Ga12 and Ga13 Stimulate Rho-dependent Tyrosine Phosphorylation of Focal Adhesion Kinase, Paxillin, and p130 Crk-associated Substrate*

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We examined whether constitutively active mutants of the Ga proteins Ga12 and Ga13, which together comprise the Ga12 subfamily of Ga proteins, induce Rho-dependent tyrosine phosphorylation of the focal adhesion proteins p125 focal adhesion kinase, paxillin, and p130 Crk-associated substrate. We report that transient expression of the constitutively active mutants of Ga12 or of Ga13 in human embryonic kidney 293 cells stimulates tyrosine phosphorylation of a set of proteins of Mr, 110,000–130,000, 97,000, and 60,000–70,000. We identified p125 focal adhesion kinase, paxillin, and p130 Crk-associated substrate as prominent tyrosine-phosphorylated proteins in human embryonic kidney 293 cells expressing constitutively active Ga12 and Ga13. In common with the increased tyrosine phosphorylation of these proteins mediated by mitogens acting through heptahelical receptors, the Ga12- and Ga13-mediated increase in tyrosine phosphorylation is blocked by cytochalasin D, which specifically disrupts the actin cytoskeleton, and p125fak (8, 9) and the adaptor proteins paxillin (10, 11) and p130cas (12), which localize to focal adhesion plaques, have been identified as prominent tyrosine-phosphorylated proteins in agonist-stimulated Swiss 3T3 cells (3, 4, 13–20). The increases in tyrosine phosphorylation of p125fak, paxillin, and p130cas are accompanied by profound alterations in the organization of the actin cytoskeleton in Swiss 3T3 cells, leading to the formation of actin stress fibers and the assembly of focal adhesions (3, 4, 21, 22). RhoA, a member of the Ras superfamily of low molecular weight GTPases, has been shown to direct stress fiber formation and focal adhesion assembly in Swiss 3T3 cells (23, 24). We proposed that the tyrosine phosphorylation of p125fak, paxillin, and p130cas mediated by heterotrimeric G-proteins is downstream of RhoA activation and focal adhesion assembly (1, 25–28). Signaling through p125fak, paxillin, and p130cas has been implicated in the regulation of cell migration, proliferation, and transformation (20, 29–32).

The immediate mechanism(s) coupling mitogen-induced activation of GPCRs to tyrosine phosphorylation of focal adhesion proteins is not understood. Although many GPCRs couple to the pertussis toxin-sensitive Gi, Gqi does not appear to mediate increases in protein tyrosine phosphorylation (1, 33). Ga12 and Ga13, which together comprise the ubiquitously expressed Ga12 subfamily of Ga proteins, are distantly related to other G-protein α subunits and are pertussis toxin-insensitive (34, 35). There is increasing evidence indicating that Ga12 and Ga13 are involved in cell migration, proliferation, and transformation. Expression of mutationally activated Ga12 stimulates cellular entry into DNA synthesis, proliferation, and malignant transformation in NIH3T3 and Rat-1 cell lines (36–41) and promotes stress fiber formation and focal adhesion assembly in Swiss 3T3 cells (42). Furthermore, in astrocytoma cells, thrombin-induced stimulation of DNA synthesis was prevented by the microinjection of anti-Ga12 antibody (43). Gene disruption experiments have implicated Ga13 in the regulation of cell migration (44). The downstream targets through which Ga12 and Ga13 induce these effects have not been identified, although Ras-, Rac-, Rho-, and Cdc42-dependent pathways leading to cytoskeletal reorganization and to the activation of mitogen-activated protein kinase, Jun N-terminal kinase, and the Na+/H+ exchanger have been implicated (40, 45–48). However, it is not known whether activation of Ga12 and/or Ga13 can also promote the tyrosine phosphorylation of the nonreceptor protein tyrosine kinase p125fak and the adaptor proteins paxillin and p130cas.

To examine whether the Ga12 subfamily of heterotrimeric G-proteins induce protein tyrosine phosphorylation, we have transiently transfected human embryonic kidney HEK 293 cells with expression vectors encoding constitutively active Ga12 and Ga13 proteins and determined the effect of the expression of these activated Ga subunits on the tyrosine phos...
phorylation of p125\textsuperscript{akh}, paxillin, and p130\textsuperscript{csk}. Our results demon-
strate that expression of active Go\textsubscript{12} and Go\textsubscript{13} in HEK 293 cells induces tyrosine phosphorylation of these focal adhesion proteins through a pathway that requires the integrity of the actin cytoskeleton and functional Rho.

**EXPERIMENTAL PROCEDURES**

cDNA Constructs—The murine Go\textsubscript{12} subunit cDNAs in the eucary-
ocytic expression vector pcDNA-1 (In Vitrogen) were gifts from Dr. H. R.

Bronne (University of California at San Francisco, CA) and included the constitutively active mutants Go\textsubscript{12Q56L} and Go\textsubscript{13Q72L} (Go\textsubscript{12}QL and Go\textsubscript{13}QL) (37). The constitutively active mutant murine Go\textsubscript{12} was
calcium phosphate precipitation and LipofectAMINE were each opti-
mized to yield peak efficiency of transfection using an expression plas-
tide chain elongation factor 1a (EF-1\textalpha) promoter (51).

**Cell Culture and Transfection**—Cultures of transformed HEK 293 cells were maintained in DMEM supplemented with 10% fetal bovine serum in a humidified atmosphere containing 10% CO\textsubscript{2} at 37 °C. HEK 293 cells were transfected with the various plasmids by calcium phos-
phate precipitation (52) or with LipofectAMINE\textsuperscript{TM} (Life Technologies, Inc.,

for 18 h at 5% CO\textsubscript{2} according to the manufacturer’s specifications. In the cotransfection experiments, total amounts of DNA were kept constant, and equal amounts of Go\textsubscript{12} and of C3 expression constructs (or vector without an insert) were mixed together. After transfection with the phosphate or LipofectAMINE, the cells were washed and maintained in DMEM supplemented with 10% fetal bovine serum. All cultures were used for experimental purposes 4 days after plating.

**Immunoprecipitation**—Cultures (one 60-mm dish) were washed twice with DMEM and equilibrated in 5 ml of the same medium at 37 °C for 1–2 h. Some dishes were treated with inhibitors during this equilibration period, with growth factors removed 10 min at the end of this period as indicated. Cultures were lysed in 1.4 ml of ice-cold lysis buffer A (10 mm Tris-HCl, pH 7.6, 5 mm EDTA, 50 mm NaCl, 30 mm sodium pyrophosphate, 50 mm NaF, 1% Triton X-100) supplemented with 2 mm Na\textsubscript{3}VO\textsubscript{4}, 1 mm 4-(aminoethyl)-benzenesulfonyl fluoride and 0.1% SDS. Lysates were clarified by centrifugation at 20,800 \times g for 10 min at 4 °C. In some experiments, the cultures were washed and lysates described above, with the exception that DMEM without phenol red was used, and the lysates were adjusted to 1 mg protein/ml after protein tyrosine phosphorylation determination using the biocinchoninic acid protein assay (Pierce). 1 ml of the lysate was immunoprecipitated at 4 °C for 3–4 h with anti-mouse IgG-agarose-linked mAb directed against phosphotyrosine (4G10 or PY72), p125\textsuperscript{akh} (2AT), paxillin, or p130\textsuperscript{csk} with Protein A-agarose-
linked rabbit antisera directed against p125\textsuperscript{akh}.

**Western Blot Analysis**—Immunoprecipitates were washed three times by centrifugation with lysis buffer A supplemented with 1 mm Na\textsubscript{3}VO\textsubscript{4} and extracted in 1× Laemmli sample buffer containing 1× EDTA for 10 min at 100 °C. The solubilized proteins were fractionated by discontinuous SDS-polyacrylamide gel electrophoresis (53) under reducing conditions on an 8% acrylamide resolving gel. Proteins were trans-
ferred onto polyvinylidene fluoride membranes (Immobilon\textsuperscript{TM}-P) at 4 °C for 2 h at 35 V and 2 h at 70 V in 48 mm Tris, 386 mm glycine, 0.1% SDS, and 20% methanol. The membranes were then blocked with 1% polyvinylpyrrolidone, 0.05% Tween 20, 0.02% sodium azide in phos-
dephosphate-buffered saline, pH 7.4 (blocking buffer) (54) and incubated for 2 h with anti-phosphotyrosine (anti-Tyr(P)) mAb (4G10, 1 μg/ml) in blocking buffer. For some experiments, cultures were immunoprecipi-
tated with anti-paxillin and with anti-p130\textsuperscript{csk} mAb in duplicate, and parallel Western blots were probed with anti-Tyr(P) antibody and with the immunoprecipitating antibody to verify that equivalent amounts of protein were immunoprecipitated. Bound antibodies were visualized by the binding of 32P\textsuperscript{p}-labeled anti-mouse IgG (0.1 μCi/ml) followed by autoradiography. After autoradiographic detection of both anti-Tyr(P) mAbs, immunoprecipitates were re-
proteins were fractionated by SDS-polyacrylamide gel electrophoresis on 10% acrylamide resolving gels. Proteins were transferred onto Immobilon\textsuperscript{TM}-P polyvinylidene fluoride membranes at 4 °C for 3 h at 400 mA in 25 mm Tris, 192 mm glycine, and 20% methanol. After blocking the membranes as above, immunoreactive Go subunits were visualized with rabbit antiserum directed against Go\textsubscript{12}, Go\textsubscript{13}, Go\textsubscript{i-2} or Go\textsubscript{i-1} at 1:500–1:1,000 dilution, 125I-labeled Protein A (0.1 μCi/ml), and autoradiography. The Go\textsubscript{12} antiserum was raised against the synthetic peptide CLHDNLKQLMLQ (which corresponds to the carboxyl-terminal peptide 367–377 of murine Go\textsubscript{13} with an N-terminal cysteine added for coupling) cross-linked to keyhole limpet hemocyanin with N-hydroxysuccinimide (NHS)-N-hydroxysuccinimide (NHS)-
phenyl) butyrate as described by Offermanns et al. (55). The Go\textsubscript{13} anti-
serum was raised against a synthetic peptide (corresponding to amino acid residues 119–133 of murine Go\textsubscript{13}) cross-linked to keyhole limpet hemocyanin with glacial acetic acid.

**RESULTS**

**Thrombin-mediated Tyrosine Phosphorylation of Focal Adhesion Proteins in HEK 293 Cells**—Elements of the signal transduction pathways mediating the tyrosine phosphorylation of focal adhesion proteins downstream of GPCRs have been well characterized in Swiss 3T3 cells. We were interested in using a transient transfection system to analyze the ability of the Go subfamily of heterotrimeric G-proteins to induce tyros-
ine phosphorylation of focal adhesion proteins. We elected to use HEK 293 cells for these studies because these cells can be efficiently transfected. However, signal transduction pathways exhibit a degree of heterogeneity in different cell types. Consequently, we initially examined tyrosine phosphorylation induced by ligands of GPCRs in HEK 293 cells. As thrombin causes GPCR-mediated activation of Go\textsubscript{12} and
Ga$_{12}$ and Ga$_{13}$ Stimulate Protein Tyrosine Phosphorylation

Ga$_{13}$ (43, 44, 55), we examined the effects of thrombin on tyrosine phosphorylation in HEK 293 cells. Lysates from these cells treated with or without thrombin were immunoprecipitated with anti-Tyr(P) mAb PY72 or mAbs directed against p125$_{fak}$, paxillin, and p130$_{cas}$. The immunoprecipitates were then subjected to Western blot analysis with the anti-Tyr(P) mAb 4G10. As illustrated by Fig. 1 (left panel), treatment of HEK 293 cells with thrombin resulted in increased tyrosine phosphorylation of proteins with apparent $M_r$ of 110,000–130,000, 97,000, and 60,000–70,000. This pattern is identical to that elicited by GPCR agonists in Swiss 3T3 cells (3–6, 13, 14). Fig. 1 (right panels) shows that thrombin stimulated tyrosine phosphorylation of p125$_{fak}$, paxillin, and p130$_{cas}$ in HEK 293 cells. p125$_{fak}$ and paxillin exhibit a greater increase in tyrosine phosphorylation in response to thrombin than does p130$_{cas}$. Western blot analysis confirmed that similar amounts of p125$_{fak}$, paxillin, and p130$_{cas}$ protein were immunoprecipitated (data not shown). Stimulation of HEK 293 cells with LPA resulted in an identical pattern of protein tyrosine phosphorylation and in increased tyrosine phosphorylation of p125$_{fak}$, paxillin, and p130$_{cas}$ (data not shown).

**FIG. 1.** Thrombin induces tyrosine phosphorylation of p125$_{fak}$, paxillin, and p130$_{cas}$ in HEK 293 cells. HEK 293 cells were washed and incubated for 1 h in DMEM before treatment for 10 min with 1 NIH unit of thrombin/ml (+) or solvent (−). Tyrosine phosphorylation was then analyzed by immunoprecipitation (IP) with anti-Tyr(P) mAb PY72 (PY), mAb 2A7 directed against p125$_{fak}$ (FAK), mAb directed against paxillin (PAX), or mAb directed against p130$_{cas}$ (CAS) and Western blotting (Blot) the immunoprecipitates with anti-Tyr(P) mAb 4G10 (PY). Molecular mass markers (in kDa) are indicated on the left.

**FIG. 2.** Thrombin-induced tyrosine phosphorylation is prevented by cytochalasin D but is not inhibited by GF109203X, pertussis toxin, thapsigargin, or U73122. HEK 293 cells were washed and incubated in DMEM before treatment with inhibitors (+) or solvent (−) as indicated. Cells were incubated for 1 h in 3.5 μM GF109203X (GPT), for 2 h in 2 μM cytochalasin D (CytoD), for 2 h in 30 ng/ml pertussis toxin (PTx), for 30 min in 30 nM thapsigargin, and for 1.5 h in 10 μM U73122, as indicated. The cells were then lysed, and tyrosine phosphorylation was analyzed by immunoprecipitation (IP) of the cell lysates with rabbit antiserum directed against p125$_{fak}$ (FAK), mAb directed against paxillin (PAX), or mAb directed against p130$_{cas}$ (CAS) and Western blotting (Blot) the immunoprecipitates with anti-Tyr(P) mAb 4G10 (PY) as indicated. The Western blots of the p125$_{fak}$ immunoprecipitates (FAK) were reblotted with rabbit antiserum directed against p125$_{fak}$ (FAK). The positions of immunoreactive p125$_{fak}$ at apparent $M_r$ 125,000, paxillin at apparent $M_r$ 60,000–70,000, and p130$_{cas}$ at apparent $M_r$ 130,000 are indicated by the arrows to the left of each set of panels.
Ga\(_{12}\) and Ga\(_{13}\) Stimulate Protein Tyrosine Phosphorylation

Fig. 3. Ga\(_{12}\), Ga\(_{13}\), and Ga\(_{13}QL\) induce tyrosine phosphorylation of p125fak and paxillin in HEK 293 cells. HEK 293 cells were transiently transfected using lipofectAMINE with the following vectors (Ga\(_{a}\)): pcDNA1 without Ga insert (−) or pcDNA1 encoding constitutively active mutant Ga\(_{12}\)QL (12\({}^{+}\)), constitutively active mutant Ga\(_{13}\)QL (13\({}^{+}\)), constitutively active mutant Ga\(_{12}\)QL (12\({}^{-}\)), or constitutively active mutant Ga\(_{13}\)QL (13\({}^{-}\)). Three days after transfection, the cells were washed and incubated for 1 h in DMEM before treatment for 10 min with 1 NIH unit thrombin/ml (+) or solvent (−), and the cells were lysed. Upper panel, total tyrosine phosphorylation was analyzed by immunoprecipitation (IP) of the cell lysates with anti-Tyr(P) mAb PY72 (PY) and Western blotting (Blot) of the immunoprecipitates with anti-Tyr(P) mAb 4G10 (PY). The positions of the migration of the molecular weight markers (in kDa) are indicated to the left. Middle panels, tyrosine phosphorylation of individual proteins was also analyzed by immunoprecipitation (IP) of the cell lysates with anti-Tyr(P) mAb PY72 (PY) and Western blotting (Blot) of the immunoprecipitates with anti-Tyr(P) mAb 4G10 (PY). The positions of the migration of the molecular weight markers (in kDa) are indicated to the left. Lower panels, levels of expression of Ga subunits were analyzed by Western blotting (Blot) aliquots of total cell lysates with antisera directed against Ga\(_{12}\) (α12), Ga\(_{13}\) (α13), Ga\(_{i}\)/(Ga\(_{i}\)) (αi), or Ga\(_{13}\) (αq). The positions of immunoreactive p125fak and paxillin are indicated by the arrows to the left.

To examine the effects of Ga subunits on tyrosine phosphorylation, HEK 293 cells were transiently transfected with expression plasmids encoding the constitutively active Ga mutants Ga\(_{12}\)QL, Ga\(_{13}\)QL, and Ga\(_{13}\)QL, which are deficient in GTPase activity (37, 49, 50). Given that pertussis toxin does not interfere with agonist-stimulated tyrosine phosphorylation of focal adhesion proteins, Ga\(_{a}\) was tested as a negative control. Conversely, because PKC activation leads to tyrosine phosphorylation of p125fak, paxillin, and p130\(_{cas}\), which stimulates phospholipase C-mediated phosphoinositide hydrolysis and thereby PKC (61–63), was included as a positive control. The extracts of transfected cells were immunoprecipitated with the anti-Tyr(P) mAb PY72, and the immunoprecipitates were analyzed by SDS-polyacrylamide gel electrophoresis and Western blotting with the anti-Tyr(P) mAb 4G10. As illustrated by Fig. 3 (upper panel), HEK 293 cells overexpressing constitutively active mutant Ga\(_{12}\)QL, Ga\(_{13}\)QL, or Ga\(_{13}\)QL proteins exhibited increased tyrosine phosphorylation of a set of proteins with apparent M\(_{r}\) of 110,000–130,000, 97,000, and 60,000–70,000. In contrast, transient transfection of HEK 293 cells with activated Ga\(_{12}\)QL expression plasmids did not increase tyrosine phosphorylation. Overexpression of wild-type Ga\(_{12}\) and Ga\(_{12}\) in HEK 293 cells also did not induce tyrosine phosphorylation (data not shown), suggesting that the effects of Ga\(_{12}\)QL, Ga\(_{13}\)QL, and Ga\(_{13}\)QL were specific for the activated state of these Ga subunits rather than due to the modulation of \(\beta\) subunit availability. No stimulation of protein tyrosine phosphorylation was observed after treatment of HEK 293 cells with medium conditioned by cells transfected transiently with activated Ga\(_{12}\)QL or Ga\(_{13}\)QL expression plasmids (data not shown), suggesting that the increased tyrosine phosphorylation was not mediated by secreted factors.

The pattern of increased total tyrosine phosphorylation in response to the constitutively active mutants Ga\(_{12}\)QL, Ga\(_{13}\)QL, or Ga\(_{13}\)QL was identical to that induced by treatment of HEK 293 cells with thrombin, LPA, or phorbol 12,13-dibutyrate. As p125fak and paxillin are tyrosine-phosphorylated in response to these stimuli in HEK 293 cells, we investigated whether these same proteins are tyrosine phosphorylated in response to activated Ga\(_{12}\), Ga\(_{13}\), and Ga\(_{i}\) in HEK 293 cells. As illustrated by Fig. 3 (middle panel), HEK 293 cells transfected with Ga\(_{12}\)QL, Ga\(_{13}\)QL, or Ga\(_{i}\)QL subunits exhibited increased tyrosine phosphorylation of p125fak and paxillin. Western blot analysis confirmed that similar amounts of p125fak were immunoprecipitated under all conditions. As shown in Fig. 3 (lower panel), we confirmed that the cells transfected with the Ga\(_{12}\)QL, Ga\(_{13}\)QL, Ga\(_{i}\)QL, or Ga\(_{i}\)QL expression plasmids were overexpressing these Ga subunits.

Tyrosine Phosphorylation in Response to Ga\(_{12}\)QL and Ga\(_{13}\)QL Requires an Intact Actin Cytoskeleton—Agonist-stimulated tyrosine phosphorylation of p125fak, paxillin, and p130\(_{cas}\) is inhibited by treatment of Swiss 3T3 cells or HEK 293 cells with cytochalasin D, which selectively disrupts the actin cytoskeleton (3, 14, 15, 64). To determine whether the increase in the tyrosine phosphorylation of p125fak and paxillin induced by activated Ga\(_{12}\) or Ga\(_{13}\) is dependent upon an intact actin cytoskeleton, we tested the effect of cytochalasin D on tyrosine phosphorylation in HEK 293 cells transfected transiently with the Ga\(_{12}\)QL or Ga\(_{13}\)QL expression vectors. As shown in Fig. 4 (upper panels), cytochalasin D inhibited the Ga\(_{12}\)QL- and Ga\(_{13}\)QL-induced tyrosine phosphorylation of p125fak and paxillin. Western blotting of total cell lysates with antisera to the Ga subunits demonstrated that cytochalasin D treatment did not alter expression levels of the transfected Ga subunits (Fig. 4, lower panels). Cytochalasin D also inhibited Ga\(_{i}\)QL-induced tyrosine phosphorylation (data not shown).

Ga\(_{12}\)QL and Ga\(_{13}\)QL Induce Tyrosine Phosphorylation of p125fak, P2X1R, and p130\(_{cas}\) in a Rhino-dependent Manner—The C. botulinum C toxin, which specifically ADP-ribosylates RhoA, RhoB, and RhoC, resulting in the functional inhibition of these GTP-binding proteins, can be used to determine whether a cellular response requires functional Rho (65). This approach has been used to demonstrate that in Swiss-3T3 cells, bombesin-, LPA- and toxin-stimulated tyrosine phosphorylation of p125fak, paxillin, and p130\(_{cas}\) requires functional Rho (25–27). To determine whether the Ga\(_{12}\)QL or Ga\(_{13}\)QL-induced increases in the tyrosine phosphorylation of p125fak and paxillin are dependent upon Rho, we cotransfected HEK 293 cells with either the Ga\(_{12}\)QL or the Ga\(_{13}\)QL expression vector and with a C3 toxin expression vector. We then immunoprecipitated lysates from the transfected cells with the anti-Tyr(P) mAb PY72, and the immunoprecipitates were analyzed by Western
Western blotting was analyzed by immunoprecipitation (IP) of the cell lysates with mAb 2A7 directed against p125 fak (FAK) or anti-Tyr(P) mAb 4G10 (PY) and Western blotting (Blot) the immunoprecipitates with anti-Tyr(P) mAb 4G10 (PY). The Western blot of the mAb anti-p125 fak immunoprecipitates (FAK) was reprobed with rabbit antiserum directed against p125 fak (FAK). The positions of immunoreactive p125 fak at apparent Mr, 125,000 and paxillin at apparent Mr, 60,000–70,000 are indicated by the arrows on the left. Lower panels, levels of expression of Ga subunits were analyzed by Western blotting (Blot) aliquots of the total cell lysates with antisera directed against Ga12 (α12) or Ga13 (α13). The positions of immunoreactive Ga12 (α12*) and Ga13 (α13*) at apparent Mr, 43,000 are indicated by the arrows on the left.

blotting with the anti-Tyr(P) mAb 4G10. As illustrated by Fig. 5 (upper panel), the increase in the tyrosine phosphorylation of proteins with apparent Mr, 110,000–130,000, 97,000, and 60,000–70,000 induced by either Ga12QL or Ga13QL was inhibited by cotransfection with the C3 toxin expression vector. The Ga12QL-induced tyrosine phosphorylation of this set of proteins was also inhibited by cotransfection with the C3 toxin expression vector (data not shown). In addition, we immunoprecipitated lysates of cotransfected cells with mAbs directed against p125 fak, paxillin, and p130 cas and subjected the immunoprecipitates to Western blot analysis with the anti-Tyr(P) mAb 4G10. As shown in Fig. 5 (middle panels), the Ga12QL- and Ga13QL-induced tyrosine phosphorylation of p125 fak, paxillin, and p130 cas was inhibited by cotransfection with the C3 toxin expression vector. Western blotting with antiserum to the Ga subunits demonstrated that cotransfection with C3 toxin plasmid did not alter the expression levels of the transfected Ga subunits (Fig. 5, lower panels).

We verified that thrombin-induced tyrosine phosphorylation of the focal adhesion proteins p125 fak, paxillin, and p130 cas in HEK 293 cells was also inhibited by transfection with the C3 toxin expression vector. In contrast, epidermal growth factor-induced tyrosine phosphorylation of a protein of apparent Mr, 170,000 (presumably the epidermal growth factor receptor) was not inhibited by transfection of HEK 293 cells with the expression plasmid encoding C3 toxin, indicating that this toxin inhibited tyrosine phosphorylation of focal adhesion proteins in a selective manner (results not shown).

DISCUSSION

Activation of GPCRs for mitogenic neuropeptides and bioactive lipids leads to a pertussis toxin-insensitive tyrosine phosphorylation of multiple protein substrates including the nonreceptor tyrosine kinase p125 fak and the adaptor proteins paxillin and p130 cas. The mechanism(s) linking activation of GPCRs to increases in protein tyrosine phosphorylation remains poorly understood. In the present study, we have used transient transfection of HEK 293 cells with expression plasmids encoding constitutively active Ga12 and Ga13 to investigate the involvement of the G12 subfamily of Ga proteins in tyrosine phosphorylation of the nonreceptor tyrosine kinase p125 fak and of the adaptor proteins paxillin and p130 cas. We report for the first time that HEK 293 cells expressing constitutively active Ga12 and Ga13 mutants exhibit increased tyrosine phosphorylation of p125 fak, paxillin, and p130 cas. Identical increases in tyrosine phosphorylation of these focal adhesion proteins were observed in HEK 293 cells treated with thrombin. This is noteworthy with reference to the previous reports that thrombin receptors couple to Ga12 and Ga13 in platelets and to Ga12 in astrocytoma cells (43, 55) and, in particular, that fibroblasts lacking Ga13 exhibit a greatly diminished migratory
These observations suggest that GAP-ribosylates and functionally inactivates Rho (25, 26, 66). And activation of a GDP/GTP exchange factor. Activated Rho p125fak, paxillin, and p130 cas. This hypothesis warrants further experimental work.

It has been proposed that the mitogen-induced increases in tyrosine phosphorylation of p125fak, paxillin, and p130 cas are downstream of Rho activation, stress fiber formation, and focal adhesion assembly (4, 25–28, 67, 68). Activated Gα12 and Gα13 have been shown to induce Rho activation (69) and Rho-dependent biological responses, including stress fiber formation and focal adhesion assembly (42, 70, 71). These results suggest that these G-proteins may couple GPCRs to increases in tyrosine phosphorylation of p125fak, paxillin, and p130 cas.

There is increasing evidence indicating that Gα12 and Gα13 are involved in cell migration, proliferation, and transformation (36, 38–40, 43, 44, 48). Downstream targets through which Gα12 and Gα13 act may include Ras-, Rac-, Rho-, and Cdc42-dependent pathways leading to cytoskeletal reorganization and to the activation of mitogen-activated protein kinase, Jun N-terminal kinase, the Na+–H+ exchanger, and the c-fos serum response element (40–42, 46–48). In this context, our results revealing that activated Gα12 and Gα13 induce increases in the level of tyrosine phosphorylation of p125fak, paxillin, and p130 cas suggest novel mechanisms of action of these Gα subunits. These findings assume an added importance in view of increasing evidence implicating p125fak and p130 cas in cell migration, proliferation, and transformation. Gene disruption experiments have demonstrated a critical role of p125fak in embryonic development, cell migration, and turn-over of focal adhesions (29, 74), and microinjection of dominant negative fragments of p125fak prevented serum stimulation of DNA synthesis (75). The adapter protein p130 cas has also been implicated in agonist-stimulated mitogenesis and in cell transformation (12, 20, 76) and has recently been identified as a mediator of p125fak-mediated cell migration (31). Interestingly, embryonic fibroblasts lacking Gα13 also display a greatly impaired migratory response to thrombin receptor activation (44). Taken together with the results presented here, these findings raise the attractive possibility that p125fak and p130 cas are downstream targets of Gα13 in a signal transduction pathway that regulates cell migration in response to GPCR agonists. Future studies should assess the contribution of increased tyrosine phosphorylation of p125fak, paxillin, and p130 cas to the growth promoting and transforming activities of Gα12 and Gα13.

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