of Phospholipase A_2. Despite the small amount of phospholipase A_2 stimulation observed with the α subunit upon addition to the transducin-depleted ROS, addition of equimolar concentrations of the α subunit together with the $\beta\gamma$ subunits abolished the stimulation observed with the $\beta\gamma$ subunits (Fig. 3). This inhibition is presumed to result from the reassociation of the transducin subunits into a heterotrimeric complex. This is supported by the observation that GTP[γ-S], which prevents subunit reassociation (23), abolished the inhibitory effect of the α subunit. Furthermore, pertussis toxin pretreatment of the combined transducin subunits, which stabilizes this G protein in its associated state (7), prevented the activation of phospholipase A_2 by exogenous transducin in the presence or absence of light (Fig. 1).

DISCUSSION

Transducin, the major G protein of the ROS of bovine retina, mediates the light activation of cGMP phosphodiesterase (2, 11, 12). More recently, light has also been shown to stimulate phospholipase A_2 activity in bovine ROS by a transducin-dependent mechanism (13, 14). The present report demonstrates that phospholipase A_2 stimulation requires dissociation of transducin, with the increase in phospholipase A_2 occurring by action of the dissociated $\beta\gamma$ subunits of transducin (Fig. 4). This was evidenced by (*i*) the capacity for exogenous transducin to stimulate phospholipase A_2 in dark-adapted transducin-poor ROS only in the presence of light or GTP[γ-S], agents that induce subunit dissociation but have little or no stimulatory effect on the phospholipase A_2 activity of these transducin-poor membranes (Fig. 2 *Right*); (*ii*) the inability of pertussis toxin-treated transducin to increase phospholipase A_2 in transducin-poor ROS in either the

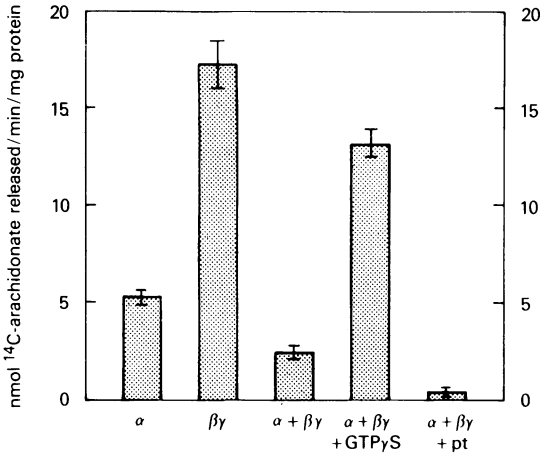

FIG. 3. Stimulation of phospholipase A_2 activity in dark-adapted transducin-poor bovine ROS by the $\beta\gamma$ subunits of transducin. Transducin was isolated from light-activated bovine ROS and the α and $\beta\gamma$ subunits were separated by chromatography over either heptyl Sepharose or CL-6B Sepharose as described in *Materials and Methods*. Equimolar concentrations of the isolated subunits (5 μg) were added either separately or together to dark-adapted transducin-depleted ROS membranes (20 μg) and phospholipase A_2 activity was measured as described in the legend to Fig. 1. The inhibitory effect of the α subunit on the stimulation of phospholipase A_2 by the $\beta\gamma$ subunits was examined in the presence and absence of GTP[γ-S] (100 μM) or after pretreatment of the subunits with activated pertussis toxin (pt, 10 ng/ml) as described in *Materials and Methods*. Values from individual experiments were normalized to a transducin content of 0.8 ± 0.15 pmol per mg of membrane protein as described above (Fig. 1). The values presented represent mean ± SEM from three experiments performed in triplicate after subtraction of the phospholipase A_2 activity of the dark-adapted transducin-depleted ROS (18 ± 2 nmol per min per mg of membrane protein).

presence or absence of light; and (*iii*) the ability of isolated purified $\beta\gamma$ subunits of transducin to induce a marked increase in the phospholipase A_2 of dark-adapted transducin-poor ROS (Fig. 3).

The α subunit, while slightly stimulating phospholipase A_2 when added alone to dark-adapted transducin-poor ROS membranes, inhibited the stimulatory effect of the $\beta\gamma$ subunits. This inhibitory effect of the α subunit appears to result from its reassociation with the $\beta\gamma$ subunits. This is based on the observation that GTP[γ-S], which prevents subunit reassociation (23), abolished the inhibitory effect of the α subunit (Fig. 3), whereas pertussis toxin pretreatment of the combined α and $\beta\gamma$ subunits, a treatment that stabilizes the subunits in their associated state (7), further inhibited the $\beta\gamma$-induced stimulation of phospholipase A_2.

These findings indicate a different role for the α and $\beta\gamma$ subunits of transducin. Not only is transducin shown to regulate an enzyme other than cGMP phosphodiesterase, but the role of the α and $\beta\gamma$ subunits in the modulation of phospholipase A_2 activity is the converse of their role in the regulation of cGMP phosphodiesterase. The increase in cGMP phosphodiesterase activity occurs by action of the dissociated α subunit of transducin (Fig. 4) (2, 11), while the $\beta\gamma$ subunits stimulate the GTPase activity of the dissociated transducin α subunit, thereby facilitating reassociation of the inactive heterotrimer and the termination of cGMP phosphodiesterase activity (9). These results indicate that the α and $\beta\gamma$ subunits of transducin may each function in the modulation of both cyclic nucleotide and phospholipid metabolism. Furthermore, each subunit may act in either a stimulatory or an inhibitory manner, depending on the state of subunit association/dissociation.

The stimulatory effect of the $\beta\gamma$ subunits on phospholipase A_2 activity is not limited to the ROS membranes. Preliminary

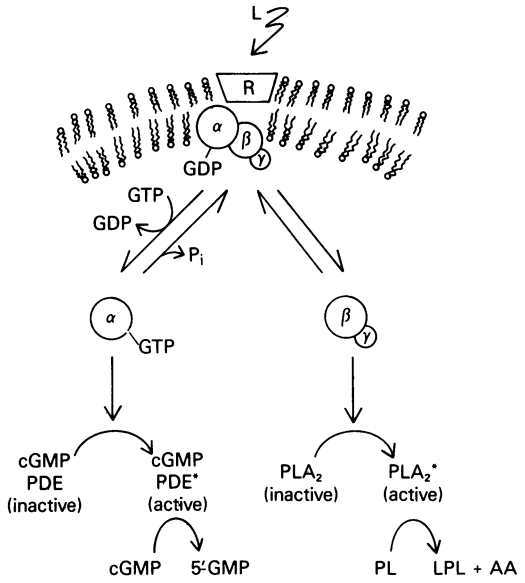

FIG. 4. The α and $\beta\gamma$ subunits of transducin may each function in both a stimulatory and an inhibitory capacity. Upon activation of rhodopsin (R) by light (L), transducin dissociates into its α and $\beta\gamma$ subunits. In this dissociated state, the GTP-bound α subunit activates cGMP phosphodiesterase (cGMP PDE), resulting in the hydrolysis of cGMP. The dissociated $\beta\gamma$ subunits in turn activate phospholipase A_2 (PLA_2), resulting in the hydrolysis of phospholipids (PL) and release of arachidonic acid (AA) coincident with formation of lysophospholipids (LPL). Both transducin subunits thus function in the activation of different effector proteins. At the same time, each subunit may also function in an inhibitory capacity by facilitating the reassociation of the inactive heterotrimeric complex. The capacity for the $\beta\gamma$ subunits to inhibit the stimulation of cGMP phosphodiesterase mediated by the α subunits has been demonstrated (10). In the modulation of phospholipase A_2 activity, the α subunit has been found to inhibit the activation of phospholipase A_2 by the $\beta\gamma$ subunits. Each of the G protein subunits thus appears to have both a stimulatory and an inhibitory role. While not depicted, both transducin subunits may stimulate their respective effector systems by inactivating inhibitors. The inhibitor of cGMP phosphodiesterase activity appears to be a regulatory subunit of the enzyme (24), while the inhibitor of phospholipase A_2 activity is not known.

experiments indicate that the $\beta\gamma$ subunits of transducin also stimulate phospholipase A_2 activity in membranes of FRTL-5 thyroid cells and RAW 264.7 macrophages (C.L.J., R. Burch, and J.A., unpublished results) to an extent comparable to that achieved upon addition of the $\beta\gamma$ subunits to dark-adapted transducin-poor ROS in the absence of light. Since the β subunit of transducin is identical to the larger of the two known β subunits (35 and 36 kDa) associated with other G proteins (16), it is suggested that G proteins other than transducin may modulate phospholipase A_2. Due to the inability to separate the β and γ subunits without denaturation, it is unclear whether phospholipase A_2 stimulation is affected by the transducin β and/or γ subunit(s). The similarity or uniqueness of the γ subunit of transducin to the γ subunits of other G proteins also remains a subject of debate (23, 25–27). The $\beta\gamma$ subunits of transducin inhibit adenylate cyclase in a reconstituted system (25). Preliminary experiments with the $\beta\gamma$ subunits from bovine brain (having both the 35- and 36-kDa forms) have revealed a similar capacity to stimulate the phospholipase A_2 activity of dark-adapted transducin-poor ROS (C.L.J. and R. Kahn, unpublished results). These preliminary findings support a more general role for the $\beta\gamma$ subunits of transducin in the modulation of phospholipase A_2.

Stimulation of phospholipase A_2 activity has been linked to G proteins other than transducin in a variety of systems (28–31). In the neutrophils, the increase in phospholipase A_2

was considered to occur secondary to the GTP-dependent stimulation of phospholipase C (28) that has recently been linked to G_i and G_o (based on the ability of exogenous G_i or G_o to stimulate the enzyme in pertussis toxin-treated neutrophil membranes) (31). In a preliminary report (13), the stimulatory effect of the $\beta\gamma$ subunits of transducin has been shown to be specific to phospholipase A_2. The $\beta\gamma$ subunits had no effect on phospholipase C when added to dark-adapted transducin-poor ROS even though light also stimulates phospholipase C in dark-adapted bovine (13, 32) and *Xenopus* (33) ROS. The differential effects of pertussis and cholera toxin on activated phospholipases A_2 and C in both the ROS membranes (13, 14, 32) and in thyroid cells (30) led to the suggestion that these two phospholipases are modulated by different G proteins (30). Our findings, however, suggest that stimulation of phospholipases A_2 and C may occur by action of different G protein subunits and need not involve different G proteins.

Relatively high levels of exogenous transducin or transducin $\beta\gamma$ subunits are required to stimulate the phospholipase A_2 activity of transducin-depleted ROS. To obtain phospholipase A_2 activities comparable to those observed upon light activation of transducin-rich ROS, 5 μg of exogenous transducin is required per 20 μg of transducin-poor ROS. This is equivalent to an assay concentration of 0.6 μM transducin, $\approx$25 times the amount of bovine brain $\beta\gamma$ subunit required to affect adenylate cyclase in cyc^- membranes. The concentration of exogenous transducin used in our experiments is, however, well within physiological limits since the molar concentration of transducin in the ROS has been calculated to reach 500 μM (34).

The limited amplification of phospholipase A_2 activity achieved upon light activation of transducin-rich ROS or addition of transducin to transducin-poor ROS is in marked contrast to the hundredfold amplification observed in cGMP phosphodiesterase activity upon dissociation of the α subunit of transducin (2, 11). The relatively smaller stimulation of phospholipase A_2 induced by the $\beta\gamma$ subunit of transducin may, however, trigger a cascade of biochemical events distal to the effector enzyme. In the stimulation of phospholipase C by G proteins, amplification of the signal appears to occur secondary to action on the enzyme itself, by the production of several second messengers such as inositol triphosphate and diacylglycerol (35). Amplification of the $\beta\gamma$-mediated effect on phospholipase A_2 may similarly occur secondary to the release of arachidonate and the formation of lysophospholipids. Arachidonic acid and/or its metabolites can, for example, increase cytosolic Ca^{2+} (36, 37), protein kinase C (38), and phospholipase C (39), while lysophospholipids have been shown to stimulate cyclic nucleotide phosphodiesterase activity (40).

The mechanism whereby the $\beta\gamma$ subunits stimulate phospholipase A_2 activity remains to be determined. Phospholipase A_2 stimulation could occur by direct activation of the enzyme or by inactivation of a phospholipase A_2 inhibitor. Two types of phospholipase A_2 inhibitors are known: the lipocortins (41) and, as suggested in this report, G protein α subunits. While the transducin α subunit does not itself have phospholipase A_2 inhibitory activity, it blocks the stimulation of phospholipase A_2 by the $\beta\gamma$ subunits. In addition, there appears to be a G protein-dependent phospholipase A_2 inhibitory activity present in the ROS, as demonstrated by the GTP-induced inhibition of phospholipase A_2 in both dark-adapted transducin-poor ROS (Fig. 2 *Right*) and in light-activated transducin-rich ROS (14). This GTP-dependent inhibition of phospholipase A_2 in the ROS suggests that one mechanism for the $\beta\gamma$-induced stimulation of phospholipase A_2 is by inactivation of a phospholipase A_2 inhibitor. It remains to be determined whether this GTP-dependent phospholipase A_2 inhibitor is itself a G protein subunit or a lipocortin-like protein subject to regulation by G proteins. The possibility of a direct stimulatory effect of the $\beta\gamma$ subunits on phospholipase A_2 is also unresolved.

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