Arachidonate and Related Unsaturated Fatty Acids Selectively Inactivate the Guanine Nucleotide-binding Regulatory Protein, G_{z}.*

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G_{z} is a member of the family of trimeric guanine nucleotide-binding regulatory proteins (G proteins), which plays a crucial role in signaling across cell membranes. The expression of G_{z} is predominately confined to neuronal cells and platelets, suggesting an involvement in a neuroendocrine process. Although the signaling pathway in which G_{z} participates is not yet known, it has been linked to inhibition of adenyl cyclase. We have found that arachidonate and related unsaturated fatty acids suppress guanine nucleotide binding to the α subunit of G_{z}. This inhibition of nucleotide binding by cis-unsaturated fatty acids is specific for G_{zα}; other G protein α subunits are relatively insensitive to these lipids. The IC_{50} for inhibition by the lipids closely corresponds to their critical micellar concentrations, suggesting that the interaction of the lipid micelle with G_{zα} is the primary event leading to inhibition. The presence of the acidic group of the fatty acid is critical for inhibition, as no effect is observed with the corresponding fatty alcohol. While arachidonic acid produces near-complete inhibition of both GDP and guanosine 5′-[(3-O-thio)triphosphate binding by G_{zα}, release of GDP from the protein was unaffected. Furthermore, the rate of inactivation of G_{zα} by arachidonate is essentially identical to the rate of GDP release from the protein, indicating that GDP release is required for inactivation. These observations indicate that the mechanism of inactivation of G_{zα} by unsaturated fatty acids is through an interaction of an acidic lipid micelle with the nucleotide-free form of the protein. Although the physiologic significance of this finding is unclear, similar effects of unsaturated fatty acids on other proteins involved in cell signaling indicate potential roles for these lipids in signal modulation. Additionally, the ability of arachidonate to inactivate this adenylyl cyclase-inhibitory G protein provides a molecular mechanism for previous findings that treatment of platelets with arachidonate results in elevated cAMP levels.

G proteins are classified through the identity of their α subunit. The high sequence homology among these polypeptides has led to the dosing of several forms for which precise physiologic roles have not yet been ascribed. One such isotope is G_{zα}, (5, 6). The distribution of G_{zα} is limited primarily to platelets and neurons, implicating this G protein in some specific role in these tissues (5–8). The protein has been purified from bovine brain as well as a bacterial expression system and shown to possess biochemical properties distinct from other G protein α subunits (9). For example, nucleotide exchange by G_{zα} is highly dependent on free magnesium concentrations. At free magnesium concentrations greater than 10^{-5} M, GTP binding by G_{zα} is nearly completely suppressed. This effect is not seen with other G proteins; in fact, the presence of high magnesium concentrations generally stimulates their rates of nucleotide exchange (2). Magnesium-dependent suppression of nucleotide exchange is observed, however, with members of the monomeric family of GTP-binding proteins, e.g. Ras (10). G_{zα} also has a very slow intrinsic rate of GTP hydrolysis, more similar to that of Ras and Ras-related proteins than α subunits (9). Although G_{z} is formally a member of the Gi family, it is insensitive to ADP-ribosylation catalyzed by pertussis toxin (9), a modification that inactivates the other members of the Gi family (11). A property that G_{zα} does share with most members of the Gi family is an ability to mediate inhibition of adenylyl cyclase (12, 13). In addition, G_{zα} serves as an excellent substrate for activated protein kinase C both in vitro and in intact platelets (14), and evidence has been obtained that this phosphorylation blocks subunit interactions of this G protein (15).

Several reports have appeared recently, indicating that particular biogenically active lipids can interact in vitro with signaling proteins and modulate their activities. For example, arachidonate and related unsaturated fatty acids physically associate with, and inhibit the activity of, the Ras GTPase activating protein known as GAP (16, 17). Such lipids can also regulate the association of the Ras-related protein, Rac, with a specific GDP dissociation inhibitor (18). Similarly, cis-unsatur-
lated fatty acids such as oleate and arachidone have been shown to activate protein kinase C (19). While the mechanism by which lipids modulate the activities of these proteins is not completely defined, their interaction raises interesting possibilities for the role of lipids in cellular regulation. In this study, we demonstrate that cis-unsaturated fatty acids block GTP·S binding by Gαs. The mechanism of inactivation involves a specific effect of lipid micelles on the nucleotide-free form of the protein. These observations are of particular interest since the tissues in which Gαs is found are known to accumulate significant levels of arachidonic acid in response to certain activating stimuli (20, 21), and thus the potential exists for cross-talk between arachidonate-producing pathways and those controlled by Gαs.

EXPERIMENTAL PROCEDURES

Production and Purification of Recombinant G Protein α Subunits—Recombinant Gαs was expressed in Escherichia coli and purified as described previously (9). The protein was stored at −80 °C in 50 mM HEPES, 1 mM EDTA, 1 mM DTT, and 5 mM MgCl2, supplemented with 2 mg/ml bovine serum albumin. Recombinant Gαs was purified from a baculovirus system as described (22). Recombinant Gαs, Gαi, and Gαq were generous gifts of Maurine Linder (Washington University School of Medicine, St. Louis MO) (23).

Lipid Storage and Micelle Preparation—All lipids were purchased from Sigma, dissolved in ethanol at final concentrations of 50 mM, and stored under N2 at −80 °C. For the preparation of pure micelles, the required amount of ethanolic lipid solution was dried under vacuum, and suspended in 50 mM HEPES, pH 8.0, 1 mM EDTA, and 1 mM DTT. This suspension was subjected to bath sonication until homogenous. Mixed micelles were prepared by dissolving the dried lipids in the same buffer containing 0.1% Lubrol (ICN) at 30 °C with vortexing or brief sonication.

GαNucleotide Binding Assays—G protein nucleotide binding by G protein α subunits was quantitated as described previously (24). Briefly, 1–5 pmol of the protein to be analyzed was diluted to 30 μl with 50 mM HEPES, pH 7.6, 1 mM EDTA, 1 mM DTT, and, where indicated, 0.1% Lubrol. 30 μl of GTP·S binding mix consisting of 50 mM HEPES, pH 7.6, 1 mM EDTA, 1 mM DTT, and 2 μM [35S]GTP·S (specific activity, ~10,000 cpm/pm) was then added. In experiments investigating competition between arachidonate and GTP·S, the concentration of the nucleotide was varied as indicated in the appropriate figure legend. Reactions were initiated by addition of protein, and, unless otherwise indicated, incubation conditions were set as a function of the intrinsic rates of exchange of the various α subunits. These were Gαs, 30 min at 30 °C; Gαi, and Gαq, 20 min at 30 °C; Gαs, 2 min at 20 °C; Gαi, 6 min at 20 °C. Free GTP·S concentration during incubation was 700 μM unless otherwise indicated. Reactions were terminated by the addition of 2 ml of ice-cold 20 mM Tris-Cl, pH 8.0, 25 mM MgCl2, and 100 mM NaCl. Samples were kept on ice until filtration through BA85 nitrocellulose filters. Filters were dried, and radioactivity was determined by liquid scintillation spectrophotometry.

For experiments assessing the time course of GDP dissociation from Gαs, 11 pmol of the protein were incubated at 30 °C for 60 min in the presence of 50 mM HEPES, pH 7.6, 1 mM EDTA, 1 mM DTT, 100 mM NaCl, 0.05% Lubrol, and 0.5 μM [3H]GDP (specific activity, ~26,000 cpm/pm). Arachidonic acid (300 μM) or palmitic acid (300 μM) in 50 mM HEPES, pH 7.6, 1 mM EDTA, 1 mM DTT, and 0.1% Lubrol was added, and samples were incubated for an additional 2 min. Samples were then spiked with unlabeled GDP such that the final concentration of GDP in the “chase” was 50 μM. The addition of arachidonate and GDP were of small enough volume as not to significantly perturb the relative concentration of protein or detergent. At the time points indicated in the appropriate figure, aliquots of Gαs were removed to ice-cold buffer (20 mM Tris-Cl, pH 7.7, 100 mM NaCl, 25 mM MgCl2) and stored on ice until filtration through BA85 nitrocellulose filters. Filters were washed, and radioactivity was determined by liquid scintillation spectrophotometry.

For experiments demonstrating the recovery of binding activity with time, Gαs (7.6 pmol) was incubated under the standard reaction conditions plus 300 μM arachidone. After 5 min, the reaction was diluted 10-fold with 50 mM HEPES, 1 mM EDTA, 1 mM DTT, 0.05% Lubrol, and 2 μM GTP·S. At the times indicated in the appropriate figure, aliquots were removed from the incubation into ice-cold buffer (20 mM Tris-Cl, pH 7.7, 100 mM NaCl, 25 mM MgCl2), and bound nucleotide was determined.

Fluorimetric Determination of Critical Micellar Concentrations of Lipids—CMC values for lipids were determined by fluorescence spectroscopy as described by Chattopadhyay and London (25) using the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene. A stock 500 μM suspension of lipid (see above) was diluted to appropriate concentrations in 500 μl of 50 mM HEPES, pH 7.6, 1 mM EDTA, 1 mM DTT, 5 mM MgCl2, and 1.5 mg/ml bovine serum albumin, and this mixture was then added to 500 μl of 50 mM HEPES, pH 7.6, 1 mM EDTA, 1 mM DTT, and 1 μM GTP. Following equilibration to room temperature, 1,6-diphenyl-1,3,5-hexatriene (2 μl of a 1 mM solution in tetrahydrofuran) was added, and the tubes were incubated in the dark for at least 30 min. Fluorescence measurements were performed on a Perkin-Elmer 650–40 fluorescence spectrophotometer set at excitation and emission wavelengths of 358 and 430 nm, respectively.

RESULTS

Cis-unsaturated Fatty Acids Inhibit GTP·S Binding by Gαs—Reports of the effects of arachidonic acid on proteins involved in signal transduction prompted us to evaluate arachidonate and related lipids for potential effects on G protein activities. The initial experiments focused on Gαs, since as noted in the Introduction, this G protein is predominantly expressed in tissues with highly active phospholipase A2 pathways. We first tested various fatty acids for their effect on the ability of Gαs to bind GTP·S, a non-hydrolyzable analog of GTP. As shown in Fig. 1, the 20-carbon unsaturated fatty acid, arachidonic acid, dramatically inhibited the ability of Gαs to bind guanine nucleotide. GTP·S binding by Gαs was suppressed in a dose-dependent fashion, and suppression was nearly complete at a concentration of 120 μM arachidonic acid. Essentially identical results were obtained when the binding of [3H]GDP, rather than GTP·S, was examined (results not shown). The carboxyl group on the fatty acid was essential for inhibition of nucleotide binding, as the equivalent fatty alcohol, arachidonyl alcohol, was not inhibitory. Interestingly, arachidic acid, the saturated 20-carbon fatty acid, also had no effect on GTP·S binding by Gαs, indicating the requirement for the double bonds for inhibition.

The ability of arachidonate to suppress GTP·S binding by Gαs prompted us to examine whether other unsaturated fatty acids exert the same effect. This was indeed found to be the case. Oleic acid, linoleic acid, and linolenic acid all suppressed nucleotide binding by Gαs in the same dose-dependent fashion as arachidonic acid (Fig. 1). Oleic acid and linoleic acid both completely suppressed GTP·S binding by Gαs, at a concentration of 175 μM, and linolenic acid was completely inhibitory at 250 μM. However, the trans-unsaturated fatty acid, elaidic acid, was only slightly inhibitory at these concentrations (data not shown).

The steepness of the inhibition curves for the unsaturated fatty acids indicated that the inhibition was not due to a simple binding event but rather to some sort of cooperative process. One such process, which is quite obvious when working with lipids, is the formation of micelles, which is a highly cooperative aggregation event. Accordingly, we determined the CMC for the lipids under the same conditions (e.g. ionic strength, Mg2+ concentration) as for the GTP·S binding experiments. CMC values for the various lipids were determined using a fluorescence technique (24), and it was found that the CMC
values corresponded nearly identically to the observed IC₅₀ for the inhibition of GTP-γS binding (Table I). For example, the observed IC₅₀ and the CMC for arachidonic acid were 60 and 73 μM, respectively. These observations provide strong evidence that the abilities of the unsaturated fatty acids to suppress GTP-γS binding by Gzₐ is micelle dependent; i.e., it is an interaction of the protein with an anionic lipid micelle, which is responsible for the inhibition.

To facilitate manipulation of the lipid in subsequent studies, we assessed whether the inhibition by arachidonate of the ability of Gzₐ to bind GTP-γS occurred when the fatty acid was present in a mixed micelle. The data in Fig. 2 show that this is the case, as the same type of inhibition is observed in response to increasing arachidonic acid when the fatty acid is present in a mixed micelle with the non-ionic detergent, Lubrol. The dose-response curve is shifted substantially to the right as would be expected for a process that depends on the mole fraction of lipid in the micelle (26). In fact, the IC₅₀ for inhibition of GTP-γS binding by Gzₐ shifts in proportion to the mole fraction of the lipid (results not shown).

Inhibition of Nucleotide Binding by Arachidonate's Specific for Gzₐ—We next determined the specificity of G protein-arachidonate interactions by determining the effect of the fatty acid on GTP-γS binding by other G protein α subunits. As in the previous experiments, GTP-γS binding by Gzₐ was inhibited 50% at 150 μM arachidonate, while at 300 μM, nucleotide binding was nearly completely suppressed (Fig. 3). GTP-γS binding by Gzₐ, a member of the Gs subfamily to which Gzₐ belongs, was not significantly inhibited at 150 μM arachidonate but was inhibited ~50% at 300 μM. The other G protein α subunits tested, including that of the closely related Gs, as well as Gα and Gα, were not inhibited by arachidonate at any concentration tested. This specificity for Gzₐ over other G protein α subunits suggests a potential role for arachidonate in Gzₐ signaling and prompted us to investigate the mechanism of this lipid effect on GTP-γS binding by Gzₐ.

Mechanism of Action—The binding of GTP-γS by G proteins is a two-step process involving GDP release (the rate-limiting step) and subsequent diffusion-controlled GTP-γS binding by the nucleotide-free protein (2). To explore the mechanism by which arachidonate inhibits GTP-γS binding, we first assessed the effect of arachidonate on the rate of GDP release from the protein. As release of bound GDP from α subunits is the rate-limiting step in nucleotide exchange, we expected that this process would be inhibited by arachidonate in a fashion similar to that of GTP-γS binding. Quite surprisingly, however, release of [³H]GDP from Gzₐ occurred with the same rate constant (Fig. 4) in the presence of 300 μM palmitic acid (a non-inhibitory fatty acid) or in the presence of 300 μM arachi-
Arachidonate Inactivation of \( \text{G}_{\text{z\alpha}} \)

A concentration that essentially completely suppressed GTP-\( \gamma \)-S binding to the protein (see Fig. 2). These data indicate that the effect of arachidonate is exerted at the step of GTP-\( \gamma \)-S binding. This would be highly unusual since GTP binding by G proteins is normally diffusion controlled, and thus its rate would have to be reduced by many orders of magnitude before an effect on the overall binding reaction would be observed.

One possibility for the selective effect of arachidonate on the GTP-\( \gamma \)-S binding step is that the lipid micelle could interact specifically with the unoccupied nucleotide binding site on \( \text{G}_{\text{z\alpha}} \) and effectively compete for GTP-\( \gamma \)-S binding. If this were the case, inhibition of nucleotide binding by arachidonic acid should be reduced by increasing the concentration of competing nucleotide. To explore this possibility, we measured the effect of arachidonic acid on GTP-\( \gamma \)-S binding in the presence of increasing GTP-\( \gamma \)-S concentrations. However, assessment of the arachidonate-mediated inhibition over a 50-fold range of GTP-\( \gamma \)-S revealed that binding was nearly completely suppressed at all concentrations of competing nucleotide (Fig. 5). Since inhibition of nucleotide binding by arachidonic acid was unaffected at GTP-\( \gamma \)-S concentrations as high as 25 \( \mu \text{M} \), which is >1000-fold above the \( K_d \) of G protein \( \alpha \) subunits for GTP-\( \gamma \)-S (2), it is considered highly unlikely that the lipid micelle is competing for the nucleotide binding site of the protein.

An alternative explanation for the effect of arachidonate on GTP-\( \gamma \)-S binding, but not on GDP release, by \( \text{G}_{\text{z\alpha}} \) is that the fatty acid could somehow interact with and inactivate the nucleotide-free form of the G protein that is a transient intermediate in the exchange process. To examine this possibility, we assessed the time dependence of the inactivation of \( \text{G}_{\text{z\alpha}} \) by arachidonate. If arachidonate could exert its effect only on the nucleotide-free form of \( \text{G}_{\text{z\alpha}} \), a recovery of binding activity should be observed if the protein is exposed to high arachidonate and then is diluted to an ineffective concentration. This recovery of binding activity would then reflect the fraction of the protein that had not yet released its GDP. Furthermore, if the arachidonate is selectively inactivating the nucleotide-free form of \( \text{G}_{\text{z\alpha}} \), then the rate of inactivation of GTP-\( \gamma \)-S binding should correspond to the rate of GDP release. Indeed, the evidence indicates that this is the case (Fig. 6). In the first experiment (Fig. 6A), binding activity was measured after \( \text{G}_{\text{z\alpha}} \) was first incubated with 300 \( \mu \text{M} \) arachidonic acid for 5 min and then diluted to 30 \( \mu \text{M} \) arachidonate. While GTP-\( \gamma \)-S binding activity was detected, the level of nucleotide binding recovered was significantly less than the control levels in which only 30 \( \mu \text{M} \) fatty acid had been present throughout.

This same type of experiment was performed over a range of pre-incubation times with 300 \( \mu \text{M} \) arachidonic acid from 5 to 90 min; in each case, the quantity of \( \text{G}_{\text{z\alpha}} \) capable of binding nucleotide was assessed after a 10-fold dilution to the ineffective concentration of the lipid (i.e. 30 \( \mu \text{M} \)). The results of this analysis, shown in Fig. 6B, revealed in each case a loss of GTP-\( \gamma \)-S binding activity that was not recovered by subsequent dilution. This was not due simply to protein lability, as pre-incubation of \( \text{G}_{\text{z\alpha}} \) in the absence of arachidonate did not result in a loss of GTP-\( \gamma \)-S binding activity. An equally important finding from this experiment is that the time dependence in the loss of the binding activity of \( \text{G}_{\text{z\alpha}} \) could be fit to an exponential with a decay constant of 0.028 min\(^{-1}\), which is nearly identical to the rate constant for GDP release from \( \text{G}_{\text{z\alpha}} \) under the same conditions (9). Taken together, these data indicate that, in the presence of arachidonate, \( \text{G}_{\text{z\alpha}} \) is able to release GDP normally but is then rapidly inactivated when the lipid micelle interacts with the nucleotide-free form of the protein.

**FIG. 3.** Comparison of the effect of arachidonic acid on GTP-\( \gamma \)-S binding to G protein \( \alpha \) subunits. The indicated \( \alpha \) subunits, all purified from bacterial expression systems, were subjected to a GTP-\( \gamma \)-S binding assay as described under “Experimental Procedures.” Assays were carried out in the absence of lipid (open bars) or in the presence of either 150 \( \mu \text{M} \) (hatched bars) or 300 \( \mu \text{M} \) arachidonic acid (solid bars). The incubation conditions were adjusted for each \( \alpha \) subunit as described under “Experimental Procedures.” Data shown represent the mean of three separate determinations with the 100% control value being the binding observed in the absence of added arachidonic acid. AA, arachidonic acid.

**FIG. 4.** Effect of arachidonic acid on GDP dissociation from \( \text{G}_{\text{z\alpha}} \). Approximately 1 pmol of \( \text{G}_{\text{z\alpha}} \) was incubated at 30°C in 50 mM HEPES, 1 mM EDTA, 1 mM DTT, 100 mM NaCl, 0.05% Lubrol, and 0.5 \( \mu \text{M} \) [\( \text{H} \)]GDP (26 Ci/mmol). After a 60-min incubation, arachidonic acid (●) or palmitic acid (○), in 50 mM HEPES, 1 mM EDTA, 1 mM DTT, 0.05% Lubrol, was added to a final concentration of 300 \( \mu \text{M} \). Samples were incubated for an additional 2 min, and then unlabeled GDP was added to a final concentration of 50 \( \mu \text{M} \). At the time points indicated, 60-\( \mu \)l aliquots were removed from the reactions to 2 ml of ice-cold 20 mM Tris, pH 7.6, 25 mM MgCl\(_2\), 100 mM NaCl. Samples were filtered through BAB5 nitrocellulose filters and dried, and bound radioactivity was determined. The 100% control value is the amount of [\( \text{H} \)]GDP bound at the initial time point sampled. Data shown are from a single experiment and are typical of results from three independent experiments. AA, arachidonic acid.
The role of lipids in cellular signaling has received increasing attention in recent years (27). It is now clear that lipids such as arachidonic acid and diacylglycerol actively participate as second messengers in signaling pathways (28, 29). Examples are also beginning to emerge of arachidonate and other cis-unsaturated fatty acids directly modulating activities of signaling proteins. For example, these fatty acids can associate with and alter the activity of Ras-GAP (17, 30). Cis-unsaturated fatty acids have also been shown to regulate association between the monomeric G protein, Rac, and its GDP dissociation inhibitor (18). Arachidonate and other unsaturated fatty acids have also been shown to activate certain isozymes of the protein kinase, protein kinase C (19).

In this report, we have identified an additional effect of cis-unsaturated fatty acids on a signaling protein, that being the inactivation of a G protein \( \alpha \) subunit, specifically \( G_\alpha_\beta \). Arachidonate-dependent inactivation of GTP\( \gamma \)S binding by \( G_\alpha_\beta \) was quite specific for this \( \alpha \) subunit, as treatment of a number of other \( \alpha \) subunits had only minimal effects on their abilities to bind nucleotide. The inactivation was dependent upon the presence of an acidic group on the lipid and correlated with the formation of a lipid micelle. Several cis-unsaturated fatty acids were potent inhibitors of GTP\( \gamma \)S binding by \( G_\alpha_\beta \), with a dose dependence that matched the lipid’s respective CMC. This suggests that it is an interaction between the charged surface of a micelle with \( G_\alpha_\beta \) that is required for its inhibition. These results are similar to the results of Serth et al. (17), who observed the inhibition of Ras-GAP in the presence of fatty acids and acidic phospholipids but not in the presence of neutral lipids, and only under conditions in which the active lipids formed micellar structures.

The inhibition of GTP\( \gamma \)S binding by \( G_\alpha_\beta \) seen upon the addition of arachidonic acid could have been exerted at either of two distinct steps in the process, these being dissociation of bound GDP or association of the GTP\( \gamma \)S. The former step was initially considered the most likely, as GDP dissociation from G proteins is \( \sim 10^7 \)-fold slower than association of guanine nucleotides.
(31). To identify the step in \( G_{\alpha} \) nucleotide exchange affected by arachidonate, we directly determined the effect of arachidonic acid on the rate of GDP release. Quite surprisingly, GDP release was virtually unaffected by concentrations of arachidonic acid on the rate of GDP release. Quite surprisingly, GDP release was virtually unaffected by concentrations of arachidonate, we directly determined the effect of arachidonic acid on the ability of \( G_{\alpha} \) to bind guanine nucleotides is dependent on an association of the lipid micelle with the nucleotide-free form of the protein, resulting in an alteration of the protein that renders it inactive.

While lipid-mediated modulation of G protein activity by irreversible inactivation seems an unlikely mode of regulation in the cell, the selectivity of the process for \( G_{\alpha} \) over other \( G \) proteins, as well as the unique distribution of \( G_{\alpha} \), provides strong reasons to suspect that the process is physiologically relevant. As noted above, the cell types in which \( G_{\alpha} \) is found, such as platelets and chromaffin cells, are known to possess high levels of phospholipase \( A_2 \) activity (20, 21). These cells are also known to produce substantial levels of arachidonate in response to external stimuli (20, 32, 33). Also of note in this regard are previous studies showing that treatment of platelets with high levels of exogenous arachidonate results in increased intracellular cAMP accompanied by reduced aggregation (34, 35).

In one of these studies, treatment of platelets with an adenylyl cyclase inhibitor restored aggregation in the presence of arachidonate, indicating that the fatty acid was exerting its effect at or upstream of adenylyl cyclase (35). The finding that arachidonate can inactivate a G protein that is both present in platelets and implicated in the inhibition of adenylyl cyclase thus provides a potential molecular mechanism for these effects.

Finally, it is certainly possible that in the context of an intact cell an increase in the concentration of arachidonic acid might be only transiently inhibitory, i.e., the cellular environment could provide protection of the apoprotein form of \( G_{\alpha} \) from permanent inactivation by the lipid. Possibilities here include a protective factor in these cells that associates with \( G_{\alpha} \) or one that reverses the association between \( G_{\alpha} \) and inhibitory lipids. Identification of the pathway in which \( G_{\alpha} \) participates will likely shed some light on these results and on the possibilities for the novel means of G protein regulation they may represent.

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