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Signal Transduction Protocols

Third Edition

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Preface

Signal transduction is the process whereby a physical or chemical stimulus in the extracellular environment is detected by a receptor on the plasma membrane or in the cytosol or nucleus of a sensitive cell and translated into a chemical or electrochemical signal that produces a change in cellular metabolism. Rather than representing a series of simple linear cascades, it is increasingly clear that signal transduction is a highly organized and integrated process. Extensive crosstalk between signaling cascades, communicated directly through receptor oligomerization or indirectly through the activation of autocrine and paracrine feedback loops, enables one type of receptor to modulate activity in multiple intracellular pathways. Additional factors impose spatial or temporal constraints on signaling that influence the final cellular response by determining where within the cell, and for how long, the signal persists.

This volume focuses on experimental approaches to understand the complexity of signal transduction. Introductory chapters have been included to provide perspective on several of the challenges in signal transduction research and guidance on selecting the best approaches to various types of questions. The individual chapters provide detailed experimental protocols, beginning with the effects of ligand binding on receptor conformation and effector coupling, then moving inside the cell to capture the spatial and temporal characteristics of signaling events.

We would like to express our deepest appreciation to the coauthors of this publication. We hope that *Signal Transduction Protocols – Third Edition* will prove to be a valuable resource for future progress in the field of signal transduction research.

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Contents

Part I

Overviews

Chapter 1

Refining Efficacy: Allosterism and Bias in G Protein-Coupled Receptor Signaling

Louis M. Luttrell and Terry P. Kenakin

Abstract

Receptors on the surface of cells function as conduits for information flowing between the external environment and the cell interior. Since signal transduction is based on the physical interaction of receptors with both extracellular ligands and intracellular effectors, ligand binding must produce conformational changes in the receptor that can be transmitted to the intracellular domains accessible to G proteins and other effectors. Classical models of G protein-coupled receptor (GPCR) signaling envision receptor conformations as highly constrained, wherein receptors exist in equilibrium between single "off" and "on" states distinguished by their ability to activate effectors, and ligands act by perturbing this equilibrium. In such models, ligands can be classified based upon two simple parameters; affinity and efficacy, and ligand activity is independent of the assay used to detect the response. However, it is clear that GPCRs assume multiple conformations, any number of which may be capable of interacting with a discrete subset of possible effectors. Both orthosteric ligands, molecules that occupy the natural ligand-binding pocket, and allosteric modulators, small molecules or proteins that contact receptors distant from the site of ligand binding, have the ability to alter the conformational equilibrium of a receptor in ways that affect its signaling output both qualitatively and quantitatively. In this context, efficacy becomes pluridimensional and ligand classification becomes assay dependent. A more complete description of ligand–receptor interaction requires the use of multiplexed assays of receptor activation and screening assays may need to be tailored to detect specific efficacy profiles.

Key words: Agonist, G protein-coupled receptor, Heterotrimeric guanine nucleotide-binding protein, Pharmaceutical chemistry, Pharmacodynamics, Signal transduction

1. Introduction

Most of the basic tenets of receptor pharmacology predate our understanding of the molecular structure of receptors themselves. When Stephenson defined efficacy in 1956, he was studying the acetylcholine-like effects of a series of alkyl-trimethyl

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ammonium salts on the contraction of guinea pig ileum (1). In this work, the readout of receptor activation was a relatively simple bioassay. Although the intervening 50 years have seen an explosion in our knowledge of receptor structure and mechanisms of intracellular signaling, even today most drug discovery efforts rely on using a single readout, often in a highly artificial system engineered for high throughput automated screening, as the basis for classifying the effect of ligand binding on receptor activity. Within such systems, where receptor density is constant and activity is measured either as an integrated whole cell or tissue response, e.g., muscle contraction, or a single molecular event, e.g., influx of cytosolic calcium, the relationship between the ligand concentration and receptor activation can be adequately described by two terms; affinity, the equilibrium dissociation constant of the ligand–receptor complex; and the maximal response that can be observed (2, 3), which is a function of efficacy. In this paradigm affinity and efficacy are largely independent functions, i.e., a ligand may have high affinity but low efficacy or vice versa, and ligands are classified as full agonists if they can elicit a maximal response from the system, partial agonists if they can only generate a submaximal response, and antagonists if they lack intrinsic efficacy but interfere with the ability of agonists to evoke a response.

Although these principles provide the framework that has guided signal transduction and drug discovery research for decades, advances in our understanding of the complexity of signal transduction networks and the evolution of technology to measure receptor activation in many dimensions have unambiguously demonstrated that the nature of efficacy is far more complex than originally envisioned, and a more general model is needed to explain the action of ligands on receptors (4). Rather than functioning like simple switches that transition between tightly constrained "off" and "on" states, receptors are highly dynamic proteins capable of adopting a large number of conformational states, some subset of which is capable of coupling to variable sets of downstream effectors. Viewed in this way, it is evident that any ligand, small molecule, or other protein that contacts the receptor in a manner that alters its conformational equilibrium may initiate, attenuate, or even qualitatively change signaling. Orthosteric ligands, allosteric modulators, even other proteins contacting the receptor in the lipid bilayer or on its cytosolic face, all work in essentially the same way. In the sections that follow, we will review the changing concepts of efficacy, their implications for drug development, and the challenges arising from the need to incorporate a more complete characterization of ligand action into experimental and industrial research.

2. Two-State Models of Receptor Activation

When only a single readout of receptor activation is considered, receptors can be described as existing in either an empty "off" state that is silent in the assay or an agonist-bound "on" state that elicits a measurable response. The early model of "induced fit" advanced by Koshland in 1958 to describe enzymatic catalysis, proposed that the interaction between a substrate and amino acid residues within the active site of an enzyme changes the structure of the enzyme so as to bring the catalytic groups into proper alignment (5). In other words, for an enzyme (or receptor) that exists in a preferred low energy "inactive" state and must transition to a higher energy "active" state to function, substrate (or ligand) binding facilitates the transition by contributing energy that makes the "active state" become the new preferred low energy state. The alternative concept of "conformational selection" arises from the Monod–Wyann–Changeux model of allostery, which proposes that proteins exist in spontaneous equilibrium between different conformations and that a molecule that binds to a specific conformation will stabilize it, shifting the conformational population toward that favored state (6). The use of such allosteric models to describe membrane receptor function began in the late 1960s (7, 8). The assumption is that the probability that an unbound receptor will exist in the active state is very low, but that stabilization of this state upon ligand binding drives the equilibrium toward the "on" state by interfering with the transition back to the "off" state.

While molecular simulations favor conformational selection models for the binding of small molecules to proteins (9), selection of a relatively rare pre-existing conformation would thermodynamically resemble conformational induction (10), leaving little need to choose between them in modeling two-state receptor behavior. Structural and biophysical data demonstrate that GPCRs vary widely in their degree of conformational flexibility. One extreme is the visual photoreceptor, rhodopsin, which for many years was the only GPCR for which high-resolution X-ray crystallographic structure was available (11, 12). Given its function, it is not surprising that rhodopsin is completely inactive toward transducin in the dark adapted state, i.e., it has evolved to function as an "on–off" switch. To achieve this, it is tightly constrained in the "off" position by intramolecular interactions between the transmembrane helices, notably an "ionic lock" linking the highly conserved E/DRY sequence found at the cytoplasmic end of TM3 in 70% of class A GPCRs, to the NPxxY motif located in TM6. More recent structures of light-activated rhodopsin and of opsin, the ligand-free form of rhodopsin, bound to a C-terminal fragment of transducin, demonstrate that the upon activation the ionic lock is released, allowing a outward turn of TM6 that exposes the transducin-binding site (13, 14).