Phospholipase Cδ1 Is a Guanine Nucleotide Exchanging Factor for Transglutaminase II (Gα1) and Promotes α1B-Adrenoceptor-mediated GTP Binding and Intracellular Calcium Release

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Effectsors involved in G protein-coupled receptor signaling modulate activity of GTPases through GTPase-activating protein or guanine nucleotide exchanging factor (GEF). Phospholipase Cδ1 (PLCδ1) is an effector in tissue transglutaminase (TGII)-mediated α1B-adrenoceptor (α1B-AR) signaling. We investigated whether PLCδ1 modulates TGII activity. PLCδ1 stimulated GDP release from TGII in a concentration-dependent manner, resulting in an increase in GTPγS binding to TGII. PLCδ1 also inhibited GTP hydrolysis by TGII that was independent from the α1B-AR. These results indicate that PLCδ1 is GEF for TGII and stabilizes the GTP-TGII complex. When GEF function of PLCδ1 was compared with that of the α1B-AR, the α1B-AR-mediated GTPγS binding to TGII was greater than PLCδ1-mediated binding and was accelerated in the presence of PLCδ1. Thus, the α1B-AR is the prime GEF for TGII, and GEF activity of PLCδ1 promotes coupling efficacy of this signaling system. Overexpression of TGII and its mutants with and without PLCδ1 resulted in an increase in α1B-AR-stimulated Ca2+ release from intracellular stores in a TGII-specific manner. We conclude that PLCδ1 assists the α1B-AR function through its GEF action and is primarily activated by the coupling of TGII to the cognate receptors.

Phospholipase C (PLC)δ1 is a member of the PLC family that produces two second messengers, inositol 1,4,5-triphosphate (IP3) and diacylglycerol by hydrolyzing phosphatidylinositol 4,5-bisphosphate (PIP2) (1). Among PLCs, PLCδ1 isosymes are stimulated by guanine nucleotide-binding proteins (G protein) Gδ and its family of proteins in response to activation of cell surface receptors. PLCδ1 isosymes are activated by phosphorylation through growth factor receptors. A number of laboratories have reported that PLCδ1 is stimulated by a unique GTP-binding protein known as tissue transglutaminase (TGII, Gαδ) (2,5). TGII is a bifunctional enzyme, having GTPase and transglutaminase (TGase) activity (6,7) and is present in the plasma membrane, cytosol, and nucleus in a variety of tissues and cells (6). Exchange of GDP to GTP by TGII is facilitated by activation of the cell surface receptors (4–9). These receptors include the α1B-adrenoceptor (AR) (5,7,8), α1D-AR (8), α-thromboxane receptor (9), and oxytocin receptor (4). The coupling of TGII with these receptors appears to be receptor subtype-specific (8,9).

PLCδ1 is widely distributed and expressed highly in some tissues such as mouse heart (1,10). Stimulation of the enzyme by TGII involving α1B-AR is modulated in a concentrated fashion within the system. Thus, bimodal regulation of PLCδ1 activity has been observed depending on the Ca2+ levels and occupancy of guanine nucleotide by TGII (3,11,12). PLCδ1 is stimulated with low concentrations of Ca2+ by GTPγS-TGII (11). However, activity of the enzyme is subsequently inhibited by increasing Ca2+ concentrations where PLCδ1 is stimulated in the presence or absence of GDP. Similarly, Murthy et al. (3) have reported that GTP-TGII inhibits PLCδ1, while GDP-TGII stimulates the enzyme. The Ca2+ dependence is not clearly defined in this study. The TGII-mediated PLC stimulation is also modulated by the level of TGII expression (12). At low levels of TGII expression, the α1B-AR-stimulated PLC activity is increased, whereas the receptor-mediated PLC stimulation is inhibited when TGII is highly expressed. The PLCδ1 activity is also inhibited by IP3, competing with its substrate PIP2 for a binding site known as the pleckstrin homology domain (13–15).

Studies have also demonstrated that an increase in the intracellular concentration of Ca2+ activates PLCδ1 (13,16,17), indicating that activation of PLCδ1 occurs secondarily in response to the receptor-mediated activation of other PLCs or Ca2+ channels. A GTPase activating-protein (GAP) for the small GTPase RhoA (RhoGAP) also activates PLCδ1 by direct association (18). All of these observations suggest that the PLCδ1 activity is regulated by multiple factors.

All known GTP binding subunits (Go) of G proteins are GTPases, which hydrolyze GTP to GDP and orthophosphate (P). It is now recognized that a large number of regulators of G protein signaling (RGS) facilitate GTP hydrolysis by Go proteins (19–21). Independent from the actions of these RGS proteins, certain effectors in the G protein-coupled receptor system modulate GTP hydrolysis by Go proteins acting as GAP or guanine nucleotide exchanging factor (GEF) (22–26). For ex-
ample, PLCβ (22–24) and the γ subunit of cGMP phosphodiesterase (25) directly accelerates GTP hydrolysis by Goα and Goγ, respectively. A recent study by Scholich et al. (26) has shown that adenyl cyclase facilitates GTP binding to Goα, thereby functioning as both GEF and GAP. These findings indicate that the effector molecules modulate their cognate GTPases to terminate or facilitate the signals.

To date, none of the known heterotrimeric G proteins stimulates PLCβ1 (1, 27), and the mechanisms that regulate PLCβ1 activity remain complex and unclear. To further understand the characteristics and the interaction of PLCβ1 with TGG and its activation by TGG and the α1βAR, we investigated the roles of PLCβ1 in the modulation of TGG activities, including the α1βAR. Here, we report a distinct role of PLCβ1 in a coupling system involving the α1βAR and TGG. PLCβ1 displays two regulatory functions for TGG. One is a GEF function, and the other is the inhibition of GTP hydrolysis by TGG. The GEF function of PLCβ1 promotes the α1βAR-mediated GTP binding by TGG. Furthermore, our results also reveal that PLCβ1 is primarily activated by the activation of the α1βAR through TGG, resulting in Ca2+ release from intracellular stores.

**EXPERIMENTAL PROCEDURES**

**Materials**—Fura 2-AM was obtained from Texas Fluorescence Laboratories (Austin, TX), and G418 was obtained from Life Technologies, Inc. Radioactive materials including [α-32P]GTP (3000 Ci/mmol), [γ-32P]GTP (3000 Ci/mmol), [35S]GTP-S (1300 Ci/mmol), [3H]GDP (25–50 Ci/mmol), [3H]prazosin (79.8 Ci/mmol), and [3H]propranolol (37.5 Ci/mmol) were purchased from PerkinElmer Life Sciences. Heparinagarose, wheat germ agglutinin-agarose, GTP-agarose, guinea pig liver TGase, hygromycin, and N,N′-dimethylacelain, phospholipids were purchased from Sigma. A monoclonal TGG antibody CUB7402 was obtained from NeoMarkers ( Freemont, CA). A monoclonal PLC-β antibody was purchased from Upstate Biotechnology Inc. (Lake Forest, NY), and a polyclonal Goα11 antibody was from BIOMOL (Plymouth Meeting, PA). Fast Mono-Q Sepharose was obtained from Amersham Pharmacia Biotech. Nitrocellulose membrane BA85 was from Schleicher & Schuell. Norit A charcoal was from Serva (New York, NY).

**Purification of Proteins**—PLCβ1 was expressed in DH5α cells and purified as described (2). The purity of the PLCβ1 preparation was ≥95% as judged by silver staining, and the specific activity of the Norit A-charcoal-purified PLCβ1 was extracted with 1% sodium dodecyl sulfate (SDS) with boiling for 20 min and stored at −80 °C until use. Sf9 cells were infected with baculovirus (the virus was kindly provided by Dr. Elliott Ross at Northern Texas Laboratories (Austin, TX)) with DDT1-MF2 using LipofectAMINE method provided by the manufacturer. Nitrocellulose membrane BA85 was from Schleicher & Schuell.

**Modulation of Goα Function by PLCβ1**

The binding of Goα to TGG was determined by measurement of [32P]GTP released from intracellular stores. For each experiment, the protein preparation was incubated with [32P]GTP (100,000 cpm/nM) in the assay buffer. [32P]GTP-released by the interaction of Goα and TGG was determined by the nitrocellulose filter method (29).

**Preparation of Radiolabeled Guanine Nucleotide-bound TGG**—A complex of [γ-32P]GTP-TGG was prepared by incubating TGG (50 nM) with 50 μM [γ-32P]GTP (100,000 cpm/nM) in 300 μl of the assay buffer. After incubation at room temperature for 20 min, unbound [γ-32P]GTP and [32P]GMP, was removed by a dried Sephadex G-25 column which was preequilibrated with the assay buffer. The amount of [γ-32P]GTP-TGG complex was determined by a nitrocellulose membrane filter method (29). The standard GTPase activity was also performed by charcoal adsorption method (29).

**Measurement of GTP Hydrolysis**—Since we have found that GTP hydrolysis by TGG is temperature-sensitive, the reaction was performed at room temperature. Single turnover GTP hydrolysis was determined with the [γ-32P]GTP-TGG complex (1 nmol/tube) preparation. The complex was mixed with and without 4 μM PLCβ1 or with 4 μM heat-inactivated PLCβ1 (boiled for 20 min) in the assay buffer. At time 0, 100 μl cold GTP was added to prevent the rebinding of radiolabeled guanine nucleotide. At the indicated time, the samples were transferred to an ice-water bath and the amount of [γ-32P]GTP-TGG remaining was determined by the nitrocellulose filter method.

**Measurement of TGG Activity**—After preincubation of TGG (4 μM) with various concentrations (0–10 nM) of TGG, the TGG activity was measured in the presence of 1 μM α1βAR. The activity of TGG was determined by dephosphorylation of the 35S-labeled Goα11 antibody. A single band corresponding to the 35S-labeled Goα11 antibody was detected with autoradiography.

**Measurement of PLC Activity**—PLC activity was measured by measurement of the amounts of GTP hydrolysis, which was determined by the nitrocellulose filter method (29).

**Measurement of GTPase Activity**—GTPase activity was determined by measurement of the amount of GTP hydrolyzed by Goα. After incubation at room temperature for 20 min, the amount of unbound [γ-32P]GTP remaining was determined by charcoal adsorption method (29). The standard GTPase activity was also performed by charcoal adsorption method (29).

**Measurement of GTPase Activity**—Since we have found that GTP hydrolysis by TGG is temperature-sensitive, the reaction was performed at room temperature. Single turnover GTP hydrolysis was determined with the [γ-32P]GTP-TGG complex (1 nmol/tube) preparation. The complex was mixed with and without 4 μM PLCβ1 or with 4 μM heat-inactivated PLCβ1 (boiled for 20 min) in the assay buffer. At time 0, 100 μl cold GTP was added to prevent the rebinding of radiolabeled guanine nucleotide. At the indicated time, the samples were transferred to an ice-water bath and the amount of [γ-32P]GTP-TGG remaining was determined by the nitrocellulose filter method.

**Measurement of TGG Activity**—After preincubation of TGG (4 μM) with various concentrations (0–10 nM) of TGG, the TGG activity was measured in the presence of 1 μM α1βAR. The activity of TGG was determined by dephosphorylation of the 35S-labeled Goα11 antibody. A single band corresponding to the 35S-labeled Goα11 antibody was detected with autoradiography.
Modulation of GTPase Function by PLCδ1

FIG. 1. Modulation of GTPase activity of TGII by PLCδ1. A, hydrolysis of \([\gamma^{32}P]\)GTP by TGII in the presence and absence of PLCδ1. \([\gamma^{32}P]\)GTP-TGII complex (1 nM/tube) was mixed with and without PLCδ1 (4 nM/tube) as detailed under "Experimental Procedures." Boiled PLCδ1 was 4 nm. The reaction was performed at room temperature and stopped by transferring the samples in an ice-water bath. At time 0, amount of \([\gamma^{32}P]\)GTP-TGII was taken as 100%. B, PLCδ1 concentration-dependent GDP release from TGII. \([\gamma^{32}P]\)GDP-TGII (~1 nM) estimated by immunoblotting using TGII antibody was mixed with various concentrations of PLCδ1 (filled circle) or heat-inactivated PLCδ1 (open triangle). The GDP release was determined at 10 °C for 10 min. At time 0, amount of \([\gamma^{32}P]\)GDP-TGII without PLCδ1 was taken as 100%. C, time-dependent GDP release from TGII induced by PLCδ1. An equal amount of TGII (4 nM) and PLCδ1 (filled circle) or Ca2+-PLCδ1 (open square) was reconstituted. Filled triangle indicates \([\gamma^{32}P]\)GDP/TGII alone. The reactions were carried at 10 °C. Amount of \([\gamma^{32}P]\)GDP-TGII at time 0 was taken as 100%. The data present the means ± S.E. from one of the representative experiments in triplicate.

To determine whether PLCδ1 influences exchange of GDP to GTP by TGII, GDP release from TGII was determined (Fig. 1, B and C). To evaluate whether Ca2+-bound or unbound PLCδ1 exhibits this effect on TGII, PLCδ1 preincubated with Ca2+ (Ca2+-PLCδ1) was also tested. A \([\gamma^{32}P]\)GDP-TGII complex was reconstituted with various concentrations of PLCδ1. The results revealed that the GDP release from TGII was accelerated as a function of PLCδ1 concentration (Fig. 1B). The heat-inactivated PLCδ1 was unable to catalyze the \([\gamma^{32}P]\)GDP release, showing the specificity of PLCδ1 action on TGII. Furthermore, the \([\gamma^{32}P]\)GDP release from TGII induced by PLCδ1 was time-dependent, reaching half-maximal \([\gamma^{32}P]\)GDP release within 4 min (Fig. 1C). The samples containing \([\gamma^{32}P]\)GDP-TGII alone or \([\gamma^{32}P]\)GDP-TGII with Ca2+-PLCδ1 showed a slow \([\gamma^{32}P]\)GDP release with a similar rate. The result indicates that Ca2+-unbound PLCδ1 acts as GEF for TGII.

GEF action of PLCδ1 for TGII was further examined by determining GTPγS binding to TGII (Fig. 2, A and B). Consistent with the observations that PLCδ1 stimulated GDP release, the GTPγS binding of TGII was increased as a function of PLCδ1 concentration (Fig. 2A). At a 1:2 ratio of TGII versus PLCδ1, the GTPγS binding to TGII reached a plateau. PLCδ1 alone showed no GTPγS binding activity, indicating that the increased GTPγS binding is caused by the interaction of TGII with PLCδ1. When the GTPγS binding by TGII was determined as a function of incubation time with and without PLCδ1, the GTPγS binding in the presence of PLCδ1 was higher (~3-fold) than TGII alone and reached a maximum within 11 min (Fig. 2B). In the presence of Ca2+-PLCδ1, the GTPγS binding was similar to TGII alone, again demonstrating that Ca2+-PLCδ1 does not stimulate GTPγS binding to TGII. A slight increase of basal GTPγS binding by TGII was also observed in the presence of PLCδ1, probably due to interaction of the enzyme with TGII during preincubation.

It has also been shown that conformational changes in TGII modulate TGII activity, which are induced by the activators
The presence of 100 either TGII or G was taken as 100%. The data present the means ± S.E. from one of the representative experiments in triplicate.

To understand the mechanism of GEF activity of the α1AR versus PLCδ1 for TGII, the α1AR, TGII, and PLCδ1 were reconstituted, and the GTPγS binding activity of TGII was assessed under various conditions (Fig. 4). The α1AR-mediated GTPγS binding to TGII was evident, reaching a plateau within 6 min (Fig. 4A). When PLCδ1 was present, the receptor-mediated GTPγS binding was further increased (~47% at 2 min) and reached a plateau within 4 min. PLCδ1-mediated GTPγS binding to TGII was slow compared with α1AR-mediated GTPγS binding in both the presence and absence of PLCδ1. These data indicate that the α1AR is the prime GEF for TGII and that PLCδ1 functions secondarily. Although TGII alone showed no GTPγS binding at time zero, when the receptor and/or PLCδ1 were present, the basal level of GTPγS binding by TGII was increased. The order of the basal GTPγS binding was α1AR + TGII + PLCδ1 > α1AR + TGII > α1AR + PLCδ1. To further understand the role of PLCδ1, the receptor and TGII were reconstituted with various concentrations of PLCδ1, and GTPγS binding by TGII was determined at 2 and 4 min (Fig. 4B). At the 2-min time point, GTPγS binding was increased as a function of PLCδ1 concentration. At the 4-min
time point, GTP\(_7\)S binding was reached maximum at 1:1 ratio of TGII and PLC\(_8\), and a further increase in the concentration of PLC\(_8\) did not increase the \(\alpha_{1B}\)AR-mediated GTP\(_7\)S binding, probably due to the limited amounts of TGII. When the TGII concentrations were varied at fixed amounts of PLC\(_8\), GTP\(_7\)S binding by TGII was increased as a function of TGII concentration (Fig. 4C). Although maximum coupling efficacy was observed at 1:1 ratio of TGII and PLC\(_8\), a further increase of TGII concentration resulted in a decrease in the coupling efficacy. These results indicate that a level of each component governs the activation of GTP binding to TGII and that the \(\alpha_{1B}\)AR and PLC\(_8\) induce GTPase conformation of TGII in a concerted way. The sequence of conformational changes of TGII would be: basal state of TGII, which can function as GTPase and TGase; the second state, GTPase conformation that is induced by the receptor and can reverse to the basal state; the third state, GTPase conformation induced by the receptor and stabilized by interacting with PLC\(_8\).

Overexpression of PLC\(_8\) Enhances \([Ca^{2+}]\) by Activation of the \(\alpha_{1B}\)AR—The role of PLC\(_8\) in facilitating coupling of the \(\alpha_{1B}\)AR with TGII was further investigated using DDT1-MF2 cells stably expressing PLC\(_8\) without and with wild-type TGII (wtTG) and its mutants (Fig. 5). A TGII mutant (\(\Delta\delta\)TG), which lacks TGase activity by mutation of Cys \(_{277}\) to Ser at TGase active site (37), was utilized to delineate GTPase versus TGase activity of TGII. Moreover, if PLC\(_8\) acts as a stabilizer of GTPase conformation of TGII through its GEF/GHIF activity, wtTG would provide the same result as \(\Delta\delta\)TG does. To evaluate a specific interaction among \(\alpha_{1B}\)AR, TGII, and PLC\(_8\), two TGII mutants were utilized; m3TG in which an \(\alpha_{1B}\)AR interaction site on TGII was mutated (5), and \(\Delta\delta\)TG (\(\Delta\delta\)HG) in which a PLC\(_8\) interaction site was deleted (38). Proteins were highly expressed, and the expression levels were comparable with each other (Fig. 5, A and B). It should be noted that a fast mobility of the m3TG on SDS-PAGE was also observed when the protein was expressed in COS-1 cell (5). The reason is not clearly understood. However, differences in an apparent molecular weight were observed with TGII from different species, indicating that the mobility of TGII on SDS-PAGE is greatly affected by the primary structure of the enzyme (39). The slow mobility of \(\Delta\delta\)TG is expected, because of the deletion of 30 amino acid residues from C terminus (38). The coupling among \(\alpha_{1B}\)AR, TGII, and PLC\(_8\) was assessed by measuring \([Ca^{2+}]\), in a Ca\(_7\)-free buffer (Fig. 5C). The control cells (vector transfection with vectors (pcDNA3.1 and pCEP4) displayed an increase in the level of \([Ca^{2+}]\), in response to activation of the \(\alpha_{1B}\)AR with (-)-epinephrine. The \(\alpha_{1B}\)-agonist-evoked peak increase in \([Ca^{2+}]\), was further increased by \(\sim 59\%\) when PLC\(_8\) (vector plus PLC\(_8\)) was expressed, demonstrating that PLC\(_8\) increases the coupling efficacy of this signaling system. Since the experiments were performed in Ca\(_7\)-free buffer, the increase in \([Ca^{2+}]\), due to the release of Ca\(_7^{2+}\) from an intracellular store that is likely mediated by IP3 formed in response to PLC\(_8\) activation. Expression of wtTG or \(\Delta\delta\)TG resulted in an increase in peak \([Ca^{2+}]\), that was \(\sim 74\%\) and \(\sim 63\%\) greater than that observed in vector-transfected cells, respectively. The cells coexpressing wtTG and \(\Delta\delta\)TG-mediated Ca\(_7^{2+}\) release, because both m3TG- and \(\Delta\delta\)TG-expressing cells showed an increase in peak \([Ca^{2+}]\), which was \(\sim 53\%\) less than the cells expressing wtTG or \(\Delta\delta\)TG. Moreover, the peak \([Ca^{2+}]\), was \(\sim 20\%\) less than the control cell (vector plus PLC\(_8\)), and coexpression of PLC\(_8\) with these mutants did not significantly increase \([Ca^{2+}]\). Although a residual stimulation of Ca\(_7^{2+}\) release in activation of the \(\alpha_{1B}\)AR is most likely due to the incomplete blocking of the interaction among these three proteins, it is also possible that the increase in \([Ca^{2+}]\), in these cells is due to the coupling of the \(\alpha_{1B}\)AR with other G proteins such as the \(G_\alpha\) family of proteins. Preincubation of the cells with the \(\alpha_{1B}\)-antagonist prazosin or nonspecific PLC inhibitor U73122 completely abolished the \(\alpha_{1B}\)-agonist-mediated increase in \([Ca^{2+}]\), (data not shown). To assess endoplasmic reticulum Ca\(_7^{2+}\) content, we treated the cells with thapsigargin (an inhibitor of endoplasmic reticulum Ca\(_7^{2+}\) pump, which stimulates Ca\(_7^{2+}\) release) at the end of the experiments (Fig. 5D). The thapsigargin-induced release of \([Ca^{2+}]\), was the lowest in the cells coexpressing of PLC\(_8\) with wtTG or \(\Delta\delta\)TG and correlated with the amounts of \([Ca^{2+}]\), release induced by the activation of the \(\alpha_{1B}\)AR with the cells expressing TGII and its mutants without or with PLC\(_8\). These data further demonstrate that the increase in \([Ca^{2+}]\), is caused by release from the intracellular Ca\(_7^{2+}\) stores.

To date, an effector protein acting as both GEF and GHIF for a GTPase has not been described in either a heterotrimeric or a monomeric GTPase signaling system. Our studies on the roles of PLC\(_8\) in regulation of TGII activities reveal that PLC\(_8\) exhibits GEF and GHIF activities for GTPase function of TGII. Evidence for the GEF function of PLC\(_8\) is that the
enzyme facilitates GDP release from TGII and stimulation of GTP$\gamma$S binding (Figs. 1 and 2). The inhibition of TGase activity by PLC$\delta$1 and the GHIIF activity of the PLC$\delta$1 suggest that the interaction of PLC$\delta$1 with TGII induces and stabilizes GTP$\gamma$S conformation of TGII. The GEF/GHIIF activity of PLC$\delta$1 displays independently from the $\alpha_{1\beta}$AR. However, when the $\alpha_{1\beta}$AR is present, the receptor is the prime GEF (Fig. 4). This conclusion is based on the observations that (i) the $\alpha_{1\beta}$AR-mediated GTP$\gamma$S binding is not additively enhanced in the presence of PLC$\delta$1, (ii) PLC$\delta$1 increases the rate of GTP$\gamma$S binding mediated by the receptor, and (iii) PLC$\delta$1-mediated GTP$\gamma$S binding to TGII is slow as compared with that mediated by the $\alpha_{1\beta}$AR.

The observation that overexpression of PLC$\delta$1 results in elevation of the $\alpha_{1\beta}$AR-mediated Ca$^{2+}$ release from the intracellular Ca$^{2+}$ stores is consistent with findings that PLC$\delta$1 is the effector in TGII-mediated signaling pathway (2–4, 40). Furthermore, overexpression of wtTG and C-STG substantially enhances the $\alpha_{1\beta}$AR-mediated Ca$^{2+}$ release, and the TGII mutants m3TG and $\Delta$$\delta$1TG greatly reduce the level of the $\alpha_{1\beta}$AR-evoked Ca$^{2+}$ release with or without overexpression of PLC$\delta$1. Interestingly, the increase in [Ca$^{2+}$]$_i$ was somewhat small when PLC$\delta$1 was coexpressed with wtTG or C-STG (Fig. 5C). Although the reason for the limited Ca$^{2+}$ release is probably due to the limited number of cognate receptors, other mechanisms may be involved. Thus, the PLC$\delta$1 activity is positively and negatively regulated by TGII depending on the Ca$^{2+}$ level, expression level of PLC$\delta$1, and binding of guanine nucleotides (3, 11, 12). PLC$\delta$1 can also be inhibited by its metabolite IP$_3$ (13–15). Since coexpression of TGII with PLC$\delta$1 increases basal IP$_3$ formation (5), all of these factors would reduce the interaction capability of PLC$\delta$1 with TGII. There were no significant differences in peak [Ca$^{2+}$]$_i$ between wtTG- and C-STG-expressing cells, indicating that at the initiation of coupling of these three molecules, the increased Ca$^{2+}$ level in cell does not affect the GTP binding by TGII. These results also support the findings that Ca$^{2+}$-unbound PLC$\delta$1 is GEF for TGII (Figs. 1C and 2B). Our results also indicate that PLC$\delta$1 is catalytically activated by GTP-TGII, since the level of the endogenous PLC$\delta$1 is sufficient to increase [Ca$^{2+}$]$_i$, maximally when wtTG as well as C-STG was highly expressed (Fig. 5C).

Effectors such as PLC$\beta$1 and cGMP phosphodiesterase as well as RGS proteins terminate GTPase function to prevent further activation of the effector themselves (19–26). In contrast, PLC$\delta$1 facilitates the signaling involving the $\alpha_{1\beta}$AR and TGII through its GEF/GHIIF activity for TGII when the cognate receptors are present. This novel role of PLC$\delta$1 is probably necessary to promote the production of second messengers and to overcome the nature of its regulation by multiple factors. The coupling efficacy of the $\alpha_{1\beta}$AR with TGII in respect to the activation of the cognate PLC is poor as compared with that with Go$_q$ (7, 8, 12). In addition, two mechanisms for the regulation of PLC$\delta$1 activity have been studied intensely: (i) a supporting role of other Ca$^{2+}$-mobilizing receptor systems and (ii) an effector role in the receptor-TGII coupling system. Our results clearly support the latter mechanism; PLC$\delta$1 is activated at the basal Ca$^{2+}$ level in an intact cell by the $\alpha_{1\beta}$AR through TGII. Operation of these two mechanisms probably depends on whether the cells express the cognate receptors and TGII. It has been reported that PLC$\delta$1 is activated by the capacitative Ca$^{2+}$ entry, following bradykinin stimulation in rat pheochromocytoma (PC-12) cell, which does not express TGII (17). In these regards, further study is required to establish physiological relevance.

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REFERENCES
1. Rhee, S. G., and Bae, Y. S. (1997) J. Biol. Chem. 272, 15045–15048
2. Feng, J.-F., Rhee, S. G., and Im, M.-J. (1996) J. Biol. Chem. 271, 16451–16454
3. Murthy, S. N., Lomasney, J. W., Mak, E. C., and Lorand, L. (1999) Proc. Natl. Acad. Sci. U.S.A. 96, 11148–11153
4. Feng, J.-F., Rhee, S. G., and Im, M.-J. (1996) J. Biol. Chem. 271, 16451–16454
5. Murthy, S. N., Lomasney, J. W., Mak, E. C., and Lorand, L. (1999) Proc. Natl. Acad. Sci. U.S.A. 96, 11148–11153
6. Feng, J.-F., Rhee, S. G., and Im, M.-J. (1996) J. Biol. Chem. 271, 16451–16454
7. Murthy, S. N., Lomasney, J. W., Mak, E. C., and Lorand, L. (1999) Proc. Natl. Acad. Sci. U.S.A. 96, 11148–11153
8. Feng, J.-F., Rhee, S. G., and Im, M.-J. (1996) J. Biol. Chem. 271, 16451–16454
9. Murthy, S. N., Lomasney, J. W., Mak, E. C., and Lorand, L. (1999) Proc. Natl. Acad. Sci. U.S.A. 96, 11148–11153
4. Park, E. S., Won, J.-H., Han, K. J., Suh, P.-G., Ryu, S. H., Lee, H. S., Yun, H.-Y., Kwon, N. S., and Baek, K. J. (1998) Biochem. J. 331, 283–289
5. Feng, J.-F., Gray, C. D., and Im, M.-J. (1999) Biochemistry 38, 2224–2232
6. Im, M.-J., Russell, M. A., and Feng, J.-F. (1997) Cell. Signal. 9, 477–482
7. Nakazaki, H., Perez, D. M., Baek, K. J, Das, T., Husain, A., Misono, K., Im, M.-J., and Graham, R. M. (1994) Science 264, 1593–1596
8. Chen, S., Lin, F., Iismaa, S., Lee, K. N., Birckbichler, P. J., and Graham, R. M. (1996) J. Biol. Chem. 271, 32885–32891
9. Vezza, R., Habib, A., and FitzGerald, G. A. (1999) J. Biol. Chem. 274, 12774–12779
10. Small, K., Feng, J.-F., Lorenz, J., Donnelly, E. T., Yu, A., Im, M.-J., Dorn, G. W., II, and Liggett, S. B. (1999) J. Biol. Chem. 274, 21291–21296
11. Das, T., Baek, K. J., Gray, C., and Im, M.-J. (1993) J. Biol. Chem. 268, 27390–27397
12. Zhang, J., Tucholski, J., Lesort, M., Jope, R. S., and Johnson, G. V. (1999) Biochem. J. 343, 541–549
13. Lemmon, M. A., Ferguson, K. M., O’Brien, R., Sigler, P. B., and Schlessinger, J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10472–10476
14. Kanematsu, T., Takeya, H., Watanabe, Y., Ozaki, S., Yoshida, M., Koga, T., Iwanaga, S., and Hirata, M. (1992) J. Biol. Chem. 267, 6518–6525
15. Cifuentes, M. E., Delaney, T., and Rebecchi, M. J. (1994) J. Biol. Chem. 269, 1945–1948
16. Allen, V., Swigart, P., Cheung, R., Cockroft, S., and Katan, M. (1997) Biochem. J. 327, 545–552
17. Kim, Y.-H., Park, T.-J., Lee, Y. H., Baek, K. J., Suh, P.-G., Ryu, S. H., and Kim, K.-T. (1999) J. Biol. Chem. 274, 26127–26134
18. Hepler, J. R. (1999) Trends Pharmacol. Sci. 20, 376–382
19. Berstein, G., Blank, J. L., Jhon, D. Y., Exton, J. H., Rhee, S. G., and Ross, E. M. (1992) Cell 70, 411–418
20. Biddlecome, G. H., Berstein, G., and Ross, E. M. (1996) J. Biol. Chem. 271, 7999–8007
21. Chidiae, P., and Ross, E. M. (1999) J. Biol. Chem. 274, 19639–19643
22. Arshavsky, V. Y., and Bownds, M. D. (1992) Nature 357, 416–417
23. Scholich, K., Mullenix, J. B., Wittphoth, C., Poppleton, H. M., Pierre, S. C., Linderof M. A., Garrison, J. C., and Patel, T. B. (1999) Science 283, 1328–1331
24. Hepler, J. R., Kozasa, T., Smrcka, A. V., Simon, M. I., Rhee, S. G., Sternweis P. C., and Gilman, A. (1993) J. Biol. Chem. 268, 14367–14375
25. Feng, J.-F., Readon, M., Yadav, S. P., and Im, M.-J. (1999) Biochemistry 38, 10743–10749
26. Im, M.-J., Riek, R. P., and Graham, R. M. (1990) J. Biol. Chem. 265, 18952–18960
27. Berman, D. M., and Gilman, A. G. (1998) J. Biol. Chem. 273, 1269–1272
28. Hepler, J. R., Kozasa, T., Smrcka, A. V., Simon, M. I., Rhee, S. G., Sternweis P. C., and Gilman, A. (1993) J. Biol. Chem. 268, 14367–14375
29. Feng, J.-F., Readon, M., Yadav, S. P., and Im, M.-J. (1999) Biochemistry 38, 10743–10749
30. Iismaa, S. E., Chung, L., Wu, M. J., Teller, D. C., Yee, V. C., and Graham, R. M. (1997) Biochemistry 36, 11655–11664
31. Im, M.-J., Gray, C., and Rim, A. J. (1993) J. Biol. Chem. 267, 8887–8894
32. Castellani, S., Schwinn, D. A., Randall, R. R., Lefkowitz, R. J., Caron, M. G., and Kohlika, B. K. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 7159–7163
33. Xu, Y., Zhu, K., Kong, H., Wu, W., Baudhuin, L. M., Xiao, Y., and Damron, D. S. (2000) Nat. Cell Biol. 2, 261–267
34. Grynkiewicz, G., Poenie, M., and Yisrael, R. Y. (1985) J. Biol. Chem. 260, 3440–3450
35. Monsonego, A., Friedmann, I., Shani, Y., Eisenstein, M., and Schwartz, M. (1990) J. Mol. Biol. 202, 713–720
36. Achyuthan, K. E., and Greenberg, C. S. (1987) J. Biol. Chem. 262, 1901–1906
37. Lee, K. N., Arnold, S. A., Birckbichler, P. J., Patterson Jr, M. K., Fraij, B. M., Takekuchi, Y., and Carter, H. A. (1993) Biochim. Biophys. Acta 1202, 1–6
38. Hwang, K. C., Gray, C. D., Sivasubramanian, N., and Im, M.-J. (1995) J. Biol. Chem. 270, 27058–27062
39. Baek, K. J., Das, T., Gray, C., Antar, S., Murugesan, G., and Im, M.-J. (1993) J. Biol. Chem. 268, 27390–27397
40. Wu, J., Liu, S.-L., Zhu, J.-L., Norton, P. A., Nojiri, S., Hoek, J. B., and Zern, M. A. (2000) J. Biol. Chem. 275, 22213–22219
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