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Potent Activation of RhoA by $G\alpha_q$ and G_q-coupled Receptors*

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Heterotrimeric G proteins of the G_i, G_s, and G_q family control a wide array of physiological functions primarily by regulating the activity of key intracellular second messenger-generating systems. α subunits of the G_{12} family, $G\alpha_{12}$ and $G\alpha_{13}$, however, can promote cellular responses that are independent of conventional second messengers but that result from the activation of small GTP-binding proteins of the Rho family and their downstream targets. These findings led to the identification of a novel family of guanine-nucleotide exchange factors (GEFs) that provides a direct link between $G\alpha_{12/13}$ and Rho stimulation. Recent observations suggest that many cellular responses elicited by $G\alpha_q$ and its coupled receptors also require the functional activity of Rho. However, available evidence suggests that $G\alpha_q$ may act on pathways downstream from Rho rather than by promoting Rho activation. These seemingly conflicting observations and the recent development of sensitive assays to assess the *in vivo* levels of active Rho prompted us to ask whether $G\alpha_q$ and its coupled receptors can stimulate endogenous Rho. Here we show that the expression of activated forms of $G\alpha_q$ and the stimulation of G_q-coupled receptors or chimeric $G\alpha_q$ molecules that respond to G_i-linked receptors can promote a robust activation of endogenous Rho in HEK-293T cells. Interestingly, this response was not prevented by molecules interfering with the ability of $G\alpha_{13}$ to stimulate its linked RhoGEFs, together suggesting the existence of a novel molecular mechanism by which $G\alpha_q$ and the large family of G_q-coupled receptors can regulate the activity of Rho and its downstream signaling pathways.

G protein-coupled receptors (GPCRs)[1] represent the largest family of cell surface molecules involved in signal transmission. They owe their name to their extensively studied interaction with heterotrimeric G proteins (α, β, and γ subunits), which undergo conformational changes that lead to the exchange of GDP for GTP bound to the α subunit upon receptor activation. Consequently, GTP-bound Gα subunits of the $G\alpha_i$, $G\alpha_s$, $G\alpha_q$, and $G\alpha_{12}$ family and free $G_{\beta\gamma}$ subunits stimulate a variety of effector molecules thereby activating or inhibiting key second messenger-generating systems (1). However, recent evidence suggests that many cellular responses elicited by activation of heterotrimeric G proteins are not mediated by classical second messengers, but they involve not yet fully understood molecular mechanisms that result in the activation of small GTP-binding proteins of the Ras and Rho families. For example, it was observed that activated forms of $G\alpha_{12}$ and $G\alpha_{13}$ promote stress fiber formation, the assembly focal adhesions, the transcriptional activation of the serum responsive factor, and cellular transformation through Rho-dependent pathways without affecting conventional second messengers (2–4). These observations prompted the study of the underlying molecular mechanisms by which $G\alpha_{12/13}$ activate Rho. In this regard, recent work has revealed that a novel family of guanine-nucleotide exchange factors (GEFs) for Rho, which includes p115RhoGEF, PDZ-RhoGEF, and LARG, can provide a direct link between $G\alpha_{12/13}$ proteins and Rho activation (5–7).

Recent evidence suggests that cell surface receptors linked to the G_q family of heterotrimeric G proteins can also activate signaling routes that are dependent on the functional activity of Rho. For example, it was observed that $G\alpha_q$ and its coupled receptors stimulate the transcriptional activity of the serum response factor and that molecules inhibiting Rho can prevent this response (3, 4). However, activation of the SRE can be provoked by other members of the Rho family of GTPases such as Rac and Cdc42, which limits the specificity of this experimental approach (8). Furthermore, in some cellular systems $G\alpha_q$ has been linked to Rac activation (9) but not to Rho (9–11), which led to the hypothesis that $G\alpha_q$ may act on pathways downstream from Rho rather than by promoting Rho activation (10). These seemingly conflicting results and the recent development of highly sensitive methods to detect endogenous levels of the GTP-bound form of Rho (12, 13) prompted us to ask whether $G\alpha_q$ and its coupled receptors can promote Rho activation. We provide evidence that in HEK-293T cells the stimulation of G_q-coupled receptors as well as the expression of activated forms of $G\alpha_q$ and chimeric $G\alpha_q$ molecules engineered to be stimulated by G_i-coupled receptors can promote a robust activation of endogenous Rho. Moreover, activation of Rho-dependent pathways by G_q-coupled receptors and a GTPase-deficient $G\alpha_q$ was not prevented by molecules interfering with the ability of $G\alpha_{13}$ to stimulate its linked RhoGEFs, thus suggesting the existence of a novel mechanism by which cell surface receptors that transduce signals through G_q can regulate the activity of Rho.

EXPERIMENTAL PROCEDURES

Expression Plasmids—Expression plasmids for constitutively activated forms of $G\alpha_q$, $G\alpha_{i2}$, $G\alpha_s$, $G\alpha_{12}$, $G\alpha_{13}$, β and γ subunits of G proteins, m1 and m2 muscarinic receptors, GFP, HA-ERK2, Cdc42QL, C3 toxin, pSREmutL reporter plasmid, and β-galactosidase were described previously (6, 8, 14). The cDNA clones for activated forms of

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[1] The abbreviations used are: GPCR, G protein-coupled receptor; RGS, regulators of G protein signaling; SRE, serum response element; GEF, guanine nucleotide exchange factor; GFP, green fluorescent protein; HA, hemagglutinin; CAT, chloramphenicol acetyltransferase; GST, glutathione S-transferase; RBD, Rho-binding domain; m1, m1 muscarinic receptor; m2, m2 muscarinic receptor; HEK-293T, human embryonic kidney 293T cells; MAP kinase, mitogen-activated protein kinase; ERK, extracellular signal-related kinase.

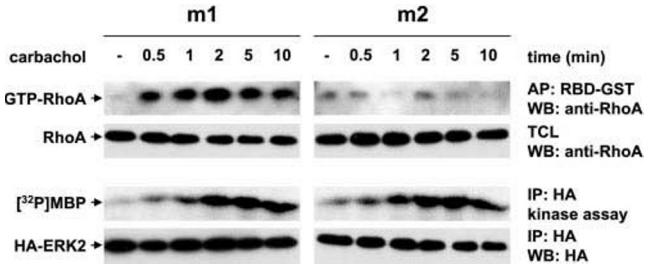

FIG. 1. **Activation of Rho by receptors coupled to G_q but not by those coupled to G_i in HEK-293T cells.** HEK-293T cells transfected with m1 or m2 muscarinic receptors were kept overnight in serum-free conditions, stimulated with carbachol (10^{-4} M) for the indicated times, and lysed. Rho affinity purified (*AP*) by binding to GST-RBD beads and that in each total cell lysate (*TCL*) were analyzed by Western blotting (*WB*) with a monoclonal antibody against RhoA. Cells transfected with an epitope-tagged MAP kinase (*HA-ERK2*) were processed in parallel. Levels of MAP kinase expression and enzymatic activity were determined in the anti-HA immunoprecipitates (*IP*) by Western blot analysis using a monoclonal antibody against HA and in immunocomplex kinase assays, respectively. Autoradiograms are from a representative experiment, which was repeated 3–5 times with similar results. The position of phosphorylated myelin basic protein ([^{32}*P*]*MBP*) and active forms of Rho (*RhoA*) are indicated.

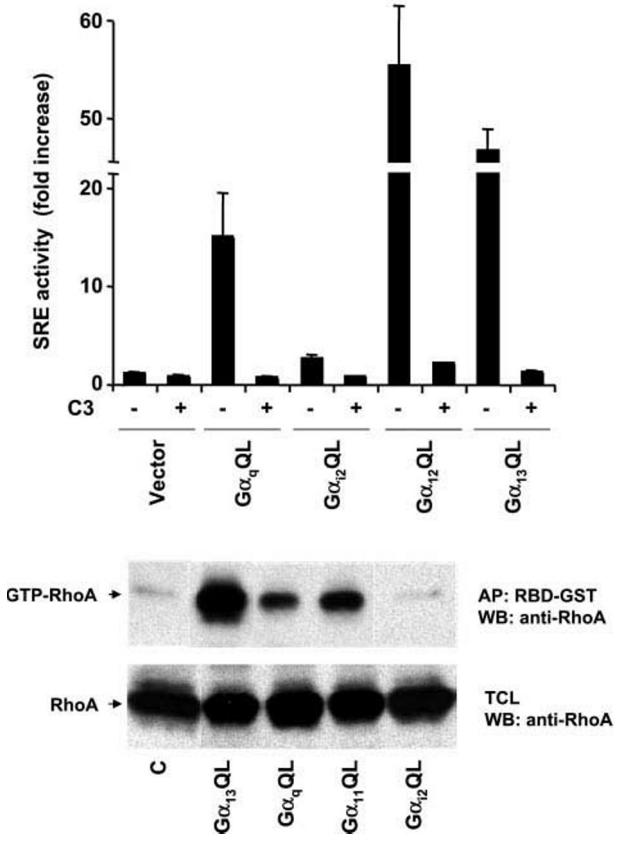

FIG. 2. **Activation of Rho by $G\alpha_q$.** *Upper panel*, HEK-293T cells were co-transfected with pSREmutL and pcDNA3-β-galactosidase in the absence (-) or presence (+) of a plasmid expressing the botulinum C3 toxin (*C3*) together with the indicated expression vectors. Cells were processed as described under "Experimental Procedures." The data represent CAT activity normalized by the β-galactosidase activity present in each cellular lysate, expressed as -fold increase relative to that observed in control, and are the mean ± S.E. of triplicate samples from a typical experiment. Similar results were obtained in three independent experiments. *Lower panel*, endogenous levels of active RhoA were determined in HEK-293T cells transfected with the indicated expression plasmids as described above. *WB*, Western blot; *TCL*, total cellular lysate; *C*, control.

human $G\alpha_{14}$ and $G\alpha_{11}$ were obtained from the Guthrie cDNA Resource Center (www.guthrie.org/AboutGuthrie/Research/cDNA). Expression plasmids for $G\alpha_q$i5 and $G\alpha_{13}$i5 chimeric proteins, in which 5 amino acids at the C terminus of corresponding $G\alpha$ proteins were replaced by the corresponding sequence of $G\alpha_{i2}$, have been described (13, 15). The plasmids expressing AU1-tagged PDZ-RhoGEF, AU1-tagged LARG, AU1-tagged p115RhoGEF, and their deletion mutants have been described previously (6, 7, 13) or generated by polymerase chain reaction amplification using their full-length plasmids as a template. An inactive Lbc/Brx mutant was prepared by the QuikChange™ mutagenesis kit (Stratagene) to replace tyrosine 769 for phenylalanine using pRSV-FLAG-Brx (16) as a template. This mutant did not bind or activate Rho (not shown), similar to the results reported for the corresponding mutation, tyrosine 2153 to phenylalanine, in AKAP-Lbc/Brx (11). pGEX expression vector encoding GST fused to the GTP-RhoA-binding domain (RBD) of rhotekin was provided by Dr. S. Narumiya.

Cell Lines, Transfections, Reporter Assays, and Biochemical Analysis—Human embryonic kidney 293T (HEK-293T) cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and transfected using LipofectAMINE Plus TM reagent (Invitrogen) according to the manufacturer's protocol as described (7). The SRE-CAT activity in the cell extracts was determined as described previously using pSREmutL and pcDNA3-β-galactosidase (6, 14). β-Galactosidase activity present in each sample was used to normalize for transfection efficiency. MAP kinase activities in cells transfected with an epitope-tagged MAP kinase (HA-ERK2) were determined as described previously (6) using myelin basic protein (Sigma) as a substrate. Western blots and immunoprecipitations were performed as described (6). The *in vivo* Rho activity was assessed by a modified method as described (12). Briefly, HEK-293T cells were transfected with the indicated plasmids, and after serum starvation for 24 h cells were lysed at 4 °C in a buffer containing 20 mM HEPES, pH 7.4, 0.1 M NaCl, 1% Triton X-100, 10 mM EGTA, 40 mM β-glycerophosphate, 20 mM $MgCl_2$, 1 mM Na_3VO_4, 1 mM dithiothreitol, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride. Lysates were incubated with GST-rhotekin-RBD previously bound to glutathione-Sepharose beads and washed four times with lysis buffer, and associated GTP-bound forms of Rho were released with protein loading buffer and revealed by Western blot analysis using a monoclonal antibody against RhoA (26C4)(Santa Cruz Biotechnology) and enhanced chemiluminescence detection (Amersham Biosciences) using goat anti-mouse (Cappel) IgGs coupled to horseradish peroxidase as a secondary antibody. Antibodies against $G\alpha_{q/11}$ (C-19) were from Santa Cruz Biotechnology. Anti-$G\alpha_{12/13}$ rabbit polyclonal serum was described previously (3). Monoclonal antibodies against AU1 and HA epitopes were purchased form COVANCE.

RESULTS AND DISCUSSION

Receptors Coupled to G_q but Not Those Coupled to G_i Stimulate Rho in HEK-293T Cells—Whereas m1 receptors are typical of those coupled through G proteins of the G_q family to phospholipase-C activation, m2 is known to couple through G_i to a number of effector pathways including the inhibition of adenylyl cyclases. Thus, we used the expression of these muscarinic receptor subtypes as a model system to examine whether G_q- and G_i-coupled receptors can stimulate Rho in HEK-293T cells. For these experiments, cells transfected with m1 and m2 receptors were stimulated with the cholinergic agonist carbachol, and cellular lysates were incubated with GST fusion protein including the RBD of rhotekin previously bound to glutathione-Sepharose beads. The levels of the GTP-bound form of Rho associated with GST-rhotekin-RBD were quantified by Western blot analysis using an anti-Rho antibody. As shown in Fig. 1, a detailed time course analysis of Rho stimulation by carbachol revealed the rapid and potent activation of Rho in m1-transfected cells, which was demonstrable as soon as 30 s after agonist addition and was attenuated within 10 min but remained above basal levels for at least 1 h (data not shown). In contrast, activation of m2 receptors did not result in Rho activation, although it stimulated ERK2 as potently as when elicited by m1 receptors, as previously reported (17), which served as an internal control. Thus, in HEK-293T cells G_q- but not G_i-coupled receptors can provoke a remarkable activation of Rho.

Activated Forms of $G\alpha_q$ Stimulate Rho—To begin exploring

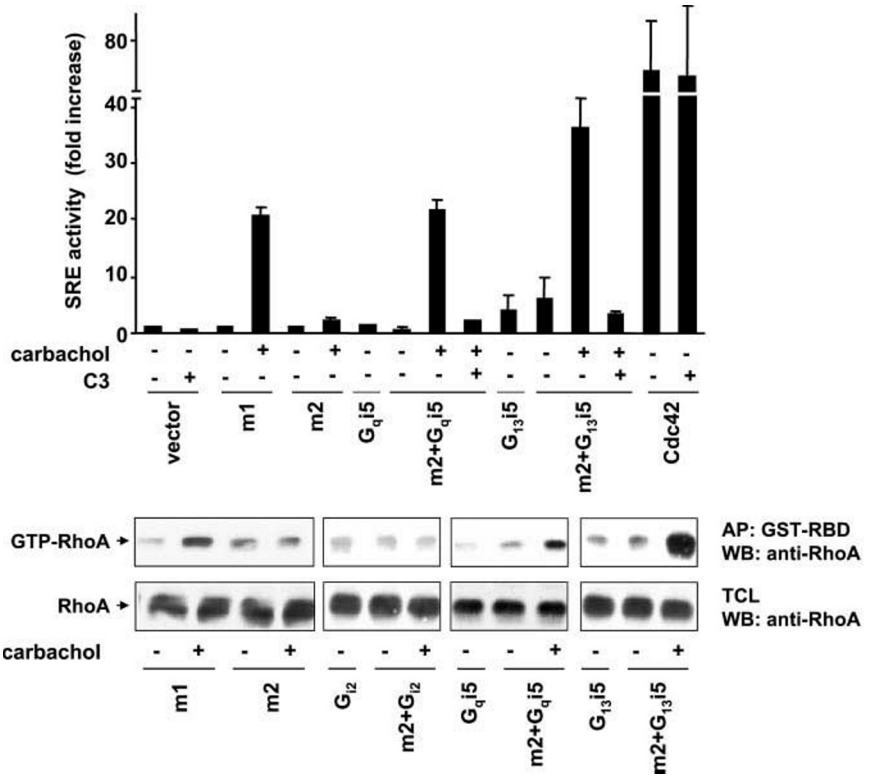

FIG. 3. **Activation of Rho by chimeric $G\alpha_q$ and $G\alpha_{13}$ proteins that can be stimulated by $G\alpha_i$-coupled receptor.** *Upper panel*, HEK-293T cells were co-transfected with pSREmutL and pCDNA3-β-gal in the absence (−) or presence (+) of a plasmid expressing the botulinum C3 toxin (*C3*) together with vector control or expression plasmids for the active form of Cdc42 (*Cdc42-QL*), m1 receptors, m2 receptors alone or in combination with $G\alpha_{i2}$, a $G\alpha_q$i5 chimera, and a $G\alpha_{13}$i5 chimera, as indicated. Cells were processed as described under "Experimental Procedures." The data represent CAT activity normalized by the β-galactosidase activity present in each cellular lysate, expressed as -fold increase relative to that observed in control cells, and are the mean ± S.E. of triplicate samples from a typical experiment. *Lower panel*, endogenous levels of active RhoA were determined in HEK-293T cells transfected with the indicated expression plasmids with (+) or without (−) stimulation with carbachol (10^{-4} M) for 3 min. Total Rho in each lysate and its active form was determined as described above. *WB*, Western blot; *TCL*, total cellular lysate.

whether G_q itself can activate Rho, we first asked whether in HEK-293T cells activated forms of $G\alpha_q$ can stimulate expression from a reporter plasmid containing a mutated SRE that eliminated the ternary complex factor-binding site and that has been shown to be potently activated by Rho (8). As shown in Fig. 2*A*, the constitutively active GTPase-deficient form $G\alpha_q$ ($G\alpha_q$-QL) activated this mutant SRE potently. Similar results were observed using activated forms of $G\alpha_{11}$, $G\alpha_{14}$, and $G\alpha_{16}$ (data not shown). Under identical conditions, activated forms of $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, and $G\alpha_s$ or overexpression of $G_{\beta\gamma}$ subunits did not induce a significant stimulation of the mutant SRE (Fig. 2, *upper panel* and data not shown), whereas activated forms of $G\alpha_{12}$ and $G\alpha_{13}$ provoked a remarkable response. These observations indicated that the ability to stimulate SRE in HEK-293T cells is specific for members of the $G\alpha_q$ and $G\alpha_{12}$ family of heterotrimeric G proteins. To explore whether Rho mediates this effect, we took advantage of the finding that the botulinum toxin C3 ADP-ribosylates Rho, thus preventing its activation (18). As shown in Fig. 2*A*, co-transfection with a C3 toxin expression plasmid abolished the stimulation of CAT activity by the activated G protein α subunits and abolished the transcriptional response provoked by m1 receptors but did not affect the SRE stimulation by an active Cdc42, which was used as a control (see below), together suggesting that G_q-coupled receptors and activated forms of $G\alpha_q$ can stimulate Rho-dependent pathways in HEK-293T cells. In line with these results, cells expressing active forms of $G\alpha_q$ and $G\alpha_{11}$ displayed an increased level of Rho in the GTP-bound form, albeit to a lesser extent than that provoked by $G\alpha_{13}$, without affecting the level of expression of Rho (Fig. 2, *lower panel*).

The Use of Chimeric α Subunits Indicates That Coupling to the G_q and $G_{12/13}$ Families of Heterotrimeric G Proteins Is Sufficient to Signal to Rho—Because persistent expression of activated $G\alpha$ subunits may promote secondary effects on intracellular signaling pathways, we took advantage of the finding that G_q/G_i chimeras, in which a C-terminal region of $G\alpha_q$ is replaced by the corresponding region of $G\alpha_i$, can be stimulated by G_i-coupled receptors and are able to transmit G_q-mediated signaling pathways (15). Thus, upon co-expression of this G_q/G_i chimera with a G_i-coupled receptor, such as an m2 receptor, on-and-off G_q-mediated signaling can now be controlled by agonist addition. As a control, we also designed a similar G_{13}/G_i chimera in which the C-terminal 5 amino acids of $G\alpha_{13}$ were replaced by the corresponding sequences of $G\alpha_i$. Expression of these proteins was confirmed by Western blot analysis (data not shown). Initially, we examined the ability of these chimeric G proteins to stimulate the transcriptional activation of the SRE-containing reporter plasmid. Activation of m2 receptors with carbachol did not activate the SRE (4), but transcription from the SRE was potently increased by m1 receptor stimulation (Fig. 3*A*). Exposure to carbachol of cells co-expressing m2 receptors with either the G_q/G_i or G_{13}/G_i chimera caused a remarkable SRE activation (Fig. 3, *upper panel*), whereas the overexpression of $G\alpha_{i2}$ did not affect the SRE activity (not shown). The ability to stimulate SRE-dependent transcription was abolished by co-transfection with C3 toxin, suggesting that these chimeric G proteins can stimulate Rho-dependent pathways. We then used this system to investigate whether $G\alpha_q$ and $G\alpha_{13}$ can stimulate Rho. As shown in Fig. 3, *lower panel*, stimulation with carbachol did not activate Rho in m2-expressing cells alone or when co-expressed with $G\alpha_{i2}$. However, in cells expressing either the G_q/G_i or G_{13}/G_i chimera, m2 receptor stimulation resulted in Rho activation. Together, these results indicate that both the G_q and $G_{12/13}$ classes of heterotrimeric G proteins can link their coupled cell surface receptors to the stimulation of signaling pathways, resulting in the activation of Rho.

$G\alpha_q$ Stimulates Rho by a Mechanism Distinct from That of $G\alpha_{13}$—The functional activity of small GTP-binding proteins of the Rho family is tightly regulated *in vivo* by proteins that control the GDP/GTP-bound state. Whereas GEFs promote the exchange of GDP for GTP, thus activating Rho proteins (19), GTPase-activating proteins increase the low intrinsic rate of GTP hydrolysis of small GTPases and are negative modulators. Many GEFs for Rho, Rac, and Cdc42 have been identified. They all share a 250-amino acid stretch of significant sequence sim-

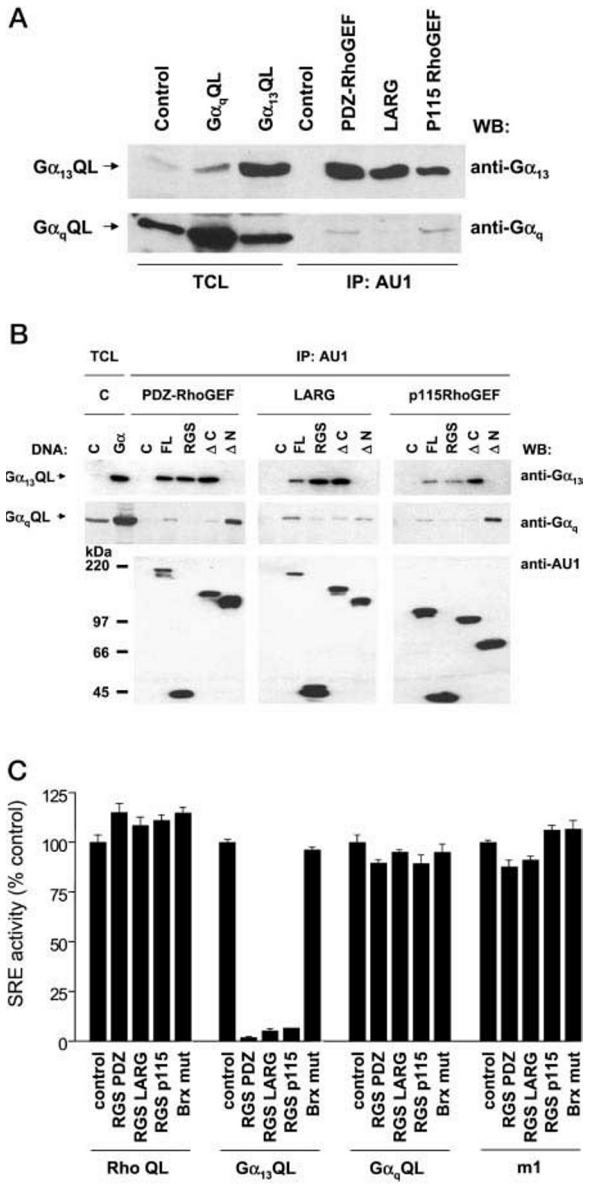

FIG. 4. **Signaling from Gα$_q$ to Rho involves a mechanism distinct from that of Gα$_{12/13}$.** *A*, HEK-293T cells were transfected with expression vector for AU1-tagged forms of GFP (*Control*), PDZ-RhoGEF, LARG, and p115RhoGEF, together with plasmids carrying cDNAs for the constitutively activated mutants of Gα$_{13}$ and Gα$_q$, as indicated. Total cellular lysate (*TCL*) and immunoprecipitates (*IP*) with anti-AU1 antibody were subjected to Western blot (*WB*) analysis using rabbit polyclonal antibodies against Gα$_{12/13}$ or Gα$_q$. *B*, similar analysis was conducted in cells transfected with constitutively activated mutants of Gα$_{13}$ and Gα$_q$ and expression vectors for AU1-tagged forms of the indicated deletion mutants of each RhoGEF, whose expression was confirmed by Western blot analysis of total cell lysates with the anti-AU1 monoclonal antibody. PDZ-RhoGEF-RGS, -ΔC, and -ΔN constructs code for amino acids 126–486, 1–1160, and 702–1523 of PDZ-RhoGEF, respectively; LARG-RGS, -ΔC, and -ΔN constructs code for amino acids 340–580, 1–1218, and 641–1544 of LARG, respectively; p115RhoGEF-RGS, -ΔC, and -ΔN constructs code for amino acids 1–252, 1–800, and 351–912 of p115RhoGEF, respectively. Molecular mass of protein standards is also depicted. Similar results were obtained in three independent experiments. *C*, HEK-293T cells were co-transfected with pSREmutL, pCDNA3-β-galactosidase, and expression vectors for RhoA-QL, Gα$_{13}$-QL, Gα$_q$-QL, and m1 receptors together with expression vectors for the isolated RGS domain from PDZ-RhoGEF, LARG, p115 RhoGEF, or an inactive mutant of Brx/Lbc. m1-transfected cells were stimulated with carbachol for 6 h. Cells were processed as described under "Experimental Procedures." The data represent CAT activity normalized by the β-galactosidase activity present in each cellular lysate, expressed as a percentage relative to that observed in the corresponding control cells co-transfected with GFP, and are the mean ± S.E. of triplicate samples from a typical experiment.

ilarity with Dbl, termed the Dbl homology domain, which is adjacent to a pleckstrin-homology domain (20) and is responsible for nucleotide exchange activity toward GTPases of the Rho family (21). One closely related family of RhoGEFs that includes p115RhoGEF, PDZ-RhoGEF, and LARG, also exhibits an area of homology to a family of proteins known as regulators of G protein signaling (5–7, 22). Recent evidence suggests that this RGS domain provides a structural feature by which G proteins of the Gα$_{12}$ family can bind and activate these RhoGEFs, thereby stimulating Rho (5–7, 22). There is limited information, however, describing how RhoGEFs mediate the activation of Rho by Gα$_q$. For example, in a recent study Gα$_q$ was found to co-immunoprecipitate with a RhoGEF known as Lbc/Brx under low detergent conditions, but this interaction did not result in Rho activation *in vivo* (10). Using a FLAG-tagged Lbc/Brx (16), we did not detect any specific binding to Gα$_q$ using standard immunoprecipitation techniques (data not shown) similar to that observed by others using the full-length AKAP-Lbc/Brx (11). Thus, we next focused our attention on the RGS-containing family of RhoGEFs.

To address the possibility that PDZ-RhoGEF, LARG, and p115RhoGEF may associate with Gα$_q$, we co-transfected expression plasmids for AU1-tagged forms of these RhoGEFs or GFP (control) together with activated forms of Gα$_{13}$ (Gα$_{13}$QL) and Gα$_q$ (Gα$_q$QL). The efficient expression of the transfected constructs was revealed by Western blot analysis (Fig. 4*A* and see below). As shown in Fig. 4*A*, PDZ-RhoGEF, LARG, and p115RhoGEF effectively co-immunoprecipitated with Gα$_{13}$QL. Instead, we detected only a limited but reproducible association with the activated form of Gα$_q$. Because the N-terminal RGS domains of these RhoGEFs are required for the physical interaction of Gα$_{13}$ with these RhoGEFs, we then explored the molecular determinants of this association with Gα$_q$ using truncated forms of PDZ-RhoGEF, LARG, and p115RhoGEF lacking N-terminal or C-terminal regions as well as their isolated RGS domains. As shown in Fig. 4*B*, the isolated RGS domains of these RhoGEFs were sufficient to bind Gα$_{13}$, and deletion of the N-terminal domains abolished the ability of these RhoGEFs to bind Gα$_{13}$. In contrast, the RGS domains of these RhoGEFs did not bind Gα$_q$, and the removal of the N-terminal domains enhanced the binding ability of PDZ-RhoGEF and p115RhoGEF. Thus, these RhoGEFs may interact physically with Gα$_q$ using distinct molecular mechanisms from those by which they interact with Gα$_{13}$.

Because we cannot rule out the possibility that G$_q$ and its coupled receptors may cause the direct or indirect activation of G$_{12}$ and G$_{13}$, we took advantage of the observation that the RGS domains of PDZ-RhoGEF, LARG, and p115RhoGEF can effectively bind Gα$_{13}$ but not Gα$_q$ to ask whether the overexpression of these RGSs can affect the transcriptional response elicited downstream from G$_q$. As expected, expression of these RGS domains abolished the ability of Gα$_{13}$ to stimulate the SRE without affecting the effect of Rho (Fig. 4*C*). In contrast, the RGS domains did not affect the activation of SRE by Gα$_q$ or m1, which further supports the emerging notion that Gα$_q$ can stimulate Rho by a distinct mechanism that is independent of the functional activity of the G$_{12/13}$ family of heterotrimeric G proteins.

Together, our study indicates that receptors transmitting signals through G$_q$ can promote Rho activation, thereby initiating the activity of intracellular pathways controlled by Rho. Similarly, we have recently observed that Gα$_q$ and G$_q$-coupled receptors can potently stimulate the Rho-related GTPase Rac1.[2] The molecular mechanism by which Gα$_q$ stimulates

[2] J.-M. Servitja, H. Chikumi, J. Vázquez-Prado, H. Miyazaki, and J. S. Gutkind, unpublished observation.

these Rho GTPases, however, is yet to be fully defined. In this regard, neither the stimulation of protein kinase C (PKC) by phorbol esters promoted Rho activation alone or in combination with agents elevating intracellular levels of calcium nor the blockade of PKCs by a variety of PKC inhibitors affected the ability of G_q-coupled receptors to stimulate Rho (data not shown). Current genomic efforts have now revealed the existence of a large number of RhoGEFs. Among them, those RhoGEFs containing an RGS domain represent good candidates to mediate $G\alpha_q$ function as they do for $G\alpha_{12}$ and $G\alpha_{13}$. Indeed, we noticed that $G\alpha_q$ may interact with these RhoGEFs, albeit to a very limited extent, and in contrast to $G\alpha_{12/13}$ removal of the N-terminal region that includes the RGS domain may enhance the association of these GEFs with $G\alpha_q$. However, the isolated Dbl-pleckstrin homology (DH-PH) or C-terminal domains did not bind $G\alpha_q$ (data not shown), suggesting that this association may require both regions, and was diminished by the presence of an intact N terminus. Whether the interaction between $G\alpha_q$ and this family of RhoGEFs has functional consequences or whether other members of the extended family of RhoGEFs can mediate the activation of Rho by $G\alpha_q$ is under current investigation.

The finding that G proteins of the G_q family can promote the activation of Rho may explain some of the remarkable biological effects of G_q and its coupled receptors, such as the ability to regulate gene expression, hypertrophy, and normal and cancerous growth (23, 24), which can only be partially explained by the activation of second messenger-generating systems. Furthermore, receptors coupled to G_q often are overexpressed in a variety of tumors where they are activated by ligands in an autocrine fashion (25). Because persistent activation of Rho often has been linked to aberrant cell growth, the ability to stimulate Rho now provides a likely mechanism by which G_q-coupled receptors may participate in tumor progression. In turn, these findings suggest that Rho and its downstream signaling molecules can be considered suitable molecular targets for therapeutic intervention in cancer as well as in many other disease states that involve the persistent activation of receptors linked to G_q.

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