GPCR-mediated 14-3-3 signaling pathway assays

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Introduction

GPCR signaling is pluridimensional and complex. Upon agonist stimulation, GPCRs activate heterotrimeric G proteins, leading to the dissociation of Gα protein from Gβγ subunits and the generation of second messengers such as cAMP, Ca2+, etc. G protein-coupled receptor kinases (GRKs) then phosphorylate activated receptors, leading phosphorylated receptors to recruit signal adaptors such as β-arrestins and 14-3-3 proteins. Interactions between GPCRs and an array of signal adaptors or cellular effectors modulate both G protein-dependent and G protein-independent signaling pathways and offers the possibility of eliciting spatiotemporal signaling cascades, inducing cross-talk with other signaling pathways, and fine tuning and specifically regulating GPCR signaling at multiple levels. 14-3-3 proteins, like β-arrestins, are ubiquitously expressed in every cells. They also act as a signal hub to interact with a vast number of phosphorylking for these interactions facilitate the formation of large molecular complexes that coordinate the responses of multiple signaling pathways to incoming stimuli, allow signal transduction among different cellular compartments, and carry out a variety of physiological functions. It is conceivable that similar to GPCR-mediated β-arrestin signaling pathway, 14-3-3 and effector interaction complex further diversify signal transduction and contribute to the overall GPCR functions.

Result

(1) Demonstrate GPCR-mediated 14-3-3 signaling pathway by LinkLight assay technology.
(2) GPCR-mediated 14-3-3 signaling could be a general phenomenon.
(3) GPCR-mediated 14-3-3 signaling can be β-arrestin-independent.
(4) Profiling agonist-activated GPCRs in 14-3-3 vs. β-arrestin signaling pathway assays.
(5) Demonstrate 14-3-3 interaction with the signaling effector Raf-1.

Summary

Physiological outcomes of GPCRs likely depend on an array of a set of signaling mechanisms. Conceptually, classic ligands can activate all pathways, while biased ligands activate one or a subset of pathways. It is clear that GPCR bias goes beyond simple bifurcation of signaling at the G protein and β-arrestin levels. A given receptor can selectively couple to multiple subtypes of G proteins, and recruit distinct conformational ensembles of β-arrestins and 14-3-3 proteins, and perhaps interact with other effectors. This coupling diversity can yield strikingly different functional outcomes. Whether a side effect or beneficial effect is associated with a specific GPCR-mediated signaling pathway has yet to be explored for different GPCRs. Historically, GPCR drug discovery research has relied on G protein signaling pathways for assessing the activity of various compounds. The discovery of G protein-independent and β-arrestin-dependent signaling pathways created an opportunity for identifying pathway-selective or signaling-biased ligands. These biased ligands may maximize clinical effectiveness and minimize unwanted side effects. Conceptually, a ligand may bind and stabilize a receptor in a conformation that may exert differential activity on the 14-3-3 signaling pathway in addition to the G protein and β-arrestin signaling pathways.

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