The Guanine Nucleotide Exchange Factor p63RhoGEF, a Specific Link between \( G_{q/11} \)-coupled Receptor Signaling and RhoA*

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The monomeric GTPase RhoA, which is a key regulator of numerous cellular processes, is activated by a variety of G protein-coupled receptors, through either \( G_{12} \) or \( G_{q/11} \) receptors. Here we report that p63RhoGEF, a recently identified RhoA-specific guanine nucleotide exchange factor, enhances the Rho-dependent gene transcription induced by agonist-stimulated \( G_{q/11} \)-coupled receptors (\( M_3 \)-cholinoceptor, histamine \( H_1 \) receptor) or GTPase-deficient mutants of \( G_\alpha_q \) and \( G_\alpha_i \). We further demonstrate that active \( G_\alpha_q \) or \( G_\alpha_{11} \), but not \( G_\alpha_{12} \) or \( G_\alpha_{13} \), strongly enhances p63RhoGEF-induced RhoA activation by direct protein-protein interaction with p63RhoGEF at its C-terminal half. Moreover, the activation of p63RhoGEF by \( G_\alpha_{11} \) occurs independently of and in competition to the activation of the canonical \( G_{q/11} \) effector phospholipase C \( \beta \). Therefore, our results elucidate a new signaling pathway by which \( G_{q/11} \)-coupled receptors specifically induce Rho signaling through a direct interaction of activated \( G_{q/11} \) subunits with p63RhoGEF.

The Rho GTPase family belongs to the Ras superfamily and comprises more than 20 distinct proteins. The best characterized members (RhoA, Rac1, and Cdc42) were first identified in the early 1990s as regulators of actin cytoskeleton rearrangements. RhoA, Rac1, and Cdc42 induce stress fiber, lamellipodia, and filopodia formation, respectively (1). Meanwhile, it became evident that Rho family proteins play a pivotal role in a variety of cellular processes, including secretion, smooth muscle contraction, migration, neurite retraction, and gene transcription (2, 3). As monomeric GTPases, Rho proteins cycle between an inactive GDP-bound and an active GTP-bound state. The activation step, i.e. the exchange of GDP by GTP, is catalyzed by a group of accessory proteins: the guanine nucleotide exchange factors (GEFs). About 60 different GEFs for Rho family members (RhoGEFs) are described so far. Most of them belong to the Dbl protein family, which share the typical tandem motif consisting of a Dbl homology (DH) and a pleckstrin homology (PH) domain (4, 5). Besides this tandem motif, RhoGEFs often contain one or more additional signal transduction domains, such as SH2, SH3, PDZ, and additional PH domains. Therefore, they often function as molecular bridges between different signal transduction pathways (4, 5).

It is well established that apart from receptor tyrosine kinases, a large variety of G protein-coupled receptors (GPCRs), particularly those coupling to the \( G_{12/13} \) type of heterotrimeric G proteins, are upstream regulators of Rho proteins (6, 7). A family of RhoA-specific GEFs, consisting of p115RhoGEF, PDZ-RhoGEF, and leukemia-associated RhoGEF (LARG), which mediates this activation process, has been identified (8–10). All these proteins contain, in addition to the DH/PH tandem motif, a regulator of G protein signaling (RGS) homology domain for direct interaction with and activation by \( G_{12/13} \) type G proteins. Recently, clear evidence has been provided that \( G_\alpha_i \) and \( G_\alpha_{11} \) as well as \( G_{q/11} \)-coupled receptors can induce potent RhoA activation (11–13). This process is apparently independent of phospholipase C \( \beta \) (PLC\( \beta \)) isozymes, the canonical \( G_{q/11} \) effectors, and their downstream signaling, i.e. Ca\(^{2+}\) mobilization and protein kinase C (PKC) activation (11, 13). Although the \( G_{12/13} \)-activated LARG has been reported to additionally mediate \( G_\alpha_q \)-induced RhoA activation (13, 14), a GEF specifically and selectively linking \( G_{q/11} \) proteins and \( G_{q/11} \)-coupled receptors to RhoA activation has not been identified so far.

We and others (15, 16) have recently identified a new RhoGEF of 580 amino acids in length and an apparent molecular mass of 63 kDa. It was therefore termed p63RhoGEF. The expression of p63RhoGEF in human heart and brain tissue was confirmed by both groups of investigators using Northern blot analysis and immunohistochemistry, and it was clearly demonstrated that p63RhoGEF activates specifically RhoA but not Rac1 or Cdc42. Sequence analysis revealed that this protein does not contain other distinct functional domains besides the typical DH/PH tandem motif.

As we tried to identify regulatory mechanisms inducing a p63RhoGEF-mediated RhoA activation, we studied the influence of the stimulation of a variety of GPCRs on p63RhoGEF activity. The data presented herein will provide evidence that p63RhoGEF links specifically \( G_{q/11} \)-coupled receptors to RhoA by a direct interaction with GTP-ligated \( G_{q/11} \) proteins. This RhoGEF therefore represents a so far unknown \( G_\alpha_q \) effector molecule.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—The construction of plasmids encoding Myc-tagged p63RhoGEF and its deletion mutants in the pCMV-Tag3B vector was reported before (15). The coding sequences of the \( M_3 \)-cholinoceptor (\( M_3 \)-R) and the histamine \( H_1 \) receptor (\( H_1 \)-R) were subcloned into the eukaryotic expression vector pcDNA3 (Invitrogen). pcDNA3-EE-GQL was from UMR cDNA Resource Center. pcIS-G\( _{\alpha_q} \), pcIS-G\( _{\alpha_R} \), pcIS-

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§ The abbreviations used are: GEF, guanine nucleotide exchange factor; DH, Dbl homology; PH, pleckstrin homology; GST, glutathione S-transferase; GST-RBD, GST fusion protein containing the Rho binding domain of rhotekin; Lac-RGS, RGS homology domain of Lac; SH3, serum-response factor; GPCR, G protein-coupled receptor; LARG, leukemia-associated RhoGEF, PLC, phospholipase C; PKC, protein kinase C; GEF, GTPase-activating protein; \( M_3 \)-R, \( M_3 \)-cholinoceptor; \( H_1 \)-R, histamine \( H_1 \) receptor; RGS, regulator of G protein signaling.
Cell Culture and Transfection—Culture of HEK-293 cells and COS-7 cells and transfection of the cells (250 ng of total DNA/well on a 48-well plate for SRF activation and up to 2 μg of DNA/6-well plate for RhoA pull-down assays) were performed as described before (15). Assays were performed 48 h after transfection in serum-starved cells.

Preparation of RNA and Reverse Transcription-PCR—Total RNA from HEK-293 and COS-7 cells was prepared with RNeasy Minikit (Qiagen, Hilden, Germany). The RNA was transcribed into cDNA using oligo(dT) primers and a first strand synthesis kit (Roche Applied Science). PCR conditions for the amplification of p63RhoGEF cDNA fragment were as follows (50 μl): primer 0.4 μM each, dNTP 0.2 mM, 1.5 mM MgCl2, Taq polymerase plus Q-Solution (Qiagen), and 2 μl of cDNA. 35 cycles (denaturation 95 °C, 30 s, annealing 56 °C, 30 s, elongation 72 °C, 90 s) followed by a final acquisition of 5 min at 72 °C were performed. Primer sequences were as follows: p63RhoGEF forward, 5′-GATGGTTGGATCATCTAAAACA-3′; p63RhoGEF reverse, 5′-GT-TACAGCTATCTTCTTCA-3′. The sequences of these primers are conserved in rat, mouse, and man.

Assay of SRF Activation—HEK-293 cells or COS-7 cells seeded on 48-well plates were co-transfected with the indicated expression plasmids together with the pSRE-L-luciferase reporter plasmid and the pRL-TK control reporter vector. 48 h after transfection, cells were washed once with phosphate-buffered saline and lysed with passive lysis buffer (Promega). Luciferase activities were determined with the Dual-Luciferase reporter assay system (Promega) as described (15, 17). The activity of the experimental reporter was normalized against the activity of the control vector.

Pull-down Assay of Activated RhoA—The cellular level of GTP-loaded RhoA was determined using a GST fusion protein containing the Rho binding domain of rhotekin (GST-RBD) (15, 18). In brief, subconfluent monolayers of HEK-293 cells were transfected with the indicated amounts of plasmid DNA or the corresponding empty vectors using Polyfect (Qiagen) and cultured for 48 h. Thereafter, the cells were lysed in a buffer containing 1% Nonidet P-40, and the particular fraction was pelleted by centrifugation. 1 μg of the GTase-containing supernatant was then incubated for 1 h at 4 °C with 40 μg of GST-RBD (expressed in and purified from Escherichia coli) bound to glutathione-Sepharose beads. After three times washing of the beads, bound proteins were eluted with sample buffer and separated by SDS-PAGE. RhoA was then detected by immunoblotting with a specific monoclonal antibody (Santa Cruz Biotechnology).

Immunoprecipitation of Gα-proteins with p63RhoGEF—HEK-293 cells were seeded in 6-well plates and transfected at a confluence of 80% with 2 μg of the indicated cDNA constructs. 48 h after transfection, the cells were solubilized in 600 μl of immunoprecipitation buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, and 1% Triton X-100). After incubation on ice for 20 min and centrifugation (26,000 g at 4 °C), 2 μg of anti-c-Myc antibody (clone 9E10, Sigma) or polyclonal anti-ε-anti-α-serum (1:150 dilution; Covance) were added to the clear supernatant (300 μg of protein) and incubated for 1 h at 4 °C. After the addition of a 40-μl 1:1 (v/v) slurry of anti-mouse-IgG-agarose conjugate (Sigma) or protein-A-Sepharose (Amersham Biosciences), the mixture was gently shaken for 4 h at 4 °C. Beads were washed three times with Tris-buffered saline, 0.1% Triton X-100, and 1 mM phenylmethylsulfonyl fluoride, and bound proteins were eluted with 25 μl of sample buffer for 5 min at 95 °C. Precipitated proteins were loaded onto a 12% polyacrylamide gel. After SDS-PAGE and transfer to nitrocellulose membranes, immunoprecipitated proteins were detected by immunoblotting with anti-c-Myc (clone 9E10, Sigma) and anti-Gα antibodies (Grahsch Laboratories, Schwabhausen, Germany).

Phospholipase C Assay—For measurement of PLC activity, transfected COS-7 cells seeded on 12-well plates were incubated with 1 of pCis-PLC (Amersham Biosciences) 48 h prior to the assay. Thereafter, the cells were washed once with Hanks’ balanced salt solution followed by the addition of fresh Hanks’ balanced salt solution supplemented with 10 mM LiCl, and if indicated, 1 mM carbachol. After a 30-min incubation at 37 °C, the reactions were stopped, and the formed [3H]inositol phosphates were determined as described (19).

Statistics—Statistical analysis was performed by analysis of variance followed by Tukey’s multiple comparison test. A p value <0.05 was considered significant. Concentration-response curves were analyzed using iterative nonlinear regression analysis (GraphPAD Prism).

RESULTS

Synergistic Activation of Rho-mediated Gene Transcription by p63RhoGEF and Stimulation of Gq11-Coupled Receptors—To study whether p63RhoGEF is activated by Gq11-coupled receptors, we coexpressed p63RhoGEF together with the M3R or the H1R in HEK-293 cells, in which the mRNA encoding p63RhoGEF could be detected by reverse transcription-PCR (Fig. 1A, inset). Both GPCRs preferentially couple to Gq11 proteins but can additionally activate other G proteins (20–22). To monitor RhoA activation in intact cells, we measured transcription of a serum-response element (SRE)-controlled reporter gene (SRE-L-luciferase). Overexpression of p63RhoGEF caused a slight increase in luciferase expression, whereas agonist (carbachol, histamine) activation of the M3R or H1R increased transcriptional activity about 35-fold (Fig. 1A). The combined expression of p63RhoGEF and the respective GPCR led to a strong enhancement of agonist-induced luciferase expression (about 90-fold). As shown in Fig. 1B, the agonist-induced transcriptional activity of the M3R and the H1R was...
further enhanced by the additional expression of Go12 but not of Go13. In addition, coexpression of Go12 but not Go13 unmasked the known constitutive activity of the H1R (23, 24). A similar effect was detected upon coexpression of the H1R with p63RhoGEF (Fig. 1A). Coexpression of RGS2, which acts as a GTPase-activating protein (GAP) and therefore as an inhibitor of Go11 but not Go12/13 proteins (25, 26), nearly eliminated the luciferase production induced by the activated M3R alone and largely suppressed the synergistic stimulation detected upon coexpression of p63RhoGEF (Fig. 1C). In contrast, the expression of the RGS domain of the mouse ortholog of p115RhoGEF Lac (Lac-RGS), which is a Go12/13-specific GAP and thereby suppresses Go12/13-mediated responses in HEK-293 cells (22), only slightly reduced (about 10–20%) the M3R-induced luciferase production. The transcriptional activity induced by the agonist-stimulated M3R coexpressed with p63RhoGEF was not affected by Lac-RGS expression. The expression of both RGS proteins was verified by immunoblotting (not shown).

Synergistic Activation of Rho-mediated Gene Transcription by p63RhoGEF and Go11 but Not Go12/13 Proteins—As the data obtained so far argued for an activation of p63RhoGEF by Go11 proteins, we studied the influence of various Go subunits on p63RhoGEF-induced luciferase expression. Overexpression of wild-type Go1 or Go11 only marginally (2–3-fold) increased luciferase production. Upon coexpression of p63RhoGEF, the transcriptional activity was increased by 20–40-fold (Fig. 2, A and B). As reported before (11, 17), overexpression of wild-type Go12 and Go13 induced strong increases in luciferase production, by 25- and 75-fold, respectively (Fig. 2C). Coexpression of p63RhoGEF with either Go12 or Go13 did not lead to further increases in transcriptional activity. The ineffectiveness of p63RhoGEF to enhance gene transcription by Go12 and Go13 was not due to a ceiling effect in the experimental setting. Expression of the GTPase-deficient mutants of Go1 (Go1-RC) and Go11 (Go11-QL) induced transcriptional activity in a range similar to that observed with Go12 or Go13 (compare Fig. 2, A and C). Upon coexpression of p63RhoGEF, the luciferase production induced by Go1-RC and Go11-QL was enhanced in a synergistic manner reaching levels of more than 100-fold. In contrast, the strong transcriptional activity (more than 100-fold) induced by the GTPase-deficient mutants of Go12 (Go12-QL) and Go13 (Go13-QL) was not enhanced but was even slightly reduced by coexpression of p63RhoGEF (Fig. 2C). As reported before (15), the p63RhoGEF-induced luciferase production, even when stimulated by Go1-RC or Go11-QL, was largely suppressed by the RhoA–C-inactivating C3 ADP-ribosyl transferase (27) (data not shown).

Activated Go11 Proteins Largely Stimulate p63RhoGEF-induced RhoA Activation—The measurements of Rho-dependent gene transcription indicated a stimulation of p63RhoGEF by activated Go11 but not by activated Go12/13 proteins. To substantiate this hypothesis, the amount of GTP-ligated RhoA was analyzed using the RhoA binding domain of rhotekin (GST-RBD) for pull-down of RhoA-GTP (18) in lysates of transfected HEK-293 cells (15). Under the conditions used (submaximally effective amounts of plasmid DNA encoding p63RhoGEF and Go subunits), expression of either p63RhoGEF, Go1-RC, or Go11-QL alone only slightly increased the amount of RhoA-GTP (Fig. 3). Combined expression of p63RhoGEF and Go1-RC or Go11-QL induced a substantial increase in RhoA-GTP. In contrast, the combined overexpression of p63RhoGEF with either Go1-QL or Go11-QL did not induce a synergistic activation of RhoA. In accordance with the data observed in the SRF activation assay, coexpression of p63RhoGEF slightly inhibited the RhoA activation by the permanent active Go12/13 mutants.
FIG. 4. Direct interaction of activated Gαq/11-proteins with p63RhoGEF. A, a schematic view of full-length p63RhoGEF (p63-FL) and its truncated mutants, p63-DH and p63-ΔN. B–D, HEK-293 cells were transfected with control vectors, p63-FL, p63-DH, or p63-ΔN and wild-type Gαq, GαqRC, EE-GαqQL, Gαq11QL, Gαq12QL, or Gαq13QL (2 μg of DNA each) as indicated. Cells were lysed 48 h after transfection, and p63RhoGEF and its variants were immunoprecipitated (IP) with the anti-c-Myc-antibody (IB and D). EE-GαqQL was precipitated with the anti-EE-antiserum (C). Precipitated proteins were separated by SDS-PAGE and immunoblotted with the anti-c-Myc antibody (upper panels) and Gα subtype-specific antibodies (lower panels). The content of the Gα subunits in the cell lysates (Lysate) is shown as a loading control. WB, Western blot.

cells. Upon precipitation of the N-terminally Myc-tagged p63RhoGEF with anti-c-Myc antibodies, the immunoprecipitates were analyzed for coprecipitated Gα proteins. As shown in Fig. 4B, the GTPase-deficient mutants GαqRC or Gαq13QL were coprecipitated by the anti-c-Myc antibody from cells transfected with the respective eukaryotic expression vectors. In contrast, Gαq was not detected in the p63RhoGEF immunoprecipitates from cells overexpressing wild-type Gαq. These data indicate that only activated Gαq and Gα11 apparently exhibit high affinity binding to p63RhoGEF and thus can be precipitated in a complex with p63RhoGEF. To verify this hypothesis, we overexpressed an EE-tagged version of Gαq,QL (EE-GαqQL) together with p63RhoGEF and precipitated EE-tagged proteins. As shown in Fig. 4C, p63RhoGEF was coprecipitated together with EE-GαqQL from lysates of cells coexpressing EE-GαqQL and p63RhoGEF.

To identify the part of p63RhoGEF in which this interaction takes place, we performed similar experiments with two truncated mutants of p63RhoGEF, i.e. p63-DH (amino acids 138–379), which mainly consists of the DH domain, responsible for the guanine nucleotide exchange activity at RhoA (15, 16) and p63-ΔN, consisting of the C-terminal half (amino acids 295–580) of p63RhoGEF with the PH domain but lacking the DH domain (Fig. 4A). The mutant p63-DH was precipitated to a similar extent as full-length p63RhoGEF (p63-FL) by the anti-c-Myc antibody but did not form a detectable complex with any of the Gαq/11 proteins. In contrast, similar to p63-FL, p63-ΔN coprecipitated GαqRC and Gαq11QL but not wild-type Gαq (Fig. 4, B and D). No coprecipitation with any p63RhoGEF construct was observed with either wild-type Gα12 and Gα13 (not shown) or their GTPase-deficient mutants, Gα12QL and Gα11QL (Fig. 4D).

The Recombinant Gαq/11 Binding Domain of p63RhoGEF Inhibits Gαq/11 and M3R-induced Rho-mediated Gene Transcription—As the N-terminally truncated mutant p63-ΔN specifically bound activated Gαq/11 proteins, we used the expression of this mutant to study the role of endogenous RhoGEFs in RhoA activation by Gα proteins and the M3R. In contrast to p63-FL, coexpression of p63-ΔN with GαqRC largely reduced the luciferase production induced by the GTPase-deficient Gαq mutant (Fig. 5A). On the other hand, p63-ΔN and p63-FL both weakly inhibited the transcriptional activity induced by Gα13. These data exclude a nonspecific inhibition of RhoA activation by p63-ΔN. Most important, expression of p63-ΔN, which by itself did not induce any transcriptional activity, potently suppressed (by up to 90%) the luciferase production induced by the carbachol-activated M3R (Fig. 5B).

p63RhoGEF Competes with PLCβ for Activated Gαq/11 Proteins—The direct interaction of p63RhoGEF with Gαq/11 proteins finally prompted us to study whether p63RhoGEF and PLCβ isozymes, which also directly interact with Gαq/11 proteins (28), may compete with each other for activation. For this, the effect of p63RhoGEF on Gαq/11 and M3R-induced luciferase production and PLC activation was examined in COS-7 cells, which are better suited to measure agonist-stimulated PLC activity in response to transient M3R expression than HEK-293.
cells. Similar to HEK-293 cells, COS-7 cells express p63RhoGEF endogenously (Fig. 1A, inset). Also, in these cells, coexpression of p63RhoGEF largely enhanced the Gaq,RC-induced transcriptional activity, by about 10-fold (Fig. 6A). On the other hand, p63RhoGEF, which had no effect on basal PLC activity, significantly reduced the PLC stimulation induced by Gaq,RC by about 40% (Fig. 6B). An even stronger inhibition, of about 70%, by p63RhoGEF was observed on the M3R-induced PLC stimulation (Fig. 6D). Vice versa, the effect of an overexpression of PLCb2 on M3R-induced PLC activity and luciferase production was studied in COS-7 cells. Overexpression of PLCb2 significantly increased the carbachol-induced inositol phosphate production from 4- to 5.5-fold (data not shown). As shown in Fig. 6C, the carbachol-induced luciferase production was reduced by about 50% in cells coexpressing PLCb2 and p63RhoGEF. To further exclude a contribution of the PLC/PKC pathway on the regulation of p63RhoGEF, we additionally studied the influence of the PLC inhibitor U-73122 and the broad range PKC inhibitor bisindolylmaleimide IX on Gaq,QL-and p63RhoGEF-induced transcriptional activity in COS-7 cells overexpressing PLCb2. Neither U-73122 (2.5 μM) nor bisindolylmaleimide IX (0.5 μM) altered the transcriptional activity induced by p63RhoGEF, Gaq,QL, or their combination (data not shown).

**DISCUSSION**

It is meanwhile well documented that activation of heterotrimeric G proteins of the Gaq,11 subfamily induce Rho activation in a variety of cells and tissues (11-13, 29). There is increasing evidence that this activation of Rho occurs independently of the PLC/PKC pathway. Most likely, so far unidentified GEFs are involved (11, 13). In this report, we presented several lines of evidence that p63RhoGEF, a novel member of the Dbl family of GEFs, might represent the specific GEF, or at least one of the GEFs, mediating this response. Firstly, p63RhoGEF largely enhanced the transcriptional activity of the primarily Gaq11-coupled M3R and H1R in a manner sensitive to RGS2, a negative regulator of Gaq, activity (25, 26), but insensitive to the G12/13-specific Lac-RGS (22). Secondly, it largely enhanced the Rho-mediated gene transcription induced by the GTPase-deficient mutants of Gaq and Ga11, but not of Gatl1 and Gatl2. This increase in transcriptional activity was completely sensitive to the RhoA-inactivating C3 ADP-ribosyl transferase of Clostridium botulinum (27). Thirdly, coexpression of p63RhoGEF and activated mutants of Gaq and Ga11 largely and synergistically increased the cellular amount of activated RhoA proteins. Finally, p63RhoGEF directly interacted with activated Gaq and Ga11 proteins. This interaction could be detected by means of communoprecipitations as well as by functional inhibition of Gaq-induced gene transcription by the Gaq binding domain of p63RhoGEF. Therefore, our data indicate that p63RhoGEF is the first known GEF that is specifically and so far exclusively regulated by activated Ga subunits of the Ga family. Furthermore, the GEF-deficient mutant p63-ΔN efficiently suppressed Rho-mediated gene transcription induced by the activated M3R and Gaq,RC, but not Ga11, indicating that p63RhoGEF is in fact specifically involved in RhoA activation by Gaq,11 proteins. This interaction is likely mediated by the Gaq,11-RhoGEF interaction domain in p63RhoGEF. Therefore, our data indicate that p63RhoGEF is the first known GEF that is specifically and so far exclusively regulated by activated Ga subunits of the Ga family. Furthermore, the GEF-deficient mutant p63-ΔN efficiently suppressed Rho-mediated gene transcription induced by the activated M3R and Gaq,RC, but not Ga11, indicating that p63RhoGEF is in fact specifically involved in RhoA activation by Gaq,11 proteins. This interaction is likely mediated by the Gaq,11-RhoGEF interaction domain in p63RhoGEF.

**FIG. 5.** p63-ΔN inhibits Gaq,RC-mediated SRF activation. A and B, luciferase production was measured in HEK-293 cells transfected with Gaq,RC or Gaq,11 (50 ng of DNA each) alone (Control) and full-length p63RhoGEF (p63-FL) or p63-ΔN as indicated (A) or with the M3R (M3,) and the indicated amounts of p63-ΔN plasmid and stimulated overnight without (Control) and with 1 mM carbachol (B). Mean ± S.D.; n = 6–18. ***, p < 0.001 versus Gaq,RC; **, p < 0.05, ***, p < 0.001 versus Gaq,11.

**FIG. 6.** Competition of p63RhoGEF (p63) and PLCβ for activated Gaq,11 proteins. Luciferase production (A and C) and inositol phosphate formation (B and D) were measured in COS-7 cells transfected with control vectors alone (Control), p63RhoGEF, Gaq,RC, PLCβ2, and the M3R (M3,) as indicated. In cells transfected with the M3R, activities were determined in the absence and presence of 1 mM carbachol. Mean ± S.D.; n = 9–15. *, p < 0.05; ***, p < 0.001; p63 or PLCβ2 versus respective control.
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G12 type G proteins, which is in line with the known G protein coupling specificity of this GPCR (21, 22). This interpretation is further corroborated by the small inhibitory effect (about 15%, Fig. 1C) of the G12/13-specific GAP Lsc-RGS (22).

Screening data bases of the human genome project and the mouse genome project revealed that p63RhoGEF, in contrast to other known RhoA-specific GEFs, has no close homolog. Besides the existence of a DH/PH tandem motif, p63RhoGEF is especially not related to the members of the p115RhoGEF family, which mediate the activation of RhoA by G12/13 proteins (8–10, 31). Accordingly, we could not detect any interaction of activated Goq family members with this domain and the subsequent Ca2+ mobilization and PKC activation (11, 14). Whether this interaction is mediated by the RGS homology domain of LARG is still a matter of debate. One report indicated a binding of activated Goq to the RGS domain of LARG and a productive coupling of activated Goq to RhoA via LARG (14). Another report showed that a LARG mutant lacking the DH domain but not the RGS homology domain interfered with Goq-induced RhoA activation (13). However, a third publication did not detect an interaction of activated Goq with the RGS homology domain of LARG, which also failed to inhibit Goq QL-induced luciferase production (11). In contrast to the data on the interaction of Goq with the LARG-RGS homology domain, the interaction of Goq family members with this domain and a productive coupling to RhoA were found by both groups (11, 14).

Another line of evidence for p63RhoGEF to be a so far unknown effector molecule for Goq proteins resulted from the experiments analyzing the influence of the canonical Goq effector molecule PLCβ (28) on the activation of p63RhoGEF and vice versa. The overexpression of p63RhoGEF inhibited Goq-stimulated PLCβ activity as well as the overexpression of PLCβ2 inhibited the Goq-induced gene transcription. These data argue for a direct competition of p63RhoGEF and PLCβ isoforms for activated Goq11 proteins. In line with the inhibitory effect of PLCβ2 and in agreement with previous observations, which indicated the existence of a Goq11-activated RhoGEF not regulated by PLC and the subsequent Ca2+ mobilization and PKC activation (11), the Mr-R- and Goq QL-induced gene transcription was not influenced by the inhibition of PLC and PKC activity, by U73122 (33) and bisindolylmaleimide IX (34), respectively.

In summary, the data presented herein define a new signaling pathway for GPCRs, with p63RhoGEF serving as a direct Goq11 effector molecule. It directly links these GPCRs to RhoA and RhoA-dependent cellular processes, apparently in competition with the canonical PLCβ/PKC pathway.

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