Signaling from G Protein-coupled Receptors to ERK5/Big MAPK 1 Involves Gaq and Gα12/13 Families of Heterotrimeric G Proteins

EVIDENCE FOR THE EXISTENCE OF A NOVEL Ras AND Rho-INDEPENDENT PATHWAY*

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The regulation of gene expression by cell surface receptors often involves the stimulation of signaling pathways including one or more members of the MAPK superfamily of serine-threonine kinases. Upon their activation in the cytosol, MAPKs can translocate to the nucleus and affect the activity of a variety of transcription factors. Recently, it has been observed that a novel member of the MAPK superfamily, ERK5, can be potently activated by transforming G protein-coupled receptors (GPCRs) and that ERK5 participates in the regulation of c-jun expression through the activation of MEF2 transcription factors. How cell surface receptors, including GPCRs, stimulate ERK5 is still poorly understood.

In this study, we have used transiently transfected COS-7 cells to begin delineating the biochemical route linking GPCRs to ERK5. We show that receptors that can couple to the Gaq and G12/13 families of heterotrimeric G proteins, m1 and thrombin receptors, respectively, but not those coupled to Ga1, such as m2 receptors, are able to regulate the activity of ERK5. To investigate which heterotrimeric G proteins signal to ERK5, we used a chimeric system by which Gaq and G12/13-mediated signaling pathways can be conditionally activated upon ligand stimulation. Using this system, as well as the expression of activated forms of G protein subunits, we show that the Gaq and G12/13 families of heterotrimeric G proteins, but not the Ga1, Gaq, and Gβy subunits, are able to regulate ERK5. Furthermore, we provide evidence that the stimulation of ERK5 by GPCRs involves a novel signaling pathway, which is distinct from those regulated by Ras and Rho GTPases.

Mitogen-activated protein kinases (MAPKs)1 are serine-threonine protein kinases that play a central role in the transduction of environmental stimuli to the nucleus, thereby regulating the expression of genes involved in a variety of cellular processes, including cell proliferation, differentiation, programmed cell death, and neoplastic transformation (1, 2). To date, MAPKs have been classified into at least six subfamilies: p44mapk and p42mapk, also called extracellular signal-regulated kinases (ERKs) 1 and 2, respectively (referred to here as MAPK); c-Jun N-terminal kinases (JNKs), also termed stress-activated protein kinases; p38 MAPKs; ERK5, also known as big MAPK 1; and the recently identified ERK7 (3), and MOK (4) (see Ref. 5 for a review). These kinases are activated by a wide variety of extracellular stimuli such as growth factors, hormones, antigens, and cytokines and can also be stimulated in response to a diverse array of cellular stresses, such as UV irradiation, oxidative stress, and heat and osmotic shock (2).

Many cell surface receptors can effectively stimulate MAPK cascades to signal to the nucleus, including the large family of receptors that transduce signals through the activation of heterotrimeric GTP-binding proteins (G proteins) (6). For example, receptors coupled to Gaq proteins can potentially stimulate MAPK (7), and that appears to be mediated primarily through the release of Gβy subunits (8) and the activation of a complex biochemical route involving phosphatidylinositol 3-kinases (9) and several nonreceptor and receptor tyrosine kinases (see Refs. 6 and 10 for reviews). In turn, activation of these tyrosine kinases leads to the phosphorylation of an adaptor protein, She, and the recruitment of the Grb2Sos complex to the plasma membrane thus stimulating the exchange of GDP for GTP on Ras GTPases. This promotes the activation of a kinase cascade including Raf and MEK, which culminates with the activation of MAPK (1, 11). Signals generated by the activation of G protein-coupled receptors (GPCRs) can also be transmitted through large MAPK 1, and by Gaq, which can activate Pyk2 and Src (15, 16), and can stimulate Raf through protein kinase C (17). Similarly, JNK and p38 MAPKs have been shown to be activated by ligands acting on GPCRs, by the release of Gβy dimers (18) and through the Gaq and Ga12/13 classes of G proteins (19, 20). However, signaling pathways from GPCRs to these MAPKs are still largely unknown, although they appear to involve the activation of the small GTPases Rac1 and Cdc42 by Gβy dimers and RhoA and Rac1 by members of the Ga12/13 class of G proteins (18, 21–23).

Recently, we have observed that a novel member of the MAPK superfamily, ERK5, can be potently activated by transforming GPCRs and provided evidence that ERK5 participates in the regulation of c-jun expression by GPCRs through the activation of members of the MEF2 class of transcription factors (24). ERK5 exhibits an extended C-terminal tail, which is absent in other types of MAPKs, suggesting that the regulation and function of this kinase might be different from that of other MAPKs (25, 26). How cell surface receptors, including GPCRs, stimulate ERK5 is still unknown. In this study, we have used transfected and endogenously expressed GPCRs and the co-

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‡ The abbreviations used are: MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; G proteins, GTP-binding proteins; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase; GPCRs, G protein-coupled receptors; HA, hemagglutinin; CRIB domain, Cdc42, Rac interactive binding domain; CAT, choleraeumphiol acetyltransferase; SRE, serum response element; GST, glutathione S-transferase; GFP, green fluorescent protein; PAR1 protease activated receptor 1.
expression of GPCRs with chimeric G protein α subunits to begin delineating the biochemical route linking GPCRs to ERK5. We show that the Goq and Go12/13 families of heterotrimeric G proteins α subunits, but not the Goq, Go13, or βγ subunits, are able to regulate ERK5 activity. Furthermore, we obtained evidence that the stimulation of the ERK5 cascade by GPCRs involves a novel pathway, which is distinct from those regulated by Ras and Rho GTPases.

**EXPERIMENTAL PROCEDURES**

**Expression Plasmids**—PAR1, kindly provided by Dr. L.F. Brass, was subcloned into the pCEFL vector as an EcoRI fragment. DNA encoding a G13/Gi chimera, in which 5 amino acids at the C terminus of Go13 were replaced by the corresponding sequence of Go13, was prepared by polynucleotide chain reaction amplification using pCDNA3 HA-Go13 (27) as a template, and the resulting DNA was subcloned into the pCEFL HA vector (24) as a Bg/II-EcoRI fragment. Sequences of mutagenic oligonucleotides will be made available upon request. Plasmids expressing epitope-tagged ERK5, MAPK, and JNK, pCFEL HA-ERK5, pCDNA HA-MAPK, and pCDNA HA-JNK, respectively, as well as expression plasmids for constitutively activated forms of Ras, Rac1, Cdc42, Go13, Go12, Goa, Go13, Go13, Go13, and Go13 subunits of G proteins, dominant negative mutants of Ras and Rho, the CRIB (Cdc42, Rac interactive binding) domain of PAK (PAK-N), m1 and m2 muscarinic receptors, a Gal4 fusion protein containing the transactivating domain of MEF2C and dominant negative and active mutants of MEK5, MEK5AA, and MEK5DD, respectively, were described previously (21, 24, 27, 28). A DNA plasmid encoding a Go13 chimeric protein, in which 5 amino acids at the C terminus of Go13 were replaced by the corresponding sequence of Go13, was a gift from Dr. B. Conklin (29). Reporter plasmids that express the chloramphenicol acetyltransferase (CAT) gene under the control of the mutant form of the serum response element (SRE) from the c-fos promoter, lacking the ternary complex factor binding site (SREmutL) as well as an expression vector for the C3 toxin were kindly provided in here as HA-MAPK and JNK (HA-JNK) according to the manufacturer’s protocol. In each experiment, the total amount of DNA was adjusted to 3–10 μg/plate with a plasmid for green fluorescent protein.

**Kinase Assays**—The ERK5 kinase activity in cells transfected with an expression plasmid for HA-ERK5 was measured as described previously (24), using 3 μg of GST-ME2F2 fusion protein containing the transactivating domain of ME2F2 as a substrate. MAPK and JNK activities in cells transfected with an epitope-tagged MAPK (HA-ERK2, referred in here as HA-MAPK) or JNK (HA-JNK) were determined as described previously (21), using myelin basic protein (Sigma) or bacterially expressed GSTTAP92/96 fusion protein as a substrate, respectively. The expression level of HA-ERK5, HA-MAPK, and HA-JNK in lysates from transfected cells was assessed by Western blot analysis according to the manufacturer’s protocol. In each experiment, the total amount of DNA was adjusted to 3–10 μg/plate with a plasmid for green fluorescent protein.

**Reporter Gene Assays**—The transactivating activity of MEF2C and the SRE activity were determined as described previously (24, 27). Briefly, for ME2F2, COS-7 cells plated in a 24-well plate were transfected with different expression plasmids together with 2 ng of pCDNAII-Gal4-ME2F2, a plasmid expressing a Gal4 fusion protein containing the transactivating domain of ME2F2 (amino acids 161–350) as well as 50 ng of pGal4-Luc and 10 ng of pRL-null (Promega). To measure the SRE activity, COS-7 cells were transfected with the indicated plasmids together with 0.1 μg of pCDNAII-I-β-galactosidase, a plasmid expressing the enzyme β-galactosidase, and 0.1 μg of pSV-β-gal, the plasmid reporter expressing a CAT gene under the control of the mutant SRE lacking a ternary complex factor binding site. After transfection, cells were cultured for 24 h in serum-free Dulbecco’s modified Eagle’s medium, then stimulated with the indicated ligands for an additional 6 h, and lysed using reporter lysis buffer (Promega). Luciferase activities in cell extracts were determined using a dual luciferase assay system (Promega). CAT activity was assayed in the cell extracts by incubation at 37 °C for 1 h in the presence of 0.25 μCi of [3H]chloramphenicol (100 μCi/mmol) (ICN) and 200 μg/ml butyryl-CoA (Sigma) in 0.25 M Tris-HCl, pH 7.4. Labeled butyrylated products were extracted using a mixture of xylene and 2,6,10,14-tetramethylpentadecane (ratio 1:2), and radioactivity was counted. β-Galactosidase activity present in each sample was assayed by a colorimetric method and was used to normalize for transfection efficiency.

**RESULTS AND DISCUSSION**

**Coupling Specificity of G Protein-linked Receptors Stimulating ERK5 Kinase Activity**—ERK5 has been recently found to participate in the regulation of the c-jun promoter by transforming GPCRs (24). To begin exploring the nature of the pathway linking these cell surface receptors to ERK5, we first investigated which classes of GPCRs are able to stimulate ERK5 kinase activity. For these experiments, COS-7 cells were transiently transfected with expression plasmids for a Ha-tagged form of ERK5, and its kinase activity was measured by an in vitro kinase assay using MEF2F2 fused to GST as a substrate. As shown in Fig. 1A, stimulation by carbachol, a cholinergic agonist, of transfected Goq-coupled m1 muscarinic receptors (31) potently activated ERK5. Similarly, stimulation with a tyrosine kinase receptor agonist, epidermal growth factor, also enhanced ERK5 kinase activity, as recently reported (32, 33). However, the stimulation of m2 muscarinic receptors, which are typical Gi-coupled receptors (34), had no effect on ERK5 activity, although it potently activated MAPK, which served as an internal control (8). ERK5 activity was also stimulated by exposure to thrombin, which acts on endogenously expressed GPCRs, and this effect was slightly enhanced by overexpression of its cognate receptors, PAR1 (Fig. 1A). Kinetics of ERK5 activation mediated by m1 and thrombin receptors were very similar, and responses were evident within 5 min after agonist addition and reached a maximal level around 10 min (Fig. 1B). As m1 and m2 muscarinic receptors couple to Goq and Go13 types of heterotrimeric G proteins, respectively, and thrombin receptors can stimulate both the Goq and Go13, as well as the Go11/13 families of G proteins (35–38), these findings suggest that receptors coupled to Goq and, possibly Go11/13, may harbor the ability to transduce a signal to ERK5, whereas Gq-coupled receptors do not.

**The MEK5-ERK5 Kinase Cascade Is Involved in the Activation of MEF2C Transcriptional Activity Mediated by G Protein-coupled Receptors**—MEF2C is a physiological substrate for ERK5 (39). Thus, we next asked whether the ability to enhance the in vitro phosphorylating activity of ERK5 by GPCRs resulted in enhanced transcriptional activity of MEF2C proteins in vivo. For these experiments, we fused the transactivation domain of MEF2C to the DNA binding domain of Gal4 and tested the ability to induce the expression from the pGal4-Luc reporter plasmid, as described previously (24). As shown in Fig. 2A, expression from the Gal4-driven luciferase reporter was induced by the stimulation of m1 and thrombin receptors, but not m2 receptors, which is consistent with their abilities to stimulate ERK5 kinase activity. Furthermore, transfection of a DNA plasmid for MEK5AA, which acts as a dominant negative mutant of MEK5 (24), completely blocked the increased transcriptional activity of MEF2C elicited by thrombin and partially inhibited m1 mediated-transcriptional activation (Fig. 2B). As a control, the activation of SRE mediated by m1 and thrombin receptors was unaffected by co-expression of MEK5AA (Fig. 2B). These findings suggested that the MEK5-ERK5 kinase pathway is functionally activated by GPCRs and that this kinase cascade is involved in the activation of MEF2C by m1 and thrombin receptors.

**Activated Forms of Goq and Go13 Stimulate ERK5**—To investigate which classes of G proteins mediate ERK5 activation induced by GPCRs, we examined the effects of activated forms of Ga subunits as well as overexpression of βγ subunits of heterotrimeric G proteins on ERK5 kinase activity. As shown in Fig. 3A, expression of Ga12QL and Go13QL could induce ERK5 activation, whereas ERK5 kinase activity was not al-
Differential activation of MAPK and ERK5 by G protein-coupled receptors exhibiting distinct coupling specificity. COS-7 cells were transfected with expression plasmids for HA-ERK5 or HA-MAPK, together with plasmids expressing GFP, m1, or PAR1 receptors, as indicated, and stimulated with vehicle (c), 100 μM carbachol (Cch), 5 units/ml thrombin (Thr), or 100 ng/ml epidermal growth factor (EGF) for 10 min (A) or for the indicated time (B). Kinase reactions were performed using anti-HA immunoprecipitates from the corresponding cellular lysates. Labeled substrates are indicated. Data shown are from a representative experiment for each assay, which was repeated three to five times with similar results. Western blot (WB) analysis was performed with anti-HA antibodies using total cellular lysates. Data represent the mean ± S.E. of three to five independent experiments expressed as fold increase with respect to unstimulated cells (control).

Fig. 1. Differential activation of MAPK and ERK5 by G protein-coupled receptors exhibiting distinct coupling specificity. COs-7 cells were transfected with expression plasmids for HA-ERK5 or HA-MAPK, together with plasmids expressing GFP, m1, or PAR1 receptors, as indicated, and stimulated with vehicle (c), 100 μM carbachol (Cch), 5 units/ml thrombin (Thr), or 100 ng/ml epidermal growth factor (EGF) for 10 min (A) or for the indicated time (B). Kinase reactions were performed using anti-HA immunoprecipitates from the corresponding cellular lysates. Labeled substrates are indicated. Data shown are from a representative experiment for each assay, which was repeated three to five times with similar results. Western blot (WB) analysis was performed with anti-HA antibodies using total cellular lysates. Data represent the mean ± S.E. of three to five independent experiments expressed as fold increase with respect to unstimulated cells (control).
Signaling from G Protein-coupled Receptors to ERK5 Does Not Involve Ras and Rho GTPases—Gi- and Gq-coupled receptors can stimulate the Ras-MAPK pathway effectively (6). However, we found that Gi-coupled m2 receptors fail to stimulate ERK5, suggesting that the pathway linking GPCRs to ERK5 is different from that which communicates these receptors to MAPK. Indeed, we observed that activated Ras causes only a very limited increase in the enzymatic activity of ERK5, although it potently stimulates MAPK (Fig. 4). On the other hand, activation of the JNK pathway is believed to be mediated by Rac and Cdc42, two members of the Rho family of GTPases (21). However, whereas expression of activated Rac and Cdc42 strongly enhanced the kinase activity of JNK, these GTPases failed to stimulate ERK5 (Fig. 4). Similarly, expression of an activated form of Ras, which stimulates the SRE-driven reporter plasmid potently (30), also failed to enhance the enzymatic activity of ERK5. Furthermore, although some minor variations in the activity of ERK5 can be observed upon expression of these GTPases, an activated form of MEK5, MEK5DD, consistently induced ERK5 activation under these experimental conditions (Fig. 4). Thus, activation of Ras, Rac, and Cdc42 may not be sufficient to stimulate the ERK5 pathway. However, it is still possible that these small GTPases are required for GPCR-mediated ERK5 activation. To address this possibility, we used the expression of dominant interfering molecules for each of these GTPases. As shown in Fig. 5A, the activation of ERK5 mediated by m1 and thrombin receptors was not affected by the expression of a dominant negative mutant of Ras, RasN17, although this inhibitory molecule effectively inhibited MAPK activation when induced by m1 stimulation (Fig. 5B) and by thrombin (data not shown), but not by phorbol esters, as previously reported (43, 44). Thus, together these data suggest that Ras is unlikely to play a prominent role in ERK5 activation by GPCRs. For Rac and Cdc42, we used the overexpression of a molecule containing the CRIB domain of PAK fused to GST, which can specifically bind the GTP-bound forms of Rac and Cdc42 thereby inhibiting these GTPases (28, 45, 46). Indeed, expression of the CRIB domain of PAK (PAK-N) significantly inhibited JNK activation evoked by the expression of activated forms of Rac and Cdc42 and by m1...
stimulation but had only a limited effect on the activation of JNK by anisomycin, which served as a control for specificity (Fig. 5C). However, PAK-N did not affect the abilities of m1 and thrombin receptors to activate ERK5 (Fig. 5A). Together, these results indicated that the small GTPases Ras, Rac, and Cdc42 are not involved in the signaling from GPCRs to ERK5.

Interestingly, both $G_{aq}$ and $G_{a12/13}$ classes of G proteins, but not $G_{ai}$, have been shown to activate Rho-dependent signaling pathways (42), and recent studies suggested that Rho-specific exchange factors such as p115-RhoGEF and PDZ-RhoGEF could be directly activated by the $G_{a12/13}$ family of G proteins (27, 47). Together, these findings suggested the possibility that Rho may participate in signaling to ERK5. However, an activated form of Rho did not enhance the kinase activity of ERK5 (see above, Fig. 4) and that of any other member of the MAPK superfamily in this cellular setting. Nonetheless, these obser-

![Fig. 4. Activation of Ras- and Rho-related GTPases is not sufficient to stimulate the ERK5 pathway.](image)

**FIG. 4.** Activation of Ras- and Rho-related GTPases is not sufficient to stimulate the ERK5 pathway. COS-7 cells were transfected with expression plasmid for HA-ERK5, HA-MAPK, or HA-JNK together with the plasmid expressing GFP or the activated mutant of H-Ras (RasV12), RhoA (RhoQL), Rac1 (RacQL), Cdc42 (Cdc42QL), or MEK5 (MEK5DD). Kinase reactions were performed using anti-HA immunoprecipitates from the corresponding cellular lysates. Labeled substrates are indicated. Data shown are from a representative experiment for each assay. Western blot (WB) analysis was performed with anti-HA antibodies using total cell lysates (HA-ERK5 and HA-MAPK) or anti-HA immunoprecipitates (HA-JNK).

![Fig. 3. Signaling to ERK5 through $G_{aq}$ and $G_{a12/13}$ families of heterotrimeric G proteins.](image)

**FIG. 3.** Signaling to ERK5 through $G_{aq}$ and $G_{a12/13}$ families of heterotrimeric G proteins. A, effects of activated mutants of G proteins $\alpha$ subunits and $\beta$ subunits on ERK5 kinase activity. COS-7 cells were transfected with an expression plasmid for HA-ERK5 together with plasmids expressing GFP, $G_{aq}$QL, $G_{a12}$QL, $G_{aq}$QL, $G_{a12}$QL, $G_{ai}$QL, or $\beta_{y2}$ subunits. Kinase reactions were performed using anti-HA immunoprecipitates from the corresponding cellular lysates. Data represent the mean ± S.E. of three independent experiments, expressed as fold increase with respect to control cells. B, COS-7 cells were cotransfected with pSREmutL and pCMV-$\beta$-galactosidase plasmid DNAs as well as with the indicated expression vectors and stimulated with vehicle (−) or 100 μM carbachol (+) for 6 h. Cells were processed as described under “Experimental Procedures.” The data represent CAT activity normalized by the $\beta$-galactosidase activity present in each cellular lysate, expressed as fold induction with respect to control cells, and are the mean ± S.E. of triplicate samples from a typical experiment. Nearly identical results were obtained in three additional experiments. C, COS-7 cells were transfected with an expression plasmid for the HA-ERK5 together with the indicated expression vectors and stimulated with vehicle (−) or 100 μM carbachol (+) for 10 min. Kinase reactions were carried out using anti-HA immunoprecipitates from the corresponding cellular lysates. Data represent the mean ± S.E. of three independent experiments, expressed as fold increase with respect to control cells.
FIG. 5. Signaling from G protein-coupled receptors to ERK5 does not require Ras- and Rho-related GTPases. A–C, COS-7 cells were cotransfected with expression plasmid for HA-ERK5 (A), HA-MAPK (B), or HA-JNK (C) as well as with expression vectors carrying cDNAs for GFP, m1, and PAR1 receptors or the activated mutant of Rac1 (RacQL) and Cdc42 (Cdc42QL), together with plasmids encoding inhibitory molecules (RasN17, RhoN19, and PAK-N). Cells were stimulated with or without 100 μM carbachol (Cch), 5 units/ml thrombin (Thr), 100 ng/ml 12-O-tetradecanoylphorbol-13-acetate (TPA), or 10 μg/ml anisomycin as indicated. Kinase reactions were performed using anti-HA immunoprecipitates from the corresponding cellular lysates. Labeled substrates are indicated. Data shown are from a representative experiment for each assay. Western blot (WB) analysis was performed with anti-HA antibodies using total cell lysates (HA-ERK5 and HA-MAPK) or anti-HA immunoprecipitates (HA-JNK). D, COS-7 cells were cotransfected with pSREmutL and pCMV-β-galactosidase plasmid DNAs as well as with expression vectors carrying cDNAs for GFP, m1 and PAR1 receptors, and the activated mutant of Cdc42 (Cdc42QL), with or without expression plasmids for C3 toxin, as indicated. The next day, the cells were stimulated with vehicle, 100 μM carbachol (Cch) or 5 units/ml thrombin (Thr) 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 fold induction with respect to control cells, and are the mean ± S.E. of triplicate samples from a typical experiment. Similar results were obtained in three separate experiments. E, COS-7 cells were transfected with expression vectors carrying DNA for HA-ERK5 and GFP, m1 or PAR1 receptors, with or without expression plasmid for C3 toxin, and stimulated with vehicle, 100 μM carbachol (Cch), 5 units/ml thrombin (Thr), or 100 ng/ml epidermal growth factor (EGF) for 10 min. Kinase reactions were performed in anti-HA immunoprecipitates from the corresponding cellular lysates. Data represent the mean ± S.E. of three independent experiments, expressed as fold increase with respect to unstimulated cells. Autoradiograms correspond to representative experiments. Western blot (WB) analysis was performed in the corresponding cellular lysates and immunodetected with the antibody to HA.
regulations cannot rule out the possibility that Rho stimulates certain MAPKs, which might not be revealed by the expression of its activated mutants for reasons such as those described for Gαq. Thus, to explore further the possibility of the existence of Gq- and G12/13-coupled receptors to stimulate ERK5, we used as a more definitive approach the expression of Clostridium botulinum C3 exoenzyme, which specifically ADP ribosylates Rho thus preventing its activation (48). As shown in Fig. 5D, a C3 toxin inhibited the activation of SRE, a typical Rho-dependent response (30), by m1 and thrombin receptors, although the Cdc42-mediated activation of SRE was unaffected and served as a control. However, this toxin did not change the abilities of m1 and thrombin receptors to activate ERK5 (Fig. 5E), strongly suggesting that the activation of ERK5 by GPCRs is independent of Rho.

In conclusion, the present study demonstrates that the ERK5 pathway can be functionally activated by the stimulation of GPCRs depending on their coupling specificity and that the Gq and G12/13 families of heterotrimeric G proteins can mediate this effect. As ERK5 regulates the activity of a growing number of nuclear transcription factors, these findings may help explain the distinct ability of Gq and G12/13-coupled receptors to promote the expression of growth-related genes. Furthermore, our observations raise the possibility of the existence of a novel signaling pathway whereby GPCRs enhance the activity of ERK5. Although the precise nature of this biochemical route is still unknown, we provide evidence that the pathway linking GPCRs to the MEK5-ERK5 kinase cascade is distinct from those utilized by these cell surface receptors to stimulate MAPK, JNK, and Rho GTPases.

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