G-protein inhibition of phospholipase C- β_1 in membranes: role of G-protein $\beta\gamma$ subunits

Irene LITOSCH

Department of Molecular and Cellular Pharmacology, University of Miami School of Medicine, Miami, FL 33101, U.S.A.

Rat liver plasma membranes reconstituted with bovine brain phospholipase C β_1 (PLC- β_1) exhibit a dual regulation of PLC- β_1 activity by G-proteins. Guanosine 5'-[γ -thio]triphosphate (GTP[S]; 0.1 nM) produced a 20–25% inhibition of PLC- β_1 activity within 7 min of incubation. The addition of vasopressin resulted in near-basal levels of activity in the presence of 0.1 nM GTP[S]. Clonidine had little effect on the net inhibition due to GTP[S]. A similar antagonism between carbachol and GTP[S] occurred in cerebral cortical membranes containing endogenous PLC- β_1 activity. $\alpha_{o/i}$ -GDP (a mixture of GDP-

liganded G_{α_x} and G_{α_z}) attenuated the GTP[S]-dependent inhibition of PLC- β_1 whereas $\alpha_{o/i}$ -GTP[S] had no effect, suggesting an involvement of G-protein $\beta\gamma$ subunits in the inhibition of PLC- β_1 . Low concentrations of $\beta\gamma$ subunits inhibited PLC- β_1 activity. Inhibition was followed by reversal to basal activity and onset of stimulation as the $\beta\gamma$ concentration was increased. Inhibition by $\beta\gamma$ was dependent on the presence of membranes. These results indicate that G-protein $\beta\gamma$ subunits constitute a mechanism by which G-proteins mediate a rapid and transient inhibition of PLC- β_1 .

INTRODUCTION

Phospholipase C β (PLC- β) constitutes part of the receptor–G-protein-controlled signalling system that regulates intracellular $[Ca^{2+}]$ and protein kinase C activity [1,2]. The α subunit of the $G_{q/11}$ family of pertussis toxin-insensitive G-proteins [3–6] and G-protein $\beta\gamma$ subunits [6–8] stimulate PLC- β through separate mechanisms. Three major PLC- β isoforms have been identified that are sensitive to stimulation by both α_q and $\beta\gamma$ subunits. The tissue distribution and different sensitivities to regulation by G-protein subunits suggests that these PLC- β isoforms might have specific roles in the regulation of cell function [7,8].

PLC- β_1 is found in high concentrations in brain and is activated by α_q and $\beta\gamma$ subunits [7,8]. PLC- β_1 activity is also negatively modulated by a G-protein mechanism. A rapid inhibition of basal and agonist-stimulated PLC activity by adenosine in GH_3 cells [9] and by α -methylhistamine in gastric cells [10] has been demonstrated. Inhibition of PLC activity occurs within seconds and is followed by a rapid return to basal levels. A rapid and transient G-protein-dependent inhibition of PLC- β_1 activity has been demonstrated in cerebral cortical membranes [11] and solubilized cerebral cortical membrane preparations [12]. Inhibition requires nanomolar concentrations of guanosine 5'-[γ -thio]-triphosphate (GTP[S]), is transient and pertussis toxin-sensitive.

Although a G_i - or G_o -like protein has been implicated in the inhibitory regulation of PLC- β_1 , the mechanisms responsible and the role of this inhibitory pathway in different cells have not been established. The present studies were undertaken to define further the mechanisms responsible for the inhibitory regulation of PLC- β_1 by using purified rat liver plasma membranes.

EXPERIMENTAL

Membrane and PLC- β_1 isolation

Rat liver plasma membranes were prepared by two different methods. Membranes containing endogenous PLC activity were prepared as described [13]. PLC activity in these membranes

corresponded to 3.95 ± 0.4 pmol/min per μ g at 30 °C and a pCa of 6.3 (approx. 33 c.p.m./min). Decreasing the ratio of rat liver to homogenization buffer from 6% to 1% resulted in rat liver membranes that were deficient in PLC activity. PLC activity in these membranes corresponded to 0.51 ± 0.03 pmol/min per μ g at 30 °C and a pCa of 6.3 (approx. 4 c.p.m./min). Experiments with these membranes, in the absence of added PLC- β_1 , were done with 2–4 μ g of protein per tube. Rat cerebral cortical membranes were prepared as described [11].

Bovine brain membranes were used as a source of PLC- β_1 . Brain membranes contain a concentration of PLC- β_1 100-fold greater than PLC- β_3 [7]. PLC- β_1 was purified 600-fold from a 2 M KCl extract of bovine brain membranes by sequential chromatography on DEAE-Sephacel, heparin-agarose, phenyl-Sepharose and Fast Flow Q (I. Litosch, unpublished work). Purification was based on published protocols [7,14]. The purified preparation was free of PLC- γ or PLC- δ as determined by immunoblotting with the BLAST Amplification Procedure (DuPont–NEN). G-protein $\beta\gamma$ subunits were purified from bovine brain as described [15,16]. Purification of $\alpha_{o/i}$ subunits [17,18] and generation of the liganded forms were as described [19].

PLC determination

Endogenous membrane PLC activity was determined in buffer containing 50 mM Hepes, pH 6.75, 0.5 mM $MgCl_2$, 12 mM LiCl and effectors, with a pCa of 6.3. [3H]Phosphatidylinositol 4,5-bisphosphate (PIP $_2$; 20 μ M, labelled plus unlabelled) was added in combination with phosphatidylethanolamine (100 μ M). Membranes were added in 10 μ l of buffer containing 25 mM Hepes, pH 7.0, and 1 mM EDTA. Incubation was at 30 °C in a total volume of 50 μ l. $[Ca^{2+}]$ was set to a pCa of 6.3 by a Ca^{2+} /EGTA buffer with 3 mM EGTA [20]. Incubation was terminated by the transfer of tubes to ice followed by the addition of 200 μ l of ice-cold 10% (w/v) trichloroacetic acid and 100 μ l of 10 mg/ml BSA as described [8]. The samples were

centrifuged, the supernatant was removed and the amount of released radioactivity was determined by liquid-scintillation counting.

In the reconstitution studies, PLC-deficient membranes (0.5–1 μg) were incubated in the presence of PLC- β_1 . An initial activity assay established the amount of PLC- β_1 needed to provide a consistent level of activity over the course of these experiments. There was some variation in the amount of PLC added in each experiment owing to slight variability in the activity of different PLC stocks. All assays were done under linear assay conditions. Incubation time was from 4 to 7 min. Assay conditions were similar to those described with membranes containing endogenous PLC activity.

Membrane protein was determined by the Bradford method (Bio-Rad) with BSA as standard. The Amido Black assay was used for G-protein subunits [21].

G-protein subunit studies

Because cholate was inhibitory in this assay, $\beta\gamma$ buffer or $\beta\gamma$ subunits diluted in 0.4% cholate, 25 mM Hepes, pH 7.5, 1 mM EDTA and 1 mM dithiothreitol was added directly to membranes. Membranes containing $\beta\gamma$ subunits were then added to the PLC assay tube. A similar protocol was used for the α subunit.

Immunoblot analysis

SDS/PAGE of PLC- β_1 and rat liver membranes was performed with 10% (w/v) gels. Proteins were transferred to nitrocellulose and incubated with the specified antibody. The blots were probed with anti-(G_β subunit) (Upstate Biotechnology), anti-($G_{q/11}$ α subunit) (Santa Cruz), anti-PLC- β_1 (Upstate Biotechnology and Santa Cruz) and anti-PLC- β_3 (Santa Cruz). Detection was by enhanced chemiluminescence with Hyperfilm (Amersham).

Materials

[^3H]PIP $_2$ was from DuPont-NEN. Antisera to the major PLC isoforms (β , γ and δ) were from Upstate Biotechnology. All other reagents were from Sigma.

RESULTS

The effect of a range of GTP[S] concentrations on rat liver plasma membrane PLC activity is shown in Figure 1. Low concentrations of GTP[S] had little effect on PLC activity, whereas stimulation was readily observed with high GTP[S] concentrations. Inhibitory effects of GTP[S] on PLC activity were not evident regardless of the incubation time, i.e. 2, 4 or 10 min (results not shown). Similar results were obtained with membranes containing low endogenous PLC activity. GTP[S] produced only stimulation of PLC activity in these membranes. Possible reasons for the lack of appreciable inhibitory regulation by GTP[S] in rat liver plasma membranes are the lack of an appropriate PLC- β isoform, the presence of unregulated PLC isoforms that obscured inhibitory regulation or the lack of appropriate G-proteins.

PLC- β_1 has been identified as an effector for the inhibitory regulatory pathway [12]. PLC- β_1 purified 600-fold from bovine brain, to resolve PLC- β from PLC- δ and PLC- γ , was used in a reconstitution approach to study the regulation of PLC- β_1 by G-protein. The PLC- β_1 preparation was unaffected by a range of GTP[S] concentrations (0.1 nM to 100 μM), indicating the lack of a GTP[S] regulatable α subunit in the preparation (results not shown).

Figure 1 Effect of GTP[S] on rat liver plasma membrane PLC activity

Rat liver membranes prepared as described in [13] (●) or as modified in the Experimental section to reduce basal activity (▽) were incubated with the indicated range of GTP[S] concentrations for 6 min. Results are shown as percentages of basal activity and are the means $\pm$ S.E.M. for three experiments. Basal activity was 4 ± 0.4 pmol/min per μg and 0.51 ± 0.03 pmol/min per μg for the control and PLC-deficient membranes respectively.

An immunoblot analysis of the PLC- β_1 preparation and rat liver membranes is shown in Figure 2. PLC- β_1 was readily evident in the PLC- β_1 preparation, whereas none was detectable in the rat liver membrane preparations. PLC- β_3 was detected in the rat liver membrane preparations but not in the brain PLC- β_1 preparation. Both G-protein $\alpha_{q/11}$ and β subunits were evident in the rat liver membrane preparation, with little difference between the two membrane preparations. Neither α_q nor β subunits were detected in the PLC- β_1 preparation.

The regulation of basal PLC- β_1 activity by GTP[S] in the presence of rat liver membranes is shown in Figure 3(A). GTP[S] inhibited PLC- β_1 activity over a narrow concentration range. Maximum inhibition occurred with 0.1 nM GTP[S]. A 20% inhibition of basal activity was evident at 7 min of incubation. The concentration dependence for inhibition was steep. In-

Figure 2 Immunoblot analysis of PLC- β_1 preparation and rat liver plasma membranes

PLC- β_1 preparation (1.1 μg) and rat liver membranes (10 μg of cholate extract of membranes) were subjected to SDS/PAGE [10% (w/v) gel], transferred to nitrocellulose and incubated with anti-PLC- β_1 , anti-PLC- β_3 , anti- β_6 and anti- $\alpha_{q/11}$. Blots were incubated with enhanced chemiluminescence (ECL) detection reagents for 1 min followed by exposure to Hyperfilm ECL for 30 s to 15 min. The exposure time for PLC- β_3 was 30 min. Lane 1, PLC- β_1 ; lane 2, PLC-depleted rat liver membranes; lane 3, rat liver membrane.

Figure 3 Effect of GTP[S] on PLC- β_1 activity in the reconstituted system

(A) Membranes plus PLC- β_1 (1–2 ng per tube) were incubated in either the absence or presence of the indicated GTP[S] concentrations. Additions to the incubation were GTP[S] (○) plus 100 nM vasopressin (●) or 10 μ M clonidine (▽). Incubation was for 7 min. Results are expressed as percentages of the basal PLC activity and are the means $\pm$ S.E.M. for six experiments with GTP[S] and three experiments with vasopressin and clonidine. Basal activity was 3 pmol/min per μ g. Basal activity corresponds to that measured in the absence of any addition. Omission of the error bar occurs where the value is smaller than the symbol. (B) Cerebral cortical membranes containing endogenous PLC- β_1 activity were incubated in the absence or presence of the indicated GTP[S] concentration. Results are shown as percentages of basal activity for GTP[S] (○) and GTP[S] plus 100 μ M carbachol (●).

creasing the GTP[S] concentration to 1 nM or greater resulted in a reversal of inhibition. These results demonstrate that the reconstitution of rat liver plasma membranes with bovine brain PLC- β_1 conferred GTP[S]-dependent inhibitory regulation on basal PLC- β_1 activity. The addition of 1 μ M guanosine 5'-[β -thio]diphosphate (GDP[S]) had little effect on basal activity (94% of control) but blocked the inhibition due to GTP[S]. Activity in the presence of 0.1 nM GTP[S] plus 1 μ M GDP[S] was 96% of basal.

Vasopressin and phenylephrine stimulate PLC- β_1 activity via the $G_{q/11}$ signalling pathway. The effect of 100 nM vasopressin on PLC- β_1 activity over a range of GTP[S] concentrations is shown in Figure 3(A). Vasopressin did not affect basal activity in the absence of guanine nucleotide. In the presence of 0.01–1 nM GTP[S], vasopressin failed to activate PLC- β_1 activity. Instead, near-basal levels of activity were maintained in the presence of 100 nM vasopressin plus 0.1–10 nM GTP[S]. Stimulation ensued with higher GTP[S] concentrations. Similar results were obtained with 100 μ M phenylephrine (results not shown). In contrast, clonidine, an α_2 -selective adrenergic agonist, which is not linked to $G_{q/11}$, did not attenuate the inhibition due to GTP[S] or affect the reversal of inhibition that occurred as the GTP[S] concentration was increased from 0.1 nM to 1 μ M. Clonidine decreased PLC- β_1 activity in the absence of GTP[S] and slightly increased the inhibition due to GTP[S].

As shown in Figure 3(B), similar results were obtained with cerebral cortical membranes containing endogenous PLC- β_1 . As reported previously, GTP[S] produced a concentration-dependent inhibition of PLC- β_1 , with maximal inhibition occurring at 1–10 nM [11]. Carbachol failed to activate PLC- β_1 activity at low GTP[S] concentrations. Near-basal levels of activity were evident in the presence of carbachol and low GTP[S] concentrations. These results indicate that the G-protein-dependent

Figure 4 Time course for G-protein-dependent inhibition and stimulation of PLC- β_1

Membranes plus PLC- β_1 (1 ng per tube) were incubated in the absence or presence of 0.1 nM GTP[S], and with or without 100 nM vasopressin, for the indicated times. Results are shown as percentages of basal activity for GTP[S] (○) and 0.1 nM GTP[S] plus 100 nM vasopressin (●), and are the means $\pm$ S.E.M. for three paired experiments. The effect of 1 μ M GTP[S] is also shown (▽). Omission of the error bar occurs where the value is smaller than the symbol.

inhibitory pathway modulates basal PLC- β_1 activity and might antagonize stimulation of PLC- β_1 activity by agonists coupled to the $\alpha_{q/11}$ signalling pathway. This occurs both in reconstituted systems as well as in native membranes. As previously shown for cerebral cortical membranes [11,12], inhibition in the liver membrane system was time-dependent. Extending the incubation time to 30 min resulted only in stimulatory regulation of PLC- β_1 , with the onset of stimulation at 10 nM GTP[S] (results not shown). The time course for inhibition of PLC- β_1 activity by 0.1 nM GTP[S] in the presence or absence of 100 nM vasopressin is shown in Figure 4. Inhibition of PLC- β_1 activity was apparent within 2–4 min of the addition of 0.1 nM GTP[S]. The level of inhibition gradually reversed to attain near-basal levels of activity at 10 min. The time dependence for the reversal of inhibition varied slightly with different membrane preparations, as previously observed with solubilized bovine brain membranes [12]. In general, the time course for inhibition was assessed with each new membrane preparation to ensure that all determinations were done at appropriate times. Vasopressin failed to stimulate PLC- β_1 activity in the presence of 0.1 nM GTP[S]. Consistent with the data shown in Figure 3(A), incubation in the presence of vasopressin and 0.1 nM GTP[S] resulted in the maintenance of near basal levels of activity. For comparison, the effect of 1 μ M GTP[S] is also shown. GTP[S] (1 μ M) stimulated PLC- β_1 activity after a 4 min delay without producing any detectable inhibition of PLC- β_1 activity. Enzymic activity in the presence of 1 μ M GTP[S] was linear for up to 8 min of incubation. The enzymic rate declined thereafter owing to substrate depletion, precluding further determination.

The stimulation due to GTP[S] varied slightly over the course of these experiments, with maximal stimulation approaching 150% of basal. The experimental conditions used in these studies were designed to optimize measurement of inhibition at short incubation times. Thus the maximal amount of PLC- β_1 activity that supported a linear enzymic rate during the incubation was

Table 1 Effect of G-protein $\alpha_{o/i}$ subunits on the GTP[S]-dependent regulation of PLC- β_1

Membranes (120 μ l) were combined with 2 μ l of buffer (25 mM Hepes, pH 7.0, 0.4 % cholate and 1 mM dithiothreitol) or 2 μ l of α subunit (19.5 ng/ μ l) diluted in the same buffer. A 10 μ l portion of the mixture was added to 40 μ l of PLC assay mixture containing PLC- β_1 (0.2 ng per tube) followed by incubation in the presence of 1 nM GTP[S] plus no added G-protein subunits, plus $\alpha_{o/i}$ -GDP or $\alpha_{o/i}$ -GTP[S] for 7 min. The basal activity of the control and that in the presence of $\alpha_{o/i}$ -GDP were 11.4 and 10.7 pmol/min per μ g respectively. The basal activity and that in the presence of $\alpha_{o/i}$ -GTP[S] were 11.1 and 10.2 pmol/min per μ g. Results are shown as the percentage of basal activity measured in the absence of GTP[S] but in the presence of buffer or G-protein subunit.

Addition	Activity (% of basal)
GTP[S]	83 $\pm$ 2.3
GTP[S] + $\alpha_{o/i}$ -GDP	94 $\pm$ 0.3
GTP[S] + $\alpha_{o/i}$ -GTP[S]	83 $\pm$ 3.3

used. Under these conditions, appreciable stimulation could result in the onset of a non-linear enzymic rate owing to substrate depletion, thereby precluding a determination of the full extent of stimulation. Although the experimental conditions are not identical, the percentage stimulation due to GTP[S] in the reconstitution system is nonetheless within the range (106–153 %) reported for rat liver plasma membranes containing endogenous PLC activity and assayed in the presence of PIP₂/phosphatidylethanolamine substrate [22,23]. The presence of phosphatidylserine in the substrate mixture has been found to be necessary for optimal expression of GTP[S]-stimulated PLC activity [23]. In a separate series of experiments with PIP₂/phosphatidylethanolamine/phosphatidylserine substrate (20 μ M:100 μ M:20 μ M), 1 μ M GTP[S] produced a 242 $\pm$ 19 % stimulation of basal activity at 7 min ($n = 3$). However, these conditions, which optimize stimulatory regulation, resulted in less inhibitory regulation and were therefore not used.

The effect of G-protein subunits on the inhibitory regulation was examined in the next series of experiments. The addition of either $\alpha_{o/i}$ -GDP (a mixture of GDP-liganded G_{o α} and G_{12 α}) or $\alpha_{o/i}$ -GTP[S] resulted in a slight decrease in basal activity (approx. 6%). Because a comparable decrease was observed with the GDP- and GTP[S]-liganded form, it was nonetheless possible to compare the effects of the two different liganded states on the inhibitory regulation. As shown in Table 1, $\alpha_{o/i}$ -GDP attenuated the inhibition due to GTP[S] whereas $\alpha_{o/i}$ -GTP[S] did not affect the degree of inhibition. Because $\alpha_{o/i}$ -GDP binds $\beta\gamma$ and thereby deactivates $\beta\gamma$ -stimulated effects whereas $\alpha_{o/i}$ -GTP[S] does not bind $\beta\gamma$ subunits as effectively, these results suggested that $\beta\gamma$ subunits might participate in the inhibitory mechanism.

The effect of a range of $\beta\gamma$ concentrations on PLC- β_1 activity in the presence of membranes is shown in Figure 5. Low concentrations of $\beta\gamma$ subunits, 0.01–1 nM, inhibited PLC- β_1 activity (Figure 5A). Increasing the $\beta\gamma$ concentration resulted in a reversal of inhibition and an onset of stimulation. The inhibitory effects of $\beta\gamma$ were dependent on the presence of membranes. Inhibition of PLC- β_1 by $\beta\gamma$ was not observed with PLC- β_1 alone or with heat-inactivated membranes (Figure 5B). In the absence of membranes, $\beta\gamma$ subunits produced only stimulation of PLC- β_1 activity. Thus whereas the $\beta\gamma$ stimulation of PLC- β_1 activity reflects a direct effect on PLC- β_1 , as has been reported [6–8], $\beta\gamma$ inhibition of PLC- β_1 required a membrane component. Inhibition due to GTP[S] and $\beta\gamma$ was not additive, suggesting that both mediate their effects through a similar mechanism (results not shown).

Figure 5 Effect of G-protein $\beta\gamma$ subunit concentration on PLC- β_1 activity

(A) Membranes (60 μ l, 0.1 μ g/ μ l) were mixed with 3 μ l of $\beta\gamma$ subunit or $\beta\gamma$ buffer, consisting of 10 mM Hepes, pH 7.0, 0.4 % cholate and 1 mM dithiothreitol. A 10 μ l portion of the mixture was then added to 40 μ l of incubation buffer containing PLC- β_1 (0.3 ng per tube) and incubation was initiated for 6 min. The $\beta\gamma$ concentration indicated refers to the concentration in the membrane mixture before addition to the PLC assay. Results are the means $\pm$ S.E.M. for three to seven experiments. Basal activity corresponds to activity measured in the presence of an equivalent amount of $\beta\gamma$ buffer, and was 12 pmol/min per μ g. (B) Experimental conditions were similar to those in (A), except that PLC- β_1 was incubated in the absence of membranes (Δ). Incubation was for 10 min with 1.5 ng of PLC- β_1 per tube. Basal activity was 4 pmol/min per μ g. The effect of incubation in the presence of heat-inactivated membranes plus $\beta\gamma$ plus PLC- β_1 is also shown ($\blacksquare$).

DISCUSSION

The present studies demonstrate that GTP[S]-dependent inhibition of PLC- β_1 occurs in a reconstitution system consisting of rat liver plasma membranes and bovine brain PLC- β_1 . Unlike cerebral cortical brain membranes, low concentrations of GTP[S] had little effect on endogenous liver membrane PLC activity. Possible explanations for the failure of GTP[S] to inhibit endogenous membrane PLC activity are the presence of unregulated PLC isoforms, different PLC- β isoforms in rat liver membranes that are insensitive to inhibitory regulation, or the lack of appropriate G-proteins. Brain contains predominantly PLC- β_1 [24], whereas liver contains little PLC- β_1 but contains PLC- β_3 [7,24,25] (Figure 2). In addition, PLC- β_3 is readily truncated in liver, generating a 110 kDa polypeptide that lacks $\alpha_{q/11}$ regulation and is markedly stimulated by $\beta\gamma$ [26]. Because these PLC- β isoforms demonstrate a different sensitivity to stimulation by α_q and $\beta\gamma$ subunits [6–8] and differences in specific activities [6–8], it is possible that these isoforms differ in their ability to be regulated by the inhibitory pathway.

Reconstitution of rat liver membranes with PLC- β_1 purified from bovine brain resulted in GTP[S]-dependent inhibition of PLC- β_1 . GTP[S] did not affect PLC- β_1 activity in the absence of membranes. Thus liver membranes contain the G-protein subunits required for inhibitory regulation of PLC- β_1 . Inhibition was rapid, transient and occurred with low GTP[S] concentrations. Importantly, the combination of low GTP[S] concentrations plus vasopressin or phenylephrine resulted in neither inhibition or stimulation of PLC- β_1 activity (Figures 3A and 4). Maintenance of basal activity was observed under these conditions. Similar results were observed in cerebral cortical membranes in the presence of carbachol (Figure 3B). This maintenance of basal activity in the presence of low concentrations of GTP[S] and agonist might reflect suppression of agonist stimulation of PLC activity by the inhibitory pathway, as has been observed in

in vivo. Activation of adenosine A_1 receptors inhibited basal inositol phosphate production and that due to thyrotropin releasing hormone in GH_3 cells [9]. Similarly, histamine H_3 receptor activation resulted in an inhibition of basal as well as carbachol-stimulated inositol phosphate production in gastric cells [10].

None of the known G-proteins has been shown to inhibit PLC- β_1 in purified reconstitution systems, suggesting that an unidentified G-protein participates in the inhibitory regulation, that the appropriate reconstitution conditions have not been used, or that regulation occurs through an indirect mechanism. The present study has identified the G-protein $\beta\gamma$ subunit as constituting one mechanism for mediating the G-protein-dependent inhibition of PLC- β_1 activity in membranes. A role for $\beta\gamma$ is supported by the finding that the GTP[S]-dependent inhibition of PLC- β_1 was antagonized by $\alpha_{o/i}$ -GDP but not by $\alpha_{o/i}$ -GTP[S]. In addition, as shown in Figure 5, low concentrations of $\beta\gamma$ subunits inhibited PLC- β_1 activity. The inhibitory effects of $\beta\gamma$ were not due to a direct effect on PLC- β_1 activity because inhibition was not observed in the absence of membranes. Thus a membrane component participates in this inhibitory mechanism. The concentration dependence for inhibition by $\beta\gamma$ is steep, presumably because higher concentrations of $\beta\gamma$ produce a direct stimulation of PLC- β_1 .

The results of this study are consistent with the hypothesis that low concentrations of GTP[S] promote the activation and dissociation of a $G_{o/i}$ -like protein that has a high affinity for guanine nucleotides. The released $\beta\gamma$ subunit produces inhibition of PLC- β_1 through interaction with a membrane component. Because one major role of G-protein $\beta\gamma$ subunits is to mediate the deactivation of α subunits [1,2,27,28], it is possible that the released $\beta\gamma$ subunits interact with membrane $\alpha_{q/11}$ to promote formation of the inactive $\alpha_{q/11}$ heterotrimer and deactivation. Inhibition by $\beta\gamma$ would be antagonized by the direct $\beta\gamma$ -mediated stimulation of PLC- β_1 activity. This could account for the steep concentration dependence for inhibition as well as the transient nature of the inhibitory regulation. Inhibition of an α_i -stimulated PLC- β activity by low concentrations of $\beta\gamma$ subunits has been shown in a liposome reconstitution system consisting of a turkey PLC- β -like enzyme and an AlF_4^- -activated turkey erythrocyte α_{11} [29]. As in the present study, inhibition was steep, followed by a reversal of inhibition and finally a stimulation of PLC- β with increasing concentrations of $\beta\gamma$. Whether inhibitory regulation by low concentrations of $\beta\gamma$ occurs in a membrane environment, its time dependence and interaction with other signalling pathways was not addressed.

Because G-protein $\beta\gamma$ subunits regulate cell function through mechanisms other than deactivation of α subunits, other mechanisms for inhibition cannot be excluded. Direct effects of $\beta\gamma$ subunits on effectors including adenylate cyclase, PLC- β and ion channels have been demonstrated [30]. In addition, $\beta\gamma$ subunits stimulate Raf-1 protein kinase [31] as well as activate mitogen-activated protein kinase through interaction with Ras [32]. It is likely that other $\beta\gamma$ -regulated proteins will be identified. Additional studies are needed to determine the mechanism by which $\beta\gamma$ inhibits PLC- β_1 and whether $\beta\gamma$ subunits constitute the only mechanism for conferring inhibitory regulation of PLC- β_1 .

As shown in Figures 3(A) and 3(B), maximal inhibition of PLC- β_1 in the liver membrane system occurred with a concentration of GTP[S] approx. one-tenth of that in cerebral cortical membranes. This difference might reflect the influence of membrane composition, G-protein population, levels of $\alpha_{q/11}$, the properties of $G_{q/11}$ or other as yet unidentified components in the inhibitory mechanism. Intrinsic differences in the properties of cerebral and hepatic $\alpha_{q/11}$ have been demonstrated [33]. In addition, the presence of PLC- β_3 in native rat liver membranes

might be a factor. PLC- β_3 is more sensitive to activation by G-protein $\beta\gamma$ subunits than is PLC- β_1 [6–8]. It is possible that different assay conditions are needed to observe inhibitory regulation in PLC- β_3 -containing membranes.

The rapid and transient inhibition of PLC- β_1 activity observed *in vivo* [9,10] and *in vitro* [11,12] has not been reported for other G-protein-regulated systems, suggesting that this might constitute an important element of the PLC signalling pathway. Differences between $\alpha_{q/11}$ and other G-protein α subunits have already been noted. First, the $\alpha_{q/11}$ family of G-proteins differs from most G-protein α subunits in that receptor-stimulated GTP[S] and GDP dissociation from $\alpha_{q/11}$ exhibits complex kinetics [33]. Secondly, purified α_q has low intrinsic GTPase activity in the absence of an activated receptor. Even in the presence of an activated receptor, the GTPase activity is considerably lower than that of other G-protein α subunits. Finally, PLC- β_1 is a GTPase-activating protein on $\alpha_{q/11}$ [34].

In summary, these studies demonstrate that G-protein $\beta\gamma$ subunits constitute one mechanism for conferring a rapid and transient inhibition of PLC- β_1 activity. The G-protein-mediated inhibitory pathway might have an important role in regulating cellular responsiveness to Ca^{2+} -mobilizing agonists.

I thank Dr. Hong Xie for purification of G-protein subunits, and Ms. Junia Joseph for technical assistance in the conduct of these experiments. This work was supported by grant DK37007 from the National Institutes of Health and a grant-in-aid from the American Heart Association, Florida Affiliate.

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Received 20 March 1996/14 June 1996; accepted 21 June 1996