Dexras1/AGS-1 Inhibits Signal Transduction from the G_{i}-coupled Formyl Peptide Receptor to Erk-1/2 MAP Kinases*

Received for publication, October 29, 2001, and in revised form, December 19, 2001
Published, JBC Papers in Press, December 21, 2001, DOI 10.1074/jbc.M110397200

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Dexras1 is a novel GTP-binding protein (G protein) that was recently discovered on the basis of rapid mRNA up-regulation by glucocorticoids in murine ACT-20 corticotroph cells and in several primary tissues. The human homologue of Dexras1, termed activator of G protein signaling-1 (AGS-1), has been reported to stimulate signaling by G_{i} heterotrimeric G proteins independently of receptor activation. The effects of Dexras1/AGS-1 on receptor-initiated signaling by G_{i} have not been examined. Here we report that Dexras1 inhibits ligand-dependent signaling by the G_{i}-coupled N-formyl peptide receptor (FPR). Dexras1 and FPR were transiently co-expressed in both COS-7 and HEK-293 cells. Activation of FPR by ligand (N-formyl-methionine-leucine-phenylalanine (f-MLF)) caused phosphorylation of endogenous Erk-1/2 that was reduced by co-expression of Dexras1. Direct effects of Dexras1 on the activity of co-expressed, epitope-tagged Erk-2 (hemagglutinin (HA)-Erk-2) were measured by immune complex in vitro kinase assay. Expression of Dexras1 alone resulted in a 1.9- to 4.9-fold increase in HA-Erk-2 activity; expression of the unliganded FPR alone resulted in a 6.2- to 8.1-fold increase in HA-Erk-2 activity. Stimulation of FPR by f-MLF produced a further 8- to 10-fold increase in HA-Erk-2 activity over the basal (non-ligand-stimulated) state, and this ligand-dependent activity was attenuated at the time points of maximal activity by co-expression of Dexras1 (reduced 31 ± 6.8% in COS-7 at 10 min and 86 ± 9.2% in HEK-293 at 5 min, p < 0.01 for each). Expression of Dexras1 did not influence protein expression of FPR or Erk, suggesting that the inhibitory effects of Dexras1 reflect a functional alteration in the signaling cascade from FPR to Erk. Expression of Dexras1 had no effect on expression of G_{i}\alpha species, but significantly impaired pertussis toxin-catalyzed ADP-ribosylation of membrane-associated G_{i}\alpha. Expression of Dexras1 also significantly decreased in vitro binding of GTP\gammaS in f-MLF-stimulated membranes of cells co-transfected with FPR. These data suggest that Dexras1 inhibits signal transduction from FPR to Erk-1/2 through an effect that is very proximal to receptor-G_{i} coupling. While Dexras1 weakly activates Erk in the resting state, more potent effects are evident in the modulation of ligand-stimulated receptor signal transduction, where Dexras1 functions as an inhibitor rather than activator of the Erk mitogen-activated protein kinase signaling cascade.

Dexras1 is the prototypic member of a recently defined group of Ras-related intermediate molecular weight, basic GTP-binding proteins (G proteins). The group, which includes Dexras1, the activator of G protein signaling 1 (AGS-1) (1), the Ras homologue enriched in striatum (2), tumor endothelial marker 2 (TEM-2) (3), and Drosophila Dexras, is characterized by highly basic net isoelectric points and molecular weights intermediate between those of other Ras family members and the heterotrimeric G protein subunits (4). The increased molecular mass of these proteins is accounted for by a unique carboxyl terminus variable domain that is highly conserved within the group and bears no known structural motifs, except for a terminal consensus sequence for isoprenylation (CAAX box) (4). Dexras1 was first identified by differential display as a murine mRNA that is rapidly and transiently up-regulated by glucocorticoids in anterior pituitary, brain, and other tissues (5). We have reported that expression of Dexras1 in AtT-20 corticotroph cells results in the inhibition of stimulus-coupled peptide hormone secretion (4). Fang et al. recently identified the rat homologue of Dexras1 through its interaction with CAPON, a targeting protein for neuronal nitric oxide synthase (nNOS), implicating Dexras1 in N-methyl-D-aspartate receptor signaling in cortical neurons (6). The mechanism by which Dexras1 exerts its biological activities and the downstream signaling pathways governed by Dexras1 are not well characterized.

The recent discovery and characterization of a human homologue of Dexras1, AGS-1, establishes G_{i} family heterotrimeric G proteins as potential signaling targets of Dexras1 (1, 7). AGS-1 and Dexras1 share 97% amino acid identity, with conserved homology in the residues that vary (4). AGS-1 was identified functionally on the basis of its ability to activate ligand-independent signal transduction by a G_{i}\alpha/G_{i} \gamma chimera in a yeast pheromone pathway-based genetic complementation screen (1). The yeast pheromone pathway (8) is analogous to the mammalian Erk-1/2 MAP1 kinase pathway that is initiated by activation of many G_{i}-coupled receptors, including the N-formyl peptide receptor (FPR), by their cognate ligand (9, 10). The Erk-1/2 MAP kinase pathway mediates diverse signaling activities of receptor-tyrosine kinases, cytokine receptors and G protein-coupled receptors, including proliferation, differentiation, survival, and metabolism (9, 11). Cismowski et al. used an indirect, luciferase-based transactivation assay to evaluate Dexras1 effects on this pathway in mammalian cells. AGS-1 expression had no effect on cAMP-response element-binding protein or Jun basal transcriptional activities in COS-7 cells but produced a significant, 2.6-fold transactivation of Elk1 (7), an ETS domain transcription factor that lies downstream of

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* This work was generously supported by the Veterans Affairs Medical Merit Review (to R. I. D. and T. E. G.) and by National Institutes of Health Grant AI43932 (to E. R. P.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: MAP, mitogen-activated protein; FPR, N-formyl peptide receptor; f-MLF, N-formyl-methionine-leucine-phenylalanine; HA, hemagglutinin; GEF, guanyl nucleotide exchange factor.
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Erk (12). These observations indicate that the effects of AGS-1 on yeast pheromone pathway signaling may translate to activation of the analogous Erk-1/2 MAP kinase pathway in mammalian cells. However, direct effects of Dexras1/AGS-1 on the phosphorylation state or kinase activity of Erk have not been demonstrated in mammalian cells. Furthermore, it is not known whether Dexras1/AGS-1 play a role in ligand-dependent signaling by G_{i/coupled receptors.

Activation of mammalian Erk MAP kinases by G␣-coupled receptors is mediated through Gβγ, analogous to Ste4 and Ste18 in the yeast pheromone pathway (8). A role for Gβγ in signaling by Dexras1 is supported by the observation that transactivation of Elk1 by Dexras1 is inhibited by overexpression of transducin-α (7), which acts as a scavenger of free Gβγ (13). Therefore, analogous to receptor-mediated activation of Gγ, the interaction between Dexras1 and Gα may promote release of Gβγ and activation of Gβγ-mediated signaling pathways. Two observations further suggest that the Dexras1-Gα interaction may functionally parallel the receptor-Gα interaction. First, Dexras1 stimulates guanylyl nucleotide exchange by Gα to an extent similar to that reported for receptors (7); second, transactivation of Elk1 by Dexras1 is sensitive to pertussis toxin (7), which modifies an important site of coupling between Gγ and receptor (14).

Activation of Gα by Dexras1 may require guanylyl nucleotide binding by Dexras1, since a mutant form of Dexras1 reported to be deficient in guanylyl nucleotide binding does not transactivate Elk1 (7). Therefore, we hypothesized that guanylyl nucleotide bound Dexras1 (Dexras1-1GTP or Dexras1-GDP) interacts with Gα in a ligand-independent fashion to effect catalytic activities similar to those associated with ligand-dependent, receptor-initiated activation of Gα. According to this hypothesis, the release of Gβγ from the Gγ heterotrimer initiated by Dexras1 would lead to ligand-independent activation of the Erk-1/2 MAP kinase pathway. Since Dexras1 potentially employs the same mechanism as receptor for activating Gγ, we further hypothesized that Dexras1 might modulate Gγ-dependent signal transduction by a ligand-stimulated receptor. In view of data suggesting a physical interaction between Dexras1 and Gγ (1, 7), we considered that a Dexras1-Gγ interaction might result in cooperative or additive enhancement of Gγ signaling, resulting in increased downstream activity of Erk following receptor stimulation. Alternatively, a Dexras1–Gγ interaction might cause competitive or antagonistic interference with receptor–Gγ signaling, resulting in inhibition of maximal Erk activity following receptor stimulation. The principle aim of this study was to distinguish between these potential modulatory effects of Dexras1 in the context of ligand-stimulated signaling by a Gγ-coupled receptor. Here we report that expression of Dexras1 increases Erk activity in COS-7 and HEK-293 cells but simultaneously attenuates further activation of Erk by ligand-stimulated FPR, acting through a mechanism that is proximal to receptor–Gγ coupling.

EXPERIMENTAL PROCEDURES

Materials—Dexras1 cDNA was kindly provided by Drs. R. Kempainen and E. Behrend (Auburn College of Veterinary Medicine, Auburn, AL) in the pZL cloning plasmid (5). The open reading frame of Dexras1 was amplified by PCR and subcloned into the pcDNA3.1-His-c mammalian expression plasmid (Invitrogen Corp., Carlsbad, CA) as described (4). The pcDNA3-FPR expression plasmid (Invitrogen Corp., Carlsbad, CA) as described (4). The pcDNA3-FPR expression plasmid (Invitrogen Corp., Carlsbad, CA) as described (4). The pcDNA3-FPR expression plasmid (Invitrogen Corp., Carlsbad, CA) was used to transfect 39 clones of random human cDNA. The expression of Dexras1 was confirmed by Western blotting with anti-Dexras1 antibodies. The relative intensities of bands on SDS-PAGE and Western blotting with phosphorylation-specific antibodies to Elk1 were performed using Lipidostat M, which shows that regulatory effects were specific for the Elk1 transactivation domain.

Immune Complex Kinase Assays—HA-Erk-2 assays against the substrate myelin basic protein were performed in vitro with anti-HA immunoprecipitates, using the technique described by Graham et al. (17). Control HA-Erk-2 was incubated with phospho-specific antibodies to Erk-1/2 and 500 ng of either pcDNA-Dexras1 or pcDNA3/His plasmids.

Elk1 Transactivation Assay—The PathDetect system (Stratagene, San Diego, CA) was used as recommended by the manufacturer to measure the transcriptional activity of an Elk1-GAL4dbd chimera against a luciferase reporter plasmid driven by the GAL4 upstream activation site (GAL4-UCAS). Luciferase activity was measured in arbitrary luminescence units as previously described (16). Transfection of reporter plasmid with a plasmid expressing GAL4dbd lacking the Elk1 domain was used as a negative control alone and under various experimental conditions to establish background transcriptional activities that were subtracted from the final numbers reported. Luciferase activities for the Elk1-GAL4dbd chimera were 35- to 100-fold greater than baseline activities under various experimental conditions, demonstrating that regulatory effects were specific for the Elk1 transactivation domain.

Membrane Preparation, GTPγS Binding, and Pertussis Toxin Labeling—COS-7 cells were transfected as described above, and isolated membranes were prepared according to the method of Manning and co-workers. (18). GTPγS binding to isolated membranes was assayed using a modification of the technique described by Seifert et al. (19), differing only in that the binding buffer contained 5 μM GDP and 1 nM [35S]GTPγS. In vitro pertussis toxin-mediated ADP-ribosylation of proteins in isolated membranes (25 μg) was performed using a standard technique (20). Membrane proteins were solubilized after completion of the ADP-ribosylation reaction by boiling in Laemmli buffer and analyzed by SDS-PAGE; [32P]ADP-ribosylation of Gα species (~41-kDa band) was imaged and quantitated by phosphorimaging.

Western Blotting—For analysis of activated, endogenous Erk-1/2, cells were treated, and lysates were prepared as for in vitro kinase assays; lysates were boiled with Laemmli buffer and analyzed by SDS-PAGE and Western blotting with phosphorylation-specific antibodies to Erk-1/2. For analysis of Gα expression in membranes, 50 μg of membrane protein was directly solubilized by boiling in Laemmli buffer and analyzed. A polyclonal antibody recognizing Gα1–3 was used to detect Gα species. Secondary reagents and chemiluminescence detection substrate were provided by the Western Blotting System (Invitrogen) and used as recommended by the manufacturer. The relative intensities of bands on gels were quantitated by autoluminography using the Kodak 440cf digital imaging system (available through NEN Life Science Products).

Analysis of Cell Surface FPR Expression—Cell surface expression of FPR was quantitated by flow cytometric analysis of fluorescent ligand binding in COS-7 cells as described (21). Briefly, cells were transfected with expression plasmids for FPR and Dexras1 or empty control plasmids.
mid in ratios comparable with those used for other assays. Cell samples were stained with 10 nM N-formyl-Leu-Leu-Phe-Nleu-Tyr-Lys-fluorescein and analyzed by flow cytometry for the fraction of cells expressing the FPR. The mean channel fluorescence was determined for the population of FPR-expressing cells and compared with the population co-transfected with control plasmid.

**Statistical Analysis**—Quantitative data were analyzed using ANOVA, with post-hoc analysis using a paired t test and pairing assigned on the basis of replicate experiments. A p value of < 0.05 defined significant variation. All experiments were performed in duplicate or triplicate, and each independent experiment was repeated at least twice. Data are expressed as means ± S.E.

**RESULTS**

Overexpression of AGS-1, the human homologue of Dexras1, has been reported by Cismowski et al. to activate Elk1, but not cAMP-response element-binding protein or Jun, as assessed by an indirect transactivation assay (PathDetect, Stratagene) (7). As shown in Fig. 1A, using the same assay, we observed a similar transactivation of Elk1 when mouse Dexras1 was overexpressed in either COS7 or HEK-293 cells. Dexras1-dependent activation of Elk1 was reduced by overnight pretreatment with pertussis toxin (89 ± 4% reduction, p < 0.01) or by transient co-expression of the β-adrenergic receptor kinase carboxyl terminus peptide (ct-β-ARK), a scavenger of free Gβγ (65 ± 4% reduction, p < 0.01) (22). We specifically tested for, but did not find, any difference in the protein expression level of Dexras1 in cells treated with pertussis toxin or co-expressing ct-β-ARK (as detected by Western blotting, data not shown). The sensitivity to pertussis toxin is consistent with the reported mechanism of Dexras1/AGS-1 to activate the Gα subunit. Release of free Gβγ following ligand-stimulated receptor activation is believed to play an important role in the activation of Erk-1/2 MAP kinases (23). Thus, the inhibitory effect of ct-β-ARK co-expression is consistent with a mechanism for Elk1 activation that involves release of free Gβγ subunits as proposed by Cismowski et al. (7) The magnitude of Elk1 activation caused by expression of Dexras1 was modest (2.3 ± 0.3-fold) and consistent with the 2.6-fold increase in Elk1 transactivation reported by Cismowski et al. (7).

Elk1 is a nuclear transcription factor whose phosphorylation and transactivation lie downstream of the Erk-1/2 MAP kinase signaling pathway in many cell types (12, 24), suggesting that Dexras1 may utilize the Raf-1/MEK/Erk-1/2 signaling cascade in the transactivation of Elk1. Nevertheless, Erk-independent pathways for activation of Elk1 have also been described (25, 26). To evaluate the effect of Dexras1 expression on more proximal signaling events in this pathway, we examined the ability of Dexras1 to influence Erk activity measured by an in vitro immune complex kinase assay utilizing co-transfected, hemagglutinin antigen-tagged Erk-2 (HA-Erk-2). As shown in Fig. 1B, *lanes 1–5*, co-transfection of COS-7 cells with an increasing quantity of expression plasmid for Dexras1 resulted in a dose-dependent increase in HA-Erk-2 activity against the myelin basic protein substrate, ranging from 1.9-fold (0.5 µg of plasmid DNA) to 4.9-fold (2.0 µg of plasmid DNA) over baseline. Overnight pretreatment of Dexras1-transfected cells with pertussis toxin or co-transfection of the ct-β-ARK peptide resulted in reductions in HA-Erk-2 activity comparable in magnitude to the inhibitory effects of these agents on Elk1 transactivation (Fig. 1B, *lanes 6 and 7*).

To evaluate the effects of Dexras1 on ligand-stimulated signaling by a Gα coupled receptor, we co-expressed Dexras1 with the Gαq/11-ARK-coupled (27) FPR in COS-7 and HEK-293 cells. Previous reports indicate that neither cell line expresses the endogenous FPR (28, 29). We examined the effects of Dexras1 on FPR signaling before and after stimulation of cells with f-MLF, a peptide ligand of the FPR. As shown in Fig. 2 (*lanes 1–3*), stimulation with f-MLF caused a 6.3-fold maximal increase in phosphorylation of endogenous Erk-1/2 in each cell type as detected by phosphoryl-specific Western blotting. f-MLF had no effect on Erk-1/2 activation in cells transfected with the control plasmid instead of FPR (data not shown), which establishes the specificity of FPR-mediated Erk-1/2 activation and is consistent with the lack of endogenous expression of FPR by either cell line. The time course to maximal production of phospho-Erk-1/2 following addition of f-MLF was 5 and 10 min for HEK-293 cells and COS-7 cells, respectively (full time course data not shown). This time course of Erk-1/2 phosphorylation is comparable with that reported for other G protein-coupled receptors (22, 30, 31), including FPR (15). Expression of Dexras1 (Fig. 2A, *lanes 4–6*) resulted in a substantial decrease in the ligand-stimulated activation of Erk-1/2 in each cell line. Surprisingly, expression of Dexras1 did not affect baseline Erk-1/2 phosphorylation in this assay. This contrasts with the findings of up to 4.9-fold activation of HA-Erk-2 measured by the in vitro kinase assay. This discrepancy likely reflects methodological differences, perhaps related to the elimination of background activity in the HA-Erk-2 in vitro kinase.
assay (Fig. 1B) that is otherwise contributed by non-transfected cells in the Western blotting assay (Fig. 2). Furthermore, the high constitutive activity of the non-ligand-stimulated FPR (discussed below) may have masked activity contributed by Dexras1 in the Western blotting assay.

As shown in Fig. 3A, lanes 1 and 3, and B, expression of FPR in the absence of ligand produced a significant increase in baseline HA-Erk-2 activity as assessed by the in vitro kinase assay. Ligand-independent signaling of Giα-coupled receptors, including FPR, to Erk-1/2 MAP kinases has been previously reported (19, 32). In the case of FPR, ligand-independent signaling appears to be selectively sensitive to inhibition by the inverse agonist, cyclosporin H (19). The magnitude of HA-Erk-2 activity stimulated by FPR expression alone and independent of ligand was greater in magnitude to that stimulated by Dexras1 expression: 6.2-fold and 8.1-fold over baseline for COS-7 and HEK-293 cells, respectively (Fig. 3A, lanes 1 and 2, and B). Interestingly, co-expression of Dexras1 with FPR but without ligand treatment produced little additional activation of HA-Erk-2 in HEK-293 cells beyond the activity resulting from expression of either Dexras1 or FPR alone (Fig. 3A, lanes 3 and 6, and B). In COS-7 cells, however, there was an approximately additive, statistically significant increase in activity under the same conditions, as shown in Fig. 3A (compare lanes 3 and 6 for COS-7) and B. Thus, Dexras1 may weakly augment the basal/constitutive signaling activity of FPR in a cell type-specific manner.

Following stimulation of FPR-transfected cells with f-MLF, HA-Erk-2 activity increased with a time course similar to that observed for Erk-1/2 phosphorylation (discussed above, Fig. 2). Peak HA-Erk-2 activities occurred at 5 and 10 min after stimulation in HEK-293 and COS-7 cells, respectively (see Fig. 3A, lanes 3–5, and B). The maximal fold increases in activity over the resting state (8- to 10-fold) were comparable with those elicited by other Gi-coupled receptors in these cell lines (22, 31). HA-Erk-2 activities in cells co-transfected with Dexras1 were compared with control cells at the points of peak ligand-stimulated; as shown in Fig. 3A (compare lanes 3–5 and lanes 6–8) and B, Dexras1 co-expression caused an 86 ± 9.2% inhibition of f-MLF-stimulated HA-Erk-2 activity in HEK-293 cells (at 5 min) and a 31 ± 6.8% inhibition in COS-7 cells (at 10 min, p < 0.01 for each cell line at those time points). The magnitude of this inhibitory effect on kinase activity was comparable with the inhibition of phosphorylation observed for endogenous Erk-1/2 in each cell line (discussed above, Fig. 2). In this series of experiments, the magnitude of the effect of Dexras1 expression on Erk activity was noticeably greater in HEK-293 cells than in COS-7 cells; this was apparent in effects of Dexras1 on both the stimulation of basal HA-Erk-2 activity and the inhibition of ligand-dependent signaling to HA-Erk-2. Since the magnitude of Dexras1 protein expression in detergent lysates or crude membrane fractions did not differ significantly between the two cell lines after correcting for total protein content (assayed by Western blotting, data not shown), we hypothesize that this difference in magnitude of effect on Erk may reflect differential regulation of Dexras1 activity or of a Dexras1–Gα interaction through cell type-specific factors that have yet to be identified. Taken together, the data presented in Figs. 1–3 indicate that expression of Dexras1 activates a basal level of ligand-independent signaling by G, but simultaneously promotes a state that is refractory to further stimulation by ligand-stimulated receptor.

Studies were performed to determine whether the inhibitory effects of Dexras1 reflected alterations in the expression level of signaling pathway components (i.e. FPR, Giα, or HA-Erk-2). Cell surface expression of FPR was monitored by flow cytometric analysis of fluorescent ligand binding in COS-7 cells co-transfected with Dexras1 or control plasmids in ratios similar
to those described for the other assays. Dexras1 did not affect the mean expression of FPR in co-transfected cells (Fig. 4B). To test the effects of Dexras1 on HA-Erk-2 expression, anti-HA immunoprecipitates were subjected to Western blotting with a non-phosphoryl-specific antibody to Erk. Co-expression of HA-Erk-2 with FPR resulted in a slight decrease in HA-Erk-2 expression, but co-expression of Dexras1 had no additional effect (see Fig. 3A, Total Erk, and Fig. 4B). The membrane expression of endogenous Gα species in COS-7 cells, as detected by Western blotting, was not affected by expression of Dexras1 (Fig. 4A, top panel, and B). Together these data indicate that the stimulatory effects of Dexras1 on basal Erk activity and the inhibitory effects of Dexras1 on ligand-stimulated Erk activity cannot be accounted for by alterations in the expression level of FPR, Gα, or HA-Erk-2.

We also evaluated the effect of Dexras1 expression on ADP-ribosylation of Gα species by pertussis toxin. In replicate experiments in isolated COS-7 cell membranes, shown in Fig. 4A, bottom panel, and B, Dexras1 expression was associated with a 53 ± 3.7% reduction in the ADP-ribosylation of a 41-kDa substrate corresponding to Gα species. Pertussis toxin requires the heterotrimERIC interaction of Gα and Gβγ to efficiently catalyze the ADP-ribosylation of Gα (33). Activation of Gα-coupled receptors or inhibition of Gγ subunit posttranslational modifications have been shown to disrupt this interaction and reduce the ADP-ribosylation of Gα in isolated membranes (33–35). The finding that Dexras1 expression causes a reduction in the ADP-ribosylation of Gα without affecting the total amount of immunoreactive Gα suggests that Dexras1 may disrupt the Gα-Gβγ interaction in a manner similar to activated Gα receptor, consistent with Dexras1-mediated activation of Gα and release of Gβγ as proposed by Cismowski, et al. (7).

To evaluate the possibility that Dexras1 may interfere, competitively or otherwise, with coupling between receptor and Gα, we co-transfected Dexras1 or control plasmids with FPR in COS-7 cells and measured the effect of each on f-MLF-stimulated guanylnucleotide exchange activity (GTPγS binding) in isolated membranes. Ligand-stimulated GTPγS binding in isolated membranes is considered one of the earliest events that can be measured following receptor-G protein coupling (36). In the case of FPR, ligand-stimulated GTPγS binding in isolated membranes has been shown to be fully sensitive to pertussis toxin, consistent with observations that both Gα2 and Gα3 selectively interact with the ligand-stimulated FPR (27). As shown in Fig. 5, stimulation of membranes derived from cells that were not transfected with the FPR resulted in no change in GTPγS binding (Fig. 5, crossbars and open triangles), consistent with the report that COS-7 cells do not express an endogenous FPR (28). In contrast, membranes expressing the FPR and stimulated with f-MLF (Fig. 5, solid squares) demonstrated significantly greater time-dependent GTPγS binding over the zero point (p < 0.001 at all time points) with a saturable kinetic. It is noteworthy that the total (molar) GTPγS binding differed from results obtained in a similar assay by Wenzel-Seifert et al. (19). The discrepancy is likely explained by the difference in experimental conditions; in the study of Wenzel-Seifert et al., S9 cells were used (rather than COS-7 cells), and G protein subunits were overexpressed with the receptor (19), a condition that would be anticipated to provide far more receptor-coupled sites for specific binding of GTPγS than in COS-7 cell membranes. Co-transfection of Dexras1 (Fig. 5, open squares) resulted in a substantial reduction in f-MLF-stimulated GTPγS binding relative to membranes transfected with FPR only (Fig. 5, closed squares, p < 0.01 at all time points). Co-transfection of Dexras1 resulted in a reduction in f-MLF-stimulated GTPγS binding comparable to magnitude to that seen in membranes derived from pertussis toxin-treated cells. At two time points in the binding assay (15 and 30 min), the magnitude of response in Dexras1-transfected membranes (Fig. 5, open squares) was significantly greater than in control membranes derived from pertussis toxin-treated cells (Fig. 5, closed circles, p < 0.05 at 15 min, and p < 0.01 at 30 min); however, the response did not differ statistically at other time points in the series (7.5, 60, 120, and 180 min). The finding that expression of Dexras1 inhibits FPR-stimulated GTPγS binding in isolated membranes indicates that Dexras1 may be
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The effects of overnight treatment with the pertussis toxin holoenzyme of cells not expressing the FPR with and without ligand stimulation are described for ligand-stimulated, G_{i}-coupled receptors. The observation suggests that Dexras1 and receptor may interact in vivo. Dexras1 and receptor domains may interact through the same structural elements of the α subunit and through a similar molecular mechanism. Based on these findings, we hypothesize that the ability of Dexras1 to selectively inhibit receptor-mediated G_{i} signal transduction and render G_{i}α species resistant to pertussis toxin-mediated ADP-ribosylation could reflect a dominant effect of Dexras1 on the activation status of G_{i}α that results in a depletion of the pool of heterotrimeric G_{i}α-βγ available to the receptor for transmitting ligand-dependent signals.

Evidence presented by Cismowski et al. (1, 7) and in this report suggests that Dexras1 modifies G_{i}-mediated signal transduction at proximal signaling events, probably including a direct effect of Dexras1 on the guanyl nucleotide binding state of G_{i}α. In vitro studies have confirmed that activation of G_{i}α by AGS-1 may involve a direct binding interaction between the two proteins that increases the guanyl nucleotide exchange rate of the α subunit and leads to the formation of G_{i}α-GTP (7). This direct interaction between a Ras family G protein and heterotrimeric G protein represents a novel paradigm for signal transduction and suggests that Dexras1/AGS-1 may be functionally classified as a guanyl nucleotide exchange factor (GEF) for G_{i}α proteins. An in vivo G_{i}-α-GEF function for Dexras1 is less clearly established, and direct in vivo interactions between Dexras1/AGS-1 and G_{i}α have not been reported. The finding that activation of Erk by Dexras1 in a transfected cell system is inhibited by pertussis toxin is consistent with a potential G_{i}α-GEF activity of Dexras1 in vivo. However, pertussis toxin-sensitive signaling from G_{i}-coupled receptors to Erk appears to depend on the release of G_{i}βγ (9), and regulation of a direct target of G_{i}α-GTP by Dexras1 (e.g. adenylate cyclase) has yet to be established. It will be important in future studies to determine whether Dexras1 also activates the α subunit-dependent arm of G_{i} signal transduction in vivo.

The novel hypothesis developed by Cismowski et al., in which a Ras family G protein directly modulates the activation of a heterotrimeric G protein subunit (7), has important physiological implications for the many systems that utilize G_{i}-coupled receptors. It will be important to discern whether Dexras1 behaves in a similar manner with other G_{i}-coupled receptors and to define the arc of upstream signals and downstream effectors that describe the Dexras1 signaling pathways. Our findings establish an inhibitory activity of Dexras1/AGS-1 on ligand-dependent signaling of a G_{i}-coupled receptor that is likely to be physiologically important and mechanistically related to ligand-independent activation of G_{i}α for which this founding member of the family of intermediate molecular weight, basic G proteins was named.

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