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Activation and nuclear translocation of ERK1/2 by the formyl peptide receptor is regulated by G protein and is not dependent on β-arrestin translocation or receptor endocytosis

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Abstract

G protein-coupled receptors (GPCRs) transmit diverse cellular signals in response to a large number of stimuli such as chemoattractants, lipids, neurotransmitters, odorants and light. The classical signaling pathway is through heterotrimeric G proteins, but GPCRs can also transmit signals through mechanisms that are not dependent on G proteins. In mammalian cells, the key component for this type of signaling is the family of scaffolding molecules called β-arrestins. They can function as scaffolds for activation of mitogen-activated protein kinases, including extracellular signal-regulated kinases 1 and 2 (ERK1/2). In this study we examined the role of G protein and β-arrestin in formyl peptide receptor (FPR)-mediated activation of chemotaxis, receptor endocytosis and ERK1/2 activation using wild type and mutant receptors. Our findings suggest that, unlike certain other GPCRs that can activate ERK1/2 without the involvement of G protein, FPR requires signaling through a G protein-mediated pathway. Previous observations have shown that ERK1/2, activated through G protein, translocates to the nucleus where it stimulates transcription factors. In contrast, the scaffolding protein β-arrestin retains the activated ERK1/2 in the cytoplasm to allow phosphorylation of cytoplasmic targets. Our experimental data show that both wild-type FPR and a mutant FPR, defective in β-arrestin binding, induce nuclear translocation of activated ERK1/2 with similar ligand concentration dependence as seen for activation of cytosolic ERK1/2. We propose that FPR-mediated activation of ERK1/2 takes place primarily through G protein and is physiologically important to ensure transcriptional activation of myeloid immunomodulators, such as cytokines.

Keywords: Formyl peptide receptor; G protein; β-arrestin; GPCR; Extracellular signal-regulated kinase; Cell signaling; Transcriptional activation; Chemotaxis

1. Introduction

The leukocyte *N*-formyl peptide receptor (FPR) is a G protein-coupled receptor (GPCR) that mediates a number of important host defense functions such as chemotaxis and killing of microorganisms by phagocytosis, oxidative burst and degranulation [1–3]. In addition, activated FPR stimulates cytokine secretion by neutrophils, eosinophils and monocytes [4,5] through activation of NF-κB [1]. Like other GPCRs, FPR signals through heterotrimeric G proteins and the signal is terminated by receptor phosphorylation and binding of cytoplasmic β-arrestin to the receptor [6]. β-Arrestin also acts as an adaptor in clathrin-mediated endocytosis [7], although in the case of FPR, β-arrestin is not required for receptor endocytosis, as shown using β-arrestin-deficient mouse embryonic fibroblasts [8]. It was previously thought that GPCRs signal through G protein only. However, recent evidence support the notion that at least one other pathway exists: Studies examining the activation of extracellular signal-regulated kinase (ERK), a member of the mitogen-activated protein kinase (MAPK) family, showed activation of ERK1/2 by mutant receptors

Abbreviations: GPCR, G protein-coupled receptor; ERK1/2, extracellular signal regulated kinases 1 and 2; FPR, *N*-formyl peptide receptor; GRK, G protein-coupled receptor kinase; *f*MLF, *N*-formyl-methionyl-leucyl-phenylalanine; CHO, Chinese hamster ovary; MAPK, mitogen-activated protein kinase; Fura 2-AM, fura 2 acetoxymethyl ester.

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that appeared to lack the capacity to signal through G protein [9,10]. Using siRNA technology, the key player in the activation of ERK1/2 was identified as β-arrestin2 [11–13]. Since ERK1/2 plays an important role in a large number of biological processes, such as cell differentiation, proliferation and survival, it is critical that the activation of ERK1/2 and its localization in the cell is differentially regulated by various cell surface receptors. Experiments utilizing β-arrestin RNA interference and an angiotensin II mutant receptor that is incapable of activating G protein, showed that G protein-dependent activation led to rapid nuclear translocation of activated ERK1/2, whereas ERK1/2 activated via β-arrestin2 accumulated in cytoplasmic endosomal vesicles colocalizing with receptor and β-arrestin [13].

In addition, the signal transduction pathway can be dictated through ligand specificity: Single amino acid substitutions within angiotensin II resulted in major differences in phospholipase C-mediated signaling and activation of ERK1/2 [14]. Similarly, a single amino acid change in a synthetic peptide that binds FPR affected calcium mobilization without inhibiting ERK1/2 activation [15].

To examine the role of G protein and β-arrestin in FPR-mediated activation of ERK1/2, we took advantage of a number of mutant receptors. We have previously characterized these mutants in stably transfected Chinese hamster ovary (CHO) cells for expression levels, ligand-binding affinity and G protein coupling [16,17]. In this study, we characterize the effect of the mutations on ligand-induced chemotaxis, β-arrestin translocation, receptor endocytosis, ERK1/2 activation, and intracellular calcium release. Based on the results, we conclude that activation of ERK1/2 through FPR occurs predominantly through G protein signaling.

2. Materials and methods

2.1. Cell culture and transfections

FPR mutagenesis and transfection of CHO cells have been described previously [16,17]. Transfected CHO cells were maintained in selection medium containing 0.5 mg/ml G418 (Calbiochem, La Jolla, CA) in α-modified Eagle's medium containing 5% fetal bovine serum (FBS), 50 U/ml Penicillin and, 50 μg/ml Streptomycin. 14 to 16 h before each experiment, increased expression of FPR was induced by adding 6 mM sodium butyrate to the medium [18].

2.2. Chemotaxis assay

Chemotaxis was assayed essentially as described previously [19]. Briefly, CHO transfectants (3×10^5 cells in 300 μl) were added to 6.5 mm diameter Transwell inserts of 8 μm pore size (Corning Costar, Cambridge, MA). 500 μl serum-free medium with various concentrations of *f*MLF (0, 1, 10 or 100 nM) were placed in the wells of a 24-well culture plate. The Transwell inserts containing the cells were moved into the wells, and chemotaxis was allowed to proceed for 4 h at 37 °C. Cells on the upper face of the insert were removed with cotton swabs; the cells that adhered to the underside of the filter were fixed with 2.5% paraformaldehyde in PBS, stained with Hematoxylin Gill's stain, and quantified using an image analyzer (M4 True Color Image Analysis System, Imaging Research, St. Catharines, ON, Canada).

2.3. Endocytosis assay

Cells were removed from tissue culture plates with PBS containing 1 mM EDTA, incubated for 1 h at 37 °C±100 nM *f*MLF and washed 3 times with PBS. To detect FPR on the cell surface, the cells were incubated with 20 nM *N*-formyl-norleucine-leucine-phenylalanine-norleucine-tyrosine-lysine-fluorescein derivative (f-NleLFNleYK-fluorescein) (Molecular Probes, Eugene, OR) ±20 μM *f*MLF (to determine background binding). The analysis was carried out using a fluorescence-activated cell scanner (FACScan) (Becton Dickinson, San Jose, CA).

2.4. Western blot analysis of β-arrestin membrane translocation

CHO transfectants on 10 cm dishes were incubated for 8 min with or without 10 μM *f*MLF, moved onto ice and rinsed with cold PBS. Cells were removed from the dishes by scraping in PBS containing 1 mM PMSF and Sigma protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). Cells were broken up by brief sonication and the samples were centrifuged 15 min at 21,000×*g* to pellet the membranes. The proteins in the supernatant (cytosol fraction) were precipitated with 70% ethanol and both pellets were resuspended in Laemmli sample buffer and heated to 95 °C for 5 min prior to electrophoresis through an SDS-polyacrylamide gel. Proteins were transferred onto nitrocellulose and β-arrestins 1 and 2 were detected using a rabbit polyclonal antibody produced using a GST fusion protein containing the 88 C-terminal amino acids of rat β-arrestin1. The antiserum was generously provided by Dr. Robert Lefkowitz (Howard Hughes Medical Institute and the Departments of Medicine and Biochemistry, Duke University Medical Center). Chemiluminescence was carried out using Western Lightning reagent (PerkinElmer Life Sciences, Boston, MA) and the relative intensity of the bands from the films was quantified using a Scion Image program (Scion Corporation, Frederick, MD) after scanning with Scanjet IIcx scanner (Hewlett-Packard, Palo Alto, CA).

2.5. ERK1/2 activation assay

The cytosol fractions from the samples described above were used for detection of phosphorylated ERK1/2 and total