**Pasteurella multocida** Toxin-induced Activation of RhoA Is Mediated via Two Families of Gα Proteins, Gαq and Gα12/13*

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**Pasteurella multocida** toxin (PMT) is a potent mitogen, which is known to activate phospholipase Cβ by stimulating the α-subunit of the heterotrimeric G protein Gq. PMT also activates RhoA and RhoA-dependent pathways. Using YM-254890, a specific inhibitor of Gq, we studied whether activation of RhoA involves G proteins other than Gq. YM-254890 inhibited PMT or muscarinic M3-receptor-mediated stimulation of phospholipase Cβ at similar concentrations in HEK293m3 cells. In these cells, PMT-induced RhoA activation and enhancement of RhoA-dependent luciferase activity were partially inhibited by YM-254890. In Gαq-deficient fibroblasts, PMT induced activation of RhoA, increase in RhoA-dependent luciferase activity, and increase in ERK phosphorylation. None of these effects were influenced by YM-254890. However, RhoA activation by PMT was inhibited by RGS2, RGS16, IscRGS, and dominant negative Gα13N17, indicating involvement of Gα13 in the PMT effect on RhoA. In Gα12/13 gene-deficient cells, PMT-induced stimulation of RhoA, luciferase activity, and ERK phosphorylation were blocked by YM-254890, indicating the involvement of Gq. Infection with a virus harboring the gene of Gα13 reconstituted the increase in RhoA-dependent luciferase activity by PMT even in the presence of YM-254890. The data show that YM-254890 is able to block PMT activation of Gαq and indicate that, in addition to Gαq, the Gα12/13 G proteins are targets of PMT.

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The 146-kDa *Pasteurella multocida* toxin (PMT)† has diverse biological effects, including potent mitogenic and osteolytic activity (1). An important biochemical effect of the toxin is the activation of PLCβ (2,3). This action is most likely caused by activation of the heterotrimeric G protein Gq (3). Besides studies with antibodies, gene deletion studies demonstrated that the Gq subunit is crucially involved in PMT action (4). Interestingly, the action of PMT is characterized by the ability to differentiate between Gαq and Gα11, although both GTPases are ~89% identical (4). Recent studies indicate that this specificity is harbored in the helical domain of the Gα subunits of the heterotrimeric G proteins (5). However, so far, the precise mechanism of the action of this toxin is not clear.

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**EXPERIMENTAL PROCEDURES**

**Materials—**[3H]-labeled inositol was obtained from PerkinElmer Life Sciences. PCR primers were from Hermann GbR Synthetische Biomoleküle (Freiburg, Germany). Inositol-free modified Eagle’s medium was purchased from Cell-concepts (Umkirch, Germany). The QuikChange kit was from Stratagene (Heidelberg, Germany). All other reagents were of analytical grade and purchased from commercial sources.

**Plasmids and Retroviral Vector Construction** The plasmid containing the construct of Gα13C4A was a kind gift of Dr. S. Offermanns (University of Heidelberg, Heidelberg, Germany). The plasmids PMD-G and PMD-G-p24 were kindly provided by Dr. R. Mulligan (Harvard Medical School, Boston, MA). The plasmid pLNCX2 was purchased from Clontech (Heidelberg, Germany). The retroviral transfer vector Gα13wt-
pLNCX2 was generated using standard cloning techniques and site-directed mutagenesis.

The cDNA constructs of RGS2, RGS16, and lscRGS were a kind gift of Dr. T. Wieland (Institut für Pharmakologie und Toxikologie, Fakultät für Klinische Medizin Mannheim, Universität Heidelberg, Heidelberg, Germany). The cDNA was cloned into the pCMV-HA mammalian expression vector, which expresses an N-terminal hemagglutinin (HA) epitope tag.

Cell Culture, Virus Production, and Transduction—Mouse embryonic fibroblasts (MEFs) derived from Gα12/13-deficient or wild-type (wt) mice were cultured as described previously (4, 18, 25). The retroviral vector was produced as described previously (26). In brief, HEK293T cells were co-transfected with pMD-G, pMD-g/p, and the retroviral transfer vector. The calcium phosphate method was used. The supernatant was collected after 4 days and centrifuged to spin down cellular debris. The virus-containing medium was filtered in the presence of Polybrene. The expression was monitored by Western blot analysis. Additional transfection of the pSRE.L-luciferase reporter plasmid and the pRL.TK control reporter vector was done with the Nucleofection system (Amaxa Biosystems, Cologne, Germany) according to the general protocol for nucleofection of mouse embryonic fibroblasts. HEK293m3 cells, which stably express the muscarinic acetylcholine M3 receptor (27), were transfected using the jetPEI-transfection kit from Biomol (Hamburg, Germany).

Assay of SRF Activation—HEK293m3 cells seeded on 96-well plates were co-transfected with the indicated expression plasmid together with the pSRE.L-luciferase reporter plasmid and the pRL.TK control reporter vector. pSRE.L encodes for firefly (Photinus pyralis) luciferase; the expression is induced by the activation of SRF. pRL.TK encodes for Renilla (Renilla reniformis) luciferase, which is expressed constitutively. This control reporter provides an internal control that serves as the base-line response. The plasmids pSRE.L and pRL.TK were a kind gift of Dr. R. Treisman (Transcription Laboratory, Imperial Cancer Research Fund Laboratories, London, England). Cα11- or Cα12/13-deficient MEFs were seeded after transfection on 12-well plates. Cultures were serum-starved for 24 h before stimulation with PMT or carbachol for an additional 20 h and lysed with passive lysis buffer (Promega, Heidelberg, Germany). Luciferase activities were determined with the Dual-Luciferase reporter assay system (Promega, Heidelberg, Germany) in accordance with the manufacturer’s instructions. The activity of the experimental reporter was normalized against the activity of the control vector.

Pull-down Experiments—The Rho-binding region, encoding the N-terminal 90 amino acids of rhotekin (rhotekin pull-down) was expressed as GST fusion proteins in Escherichia coli BL21. Overnight cultures were diluted 1:40 and grown for 1 h at 37 °C. Thereafter, 0.1 mM isopropyl-β-D-thiogalactopyranoside (final concentration) was added. 2 h after induction, cells were collected and lysed by sonication in rhotekin lysis buffer (20% sucrose, 10% glycerol, 50 mM Tris-HCl, pH 8.0, 0.2 mM sodium bisulfite, and 2 mM MgCl2) and purified by affinity chromatography with glutathione-Sepharose (Amersham Biosciences). Loaded beads were washed three times with rhotekin lysis buffer and once with buffer A (10% glycerol, 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1% Nonidet P-40, 2 mM MgCl2). Toxin-treated cells were lysed in buffer A. For analysis of the total amount of Rho in the lysates, an aliquot of the volume was taken for Western blot analysis. The lysate was incubated with protein-loaded beads for 1 h at 4 °C by head-over-head rotation. After incubation, the beads were washed once with buffer A. Thereafter, SDS sample buffer was added, and the samples were boiled and separated by SDS-PAGE. RhoA was analyzed by immunoblotting with a specific antibody.

Analysis of Total Inositol Phosphates—HEK293m3 cells, Cα12/13-deficient or wt-MEFs, were grown in 24-well plates for 2–3 days. Thereafter, the cultures were labeled with 2 μCi/ml [2-3H]inositol in serum-free medium overnight. Subsequently, PMT or carbachol at the indicated concentrations were added at the indicated times and incubated for the indicated times. LiCl (20 mM) was added 60 min before the assay was stopped to allow accumulation of inositol phosphates. Thereafter, the medium was replaced by 750 μl of ice-cold formic acid (10 mM, pH 3). After 30 min, the extract was neutralized with 3 ml of NH4OH (5 mM, pH 8–9). Analysis of total inositol phosphates was done by anion exchange chromatography as described previously (28).

Determination of ERK Phosphorylation—MEFs were seeded in 12-well plates and serum-starved for 24 h. The cells were incubated with PMT proteins (70 pm) and epidermal growth factor (10 ng/ml) at 37 °C. The cells were washed once with ice-cold phosphate-buffered saline and lysed with 30 μl/well Laemmli sample buffer. Cell lysates were separated by SDS-PAGE, and phosphorylation was determined by sub-
Sequent immunoblotting with a phosphospecific anti-ERK antibody (Santa Cruz Biotechnology). To confirm equal loading of ERK1/2, membranes were stripped and reprobed with an anti-ERK antibody (Santa Cruz Biotechnology).

**Results**

Recently, the novel compound YM-254890, a cyclic peptide, was shown to inhibit specifically the activation of Gαq/11 induced by stimulation of Gα13-coupled heptahedral receptors (29). We wanted to know whether YM-254890 is also able to inhibit PMT-induced activation of Gαq. For this purpose, HEK293m3 cells, which harbor a muscarinic acetilycholine M3 receptor, were treated with PMT (700 pM) for 4 h in the presence of increasing concentrations of YM-254890. Thereafter, inositol phosphate formation was determined. As shown in Fig. 1A, YM-254890 inhibited the PMT effect on inositol phosphate accumulation in a concentration-dependent manner. A half-maximal and maximal effect of YM-254890 occurred at ~30 and 300 nM, respectively. At maximally effective concentrations of YM-254890, the PMT-induced inositol phosphate accumulation was completely blocked, suggesting that Gαq was activated by the compound. We compared the effect of YM-254890 on PMT-induced inositol phosphate accumulation with the effect of carbachol, which activates PLCβ via M3 receptors in these cells (Fig. 1B). Inhibition of carbachol and PMT-activated PLCβ occurred at similar concentrations (IC_{50} ~100 nM) of YM-254890.

Next we used YM-254890 to analyze the signaling pathways of PMT involving RhoA. RhoA is known to be involved in regulation of the gene transcription factor SRF (30). Although SRF is regulated by various factors, Rho plays a central role. Using a luciferase reporter gene, we exploited the RhoA dependence of stimulation of the serum response element (SRE) by SRF to determine RhoA activation. At first, we studied the activation of luciferase expression in HEK293m3 cells. PMT increased luciferase activity by ~8–10-fold (Fig. 2A). Also, carbachol, which probably acts on Gα12/13 and on Gα13, caused a large stimulation of luciferase activity by ~20-fold (Fig. 2B). Similarly, as found for inhibition of the PMT-induced inositol phosphate accumulation, we observed inhibition of PMT-induced luciferase activity by YM-254890 in a concentration-dependent manner, indicating the involvement of Gαq. However, even at high concentrations of YM-254890, which were sufficient for complete inhibition of PLCβ stimulation by PMT or carbachol, a residual luciferase activity remained. This suggested involvement of PMT-stimulated pathways, which could not be blocked by YM-254890.

To get further hints for the types of G proteins involved, we studied the effects of various regulators of G protein signaling (RGS) on PMT-induced increase in luciferase activity. Therefore, RGS2 and RGS16 (which interact with Gα12 and Gα13) and IscRGS (which is suggested to

**Figure 1.** YM-254890 inhibits PMT- and carbachol-induced SRF activation. Luciferase production was measured in HEK293m3 cells transfected with pSRE.L and pRL.TK as described under “Experimental Procedures.” Cells were treated with YM-254890 for 30 min at the indicated concentrations. Thereafter, the cells were stimulated for 24 h with PMT (700 pM) or carbachol (1 mM). Shown is the fold stimulation of luciferase as compared with controls. Data are given as mean ± S.E. (n = 4). Experiments were performed at least three times with similar results.

**Figure 2.** YM-254890 inhibits PMT- and carbachol-induced SRF activation. Luciferase production was measured in HEK293m3 cells transfected with pSRE.L and pRL.TK as described under “Experimental Procedures.” Cells were treated with YM-254890 for 30 min at the indicated concentrations. Thereafter, the cells were stimulated for 24 h with PMT (700 pM) or carbachol (1 mM). Shown is the fold stimulation of luciferase as compared with controls. Data are given as mean ± S.E. (n = 4). Experiments were performed at least three times with similar results.

**Figure 3.** Expression of RGS proteins reduces PMT-induced SRF activation. A luciferase production was measured in HEK293m3 cells transfected with pSRE.L, pRL.TK, and control vectors alone (con) or hRGS2, hRGS16, or IscRGS (50 ng of DNA/well each). Before the assay, the cells were stimulated for 24 h with PMT (700 pM). Shown is the fold stimulation of luciferase as compared with controls. Data are given as mean ± S.E. (n = 4). Experiments were performed at least three times with similar results. B, immunoblot (IB) detection of HA-tagged RGS proteins.
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exclusively interact with \( \alpha_{12/13} \) were overexpressed in HEK293m3 cells (31, 32). RGS2 and RGS16 expression blocked the PMT effect by 40–60%, respectively (Fig. 3). Also, expression of IscRGS, which specifically interacts with \( \alpha_{12/13} \) reduced PMT-stimulated luciferase activity by 50%. These findings suggest that \( \alpha_{12/13} \) is involved in SRF-induced luciferase activation by PMT. Overexpression of dominant negative Ga proteins is another approach to interference with G protein-mediated signaling. We expressed dominant negative \( \alpha_{12/13} \), which is not able to release \( \beta \gamma \)-subunits (33) in HEK293m3 cells. As shown in Fig. 4, \( \alpha_{12/13} \) reduced PMT-induced increase in luciferase activity by 70%. Interestingly, the addition of the \( \gamma_{11} \) inhibitor YM-254890 further decreased inhibition.

To further clarify the roles of \( \gamma_{11} \) and \( \alpha_{12/13} \) in PMT-induced luciferase activation, we used G\( \alpha_{12/13} \)-deficient cell lines (4, 18). At first, we studied the activation of RhoA by PMT, using the Rho-binding domain of rhotein in pull-down assays with \( \alpha_{12/13} \) and \( \gamma_{11} \)-deficient cells. As shown in Fig. 5A, in \( \gamma_{11} \)-deficient cells, PMT increased pull-down of the RhoA-rhotein-Rho-binding domain complex, indicating activation of the GTPase. As a positive control, the cytotoxic necrotizing factor 1, which activates RhoA by deamidation of glutamine 63 (34), was tested. Notably, PMT and cytotoxic necrotizing factor stimulated Rho to the same extent. Also, in \( \alpha_{12/13} \)-deficient cells, PMT induced a significant activation of RhoA. However, cytotoxic necrotizing factor 1 was much more potent than PMT in RhoA activation in \( \alpha_{12/13} \)-deficient cells. By contrast, the activity of PMT on wild-type and \( \alpha_{12/13} \)-deficient cells was similar in respect to inositol phosphate accumulation, indicating that both cell types did not differ in toxin binding and/or uptake. Next, we studied the influence of YM-254890 on RhoA activation in \( \gamma_{11} \)- and \( \alpha_{12/13} \)-deficient cells. As shown in Fig. 6, in \( \gamma_{11} \)-deficient cells, increasing the concentration of YM-254890 did not affect the pull-down of active RhoA. In line with the hypothesis that YM-254890 acts only on \( \gamma_{11} \), this finding showed directly that \( \alpha_{12/13} \) is not a target for YM-254890. By contrast, in \( \alpha_{12/13} \)-deficient cells, stimulation of RhoA by PMT was clearly inhibited by YM-254890, indicating that, in these cells, activation of RhoA by the toxin depends on \( \gamma_{11} \). PMT was also shown to activate mitogen-activated protein kinase pathways (35). We tested the effect of YM-254890 on PMT-induced ERK phosphorylation in \( \gamma_{11} \)- and \( \alpha_{12/13} \)-deficient cells. In \( \alpha_{12/13} \)-deficient cells, PMT caused a strong activation of ERK phosphorylation (Fig. 7). YM-254890 blocked the ERK activation in a concentration-dependent manner. At 1 \( \mu \)M, YM-254890 ERK activation was strongly blocked. When the ERK activation was studied in \( \gamma_{11} \)-deficient cells, stimulation by PMT was also observed. However, YM-254890 did not elicit any inhibition on phosphorylation of ERK in \( \gamma_{11} \)-deficient cells. Next, we studied the effects of YM-254890 on PMT-induced luciferase expression and activation in \( \gamma_{11} \)- and \( \alpha_{12/13} \)-deficient cells. Unfortunately, in \( \gamma_{11} \)-deficient cells, PMT-stimulated luciferase activation was only 2-fold (Fig. 8). The addition of YM-254890 did not reduce the luciferase activation by PMT, indicating the specific action of YM-254890 on \( \gamma_{11} \). By contrast, when the stimulation of luciferase expression was studied in \( \alpha_{12/13} \)-deficient cells, YM-254890 completely blocked PMT-induced increase in luciferase activity, which was up to 20-fold in these cells, indicating signaling via \( \gamma_{11} \). Finally, we studied whether the introduction of \( \alpha_{12/13} \) into \( \alpha_{12/13} \)-deficient cells was able to specifically reconstitute PMT-induced stimulation of luciferase expression. For this purpose, cells were infected with virus harboring the gene of wild-type \( \alpha_{12/13} \). Thereafter, the cells were trans-
fected with the luciferase reporter gene and subsequently treated with PMT in the presence of YM-254890 to block activation of Gq/11. As shown in Fig. 9A, infection of Gαq12/13-deficient cells with virus containing the Gα13" gene reconstituted PMT-induced stimulation of luciferase activity even in the presence of YM-254890, indicating that PMT activates G13.

DISCUSSION

PMT is a potent mitogen and an efficient activator of PLCβ (2). At least the latter effect is clearly attributed to toxin-caused activation of Gq (3). Notably, PMT acts on Gαq but not on Gα11, although both proteins are ~89% identical (4). Similarly surprising is the recent finding that the helical domain of Gαq is essential for the PMT effect (5). However, several recent studies suggest that PMT not only acts on Gαq but also on other heterotrimeric G proteins. For example, it was observed that, in Gαq gene-deficient cells, PMT activates mitogen-activated protein kinase ERK and stimulates RhoA (4). Here, we showed that PMT also causes activation of G12/13-mediated signal pathways. To differentiate between Gq- and G12/13-mediated signaling, we made use of the cyclic peptide YM-254890, which is a highly specific inhibitor of receptor-mediated activation of Gq/11 (29). YM-254890 blocked completely the activation of PLCβ by PMT, which is in line with the view that inositol phosphate accumulation induced by PMT is exclusively dependent on Gq (5). It has been proposed that YM-254890 acts on the activation step of Gαq. In the model by Takasaki et al. (29), binding of GTP to Gαq is inhibited by YM-254890. Our finding that YM-254890 inhibits the effect of PMT on inositol phosphate accumulation suggests that nucleotide binding to Gαq is also crucial for the effect of PMT and indicates that the action of the protein toxin on the G protein does not block the

FIGURE 6. YM-254890 inhibits PMT-induced activation of Rho in Gα12/13-deficient MEF but not in Gαq/11-deficient MEF. The level of activated RhoA and total RhoA were determined in Gαq/11-deficient MEF (A) and Gα12/13-deficient MEF (B). YM-254890 was added at the indicated concentrations to the medium. After 30 min of incubation, PMT (70 pM) was added for a further 3 h. Thereafter, the cells were lysed and pull-down experiments were performed as described under “Experimental Procedures.” Experiments were repeated at least three times with similar results. IB, immunoblot.

FIGURE 7. YM-254890 inhibits PMT-induced activation of ERK in Gα12/13-deficient MEF (A) but not in Gαq/11-deficient MEF (B). Serum-starved MEFs were treated with the indicated concentration of YM-254890. After 30 min, incubation was continued in the presence of PMT (70 pM) for 4 h or in the presence of epidermal growth factor (10 ng/ml) for 5 min. The cells were then lysed, and activation of ERK was determined by immunoblotting with an anti-phospho-ERK antibody. As a control, the same blot was reprobed with an anti-ERK antibody. Representative blots of three independent experiments are shown.

FIGURE 8. YM-254890 inhibits PMT-induced SRF activation in Gα12/13-deficient MEF but not in Gαq/11-deficient MEF. Luciferase production was measured in Gαq/11-deficient MEF (A) and Gα12/13-deficient MEF (B). Transfected (pSRE.L and pRL.TK) and serum-starved MEFs were preincubated with or without 1 μM YM-254890 for 30 min. PMT (700 pM) was added to the medium, and incubation was continued overnight before luciferase activity was measured. Shown is the fold stimulation of luciferase as compared with controls. Data are given as mean ± S.E. (n = 3). Experiments were performed at least three times with similar results.
interaction of $\alpha_q$ with YM-254890. In line with this hypothesis is the finding that even pretreatment of cells with PMT did not prevent YM-254890-induced inhibition of inositol phosphate accumulation (data not shown). Moreover, the studies indicate that YM-254890 is a powerful tool in studying signal transduction pathways induced by PMT, because the compound allows inhibition of $\alpha_{q/11}$-mediated effects.

It has been reported by many groups that RhoA is involved in SRF-regulated gene transcription (30, 36–39). Using luciferase expression as a reporter system, we studied SRF activation in HEK293m3 cells. We observed that PMT caused a strong increase in luciferase activity in these cells. This effect may be due to activation of RhoA by the toxin. The increase in SRF activity was inhibited by YM-254890, suggesting an effect involving $\alpha_q$. Although RhoA is important for SRF activation, one has to consider that SRF is likely to be regulated by other factors including calcium (40). Therefore, PMT-induced PLC activation via $\alpha_q$ and subsequent calcium mobilization may participate in this effect.

In addition to YM-254890, we observed the inhibition of PMT-stimulated luciferase activity by overexpression of RGS2, RGS16, and lscRGS. RGS2 acts on $\alpha_q$ and $\alpha_{q/11}$ but preferentially on $\alpha_{q/11}$ (41, 42). RGS16 acts on $\alpha_q$ and $\alpha_{q/11}$ (31, 43). lscRGS, the RGS domain of the Rho guanine nucleotide exchange protein lsc, appears to be specific for $\alpha_{12/13}$. Thus, studies with RGS proteins indicated that $\alpha_{12/13}$ is involved in PMT-induced increase in luciferase activity. To further study this topic in more detail, we used cells deficient in the genes of $\alpha_{12/13}$ and $\alpha_{q/11}$, respectively.

In $\alpha_{q/11}$-deficient cells, Rho protein was strongly activated by PMT, which was shown by a rhotekin pull-down assay. In line with the proposed specificity of YM-254890 (29), this activation of RhoA was not affected by compound YM-254890. Similarly, the stimulation of RhoA-dependent luciferase activity by PMT was not affected by YM-254890 in cells, which did not possess $\alpha_{q/11}$, the targets of YM-254890. By contrast, expression of dominant negative $\alpha_{12/13}$-GA reduced Rho-dependent luciferase activity by PMT in these cells. This dominant negative construct is suggested to block activation of $\alpha_{12/13}$-GA by its inability to release the $\beta/\gamma$-subunits (33). Therefore, it is suggested that $\alpha_{12/13}$-GA binds tightly to respective $\alpha_{12/13}$-coupled heptahelical receptors thereby preventing activation of RhoA through $G_13$ and $G_{q}$ proteins (17, 44). Again, this shows that $\alpha_{12/13}$ is crucial for PMT-induced RhoA-dependent luciferase activation.

ERK activation by PMT was suggested to occur via $\alpha_{q/11}$-dependent transactivation of the epidermal growth factor receptor (35). Also, in $\alpha_{q/11}$-deficient cells, PMT caused activation of ERK, indicating that activation of the mitogen-activated protein kinase pathway occurs independent of $\alpha_{q/11}$. The finding that YM-254890 had no effect on PMT-induced ERK activation in $\alpha_{q/11}$-deficient cells is in line with this hypothesis and strongly supports the view that this compound is highly specific for $\alpha_{q/11}$.

Of special interest were studies on the effect of PMT on $\alpha_{12/13}$-deficient cells. In these cells, PMT-induced Rho activation was determined by the rhotekin pull-down assay and by activation of luciferase. YM-254890 completely blocked both effects of PMT. This indicates that the effects were mediated by $\alpha_{q/11}$. It was shown that $\alpha_{q/11}$ and $\alpha_q$-coupled receptors effectively activate RhoA (45). This activation appears not to be mediated by guanine nucleotide exchange factors, which were shown to couple $\alpha_{12/13}$ to RhoA activation similar to p115RhoGEF, PDZ-RhoGEF, and LARG (45). However, recently a novel GEF (p63RhoGEF) has been identified, which selectively interacts with $\alpha_q$ (20). This is a strong candidate for the PMT-induced activation of RhoA via $\alpha_q$. As expected, in $\alpha_{12/13}$-deficient cells, PMT caused activation of ERK. This activation by PMT was completely blocked by YM-254890. Thus, all of these findings with $\alpha_{12/13}$-deficient cells suggest that no other G proteins than $\alpha_q$ and $\alpha_{12/13}$ are targets of PMT to induce ERK and/or RhoA activation. Most importantly, when the $\alpha_q$ pathway was blocked by YM-254890, we were able to reconstitute the RhoA-dependent luciferase activation by PMT in $\alpha_{12/13}$-deficient cells after introducing $\alpha_{13}$ into the cells with virus harboring the gene for the G protein. This finding is another strong indication for the hypothesis that $\alpha_{12/13}$ is a target of PMT.

Taken together, using the novel compound YM-254890, which specifically blocks $\alpha_{q/11}$ activation, and by applying cells deficient in the genes for $\alpha_{q/11}$ or $\alpha_{12/13}$, we were able to dissect signaling pathways induced by PMT involving both subgroups of G proteins. We showed that PMT activates RhoA and RhoA-dependent pathways by stimulation of $\alpha_{q/11}$ and by stimulation of $\alpha_{12/13}$. Our findings indicate that the effect of PMT is not specific for $\alpha_q$ but also involves $\alpha_{12/13}$.

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