
Preface

A broad definition of a receptor is a specialized protein on or in a cell that recognizes and binds a specific ligand to undergo a conformational change, leading to a physiological response or change in cell function. A ligand can be an endogenous neurotransmitter, hormone, paracrine/autocrine factor, or a synthetic drug that may function as an agonist or antagonist. Receptor families include the seven-transmembrane spanning G-protein-coupled receptors (GPCRs), ligand-gated ion channels, tyrosine kinases, and nuclear hormone receptors. The GPCR superfamily can be further divided into classes. Class 1 is the largest and most diverse family. It includes over 400 receptors classified as sensory and presumed to be involved in the detection of odor, taste, or light. In addition, there are over 270 non-sensory receptors in Class 1 as well as a further 49 in Class 2 and 19 in Class 3 that are activated by established transmitters that can be studied by ligand-binding techniques. GPCRs, particularly Class 1, are targets for a third of all medicines in current use. In addition, following near completion of the human genome, over one hundred Class 1 and Class 2 “orphan” receptors are predicted to exist, so called because the endogenous ligand(s) is not yet known, but each one represents a potential new therapeutic target. Until 2007, only one crystal structure of a GPCR has been solved, the visual sensory protein rhodopsin. Receptor research has been revolutionized during the last 5 years by recent reports of the crystal structure of beta 1 and beta 2-adrenergic, adenosine A2a, the CXCR4, and histamine H1 receptors with many more in the pipeline.

The search for novel drugs for established receptors or the discovery of endogenous ligands for “orphans” receptors is facilitated by the use of computational tools which are used to efficiently mine large datasets. Chapter 1 describes in detail the computational Web sites and software for predicting ligand–receptor binding together with databases dedicated to specific families of receptors (GPCRs, ligand-gated ion channels, and nuclear hormone receptors). Ligand binding to a receptor is but the first step in understanding the pathways that regulate a biological response, and the authors conclude with an assessment of data for signal transduction pathways which can give insight into “downstream” responses resulting from ligand–receptor interactions.

The second chapter on *in silico* techniques focuses on the main features of the International Union of Basic and Clinical Pharmacology Database (IUPHAR-DB) with examples for navigating and exploring information on GPCRs, ligand-gated ion channels, and nuclear receptors. This curated relational database contains essential pharmacological, chemical, genetic, functional, and anatomical data. The content represents more than a decade of work by over 60 subcommittees (comprising about 700 international experts from academia and industry) that have been peer reviewed by the IUPHAR Nomenclature Committee (NC-IUPHAR). The database provides up-to-date recommendations on the nomenclature of receptors and ion channels, documents their properties and the ligands that are useful for receptor characterization. The database collates the latest pairing of orphan receptors with their ligands and has “hot topics” pages with commentaries on high-impact papers focusing on receptors.

Radioligand binding remains one of the most widely used techniques to characterize receptors using labeled analogues of naturally occurring transmitters, hormones, or synthetic drugs and to determine their anatomical distribution. The technique is used in drug discovery to identifying at early stages the most promising drug candidates. In Chapter 3 protocols are given for the three main assays (saturation, competition, and kinetic). The chapter includes a list of widely used radioligands and explains the use of quantitative autoradiography and image analysis to determine the anatomical distribution of receptors. Nonlinear curve-fitting programs are described to derive key binding parameters: affinity of the labeled ligand for a receptor (equilibrium dissociation constant, K_D), receptor density ($B_{\max}$), and Hill slope (nH). A limitation of conventional radioligand-based assays is the requirement to break the equilibrium binding conditions to separate bound from free radioligand. This step is not needed in scintillation proximity assays (Chapter 4). Existing binding assays can be readily converted to this format and are particularly suitable in drug screening applications where high throughput is required.

Considerable advances have been made in the development of fluorescent-labeled ligands and receptor proteins. Confocal microscopy increases optical resolution and contrast to reduce out-of-focus light in specimens that are thicker than the focal plane so that receptor pharmacology can now be visualized and quantified in a single cell. Chapter 5 describes how the binding of fluorescent ligands to receptors in tissues such as blood vessels can be analyzed and three-dimensional structures reconstructed using confocal microscopy. Importantly, ligands selective for two different receptors labeled with red or green fluorophores can be used in the same assay to address whether they colocalize to a specific cell type. In Chapter 6, bimolecular fluorescence complementation is applied to GPCR signaling particularly measuring interactions with G-proteins and β -arrestin. This emerging technique involves the division of a protein reporter into two nonfunctioning fragments, each fused to the separate partners under investigation. The association of the target proteins then drives recombination of the reporter fragments, yielding measurable functional activity, usually fluorescence excitation/emission characteristics of the parent protein. The technique has the potential to answer key questions as to how GPCRs select downstream signaling partners and how these are compartmentalized. Tagging receptors with green fluorescent protein (GFP) originally derived from the jellyfish *Aequorea victoria* has revolutionized the study of trafficking and subcellular location of receptors in living cells. Chapter 7 succinctly outlines the construction of GPCR fusion proteins and how these can be applied to receptor activation, trafficking between subcellular compartments, and to measure transport kinetics.

Classically, agonist binding to a GPCR causes a conformation change, activating associated heterotrimeric G-proteins which subsequently dissociate leading to signaling through second messenger systems, such as cAMP and calcium. Signaling is terminated with the binding of β -arrestins with desensitization followed by internalization into clathrin-coated pits. Evidence is increasing that signaling via β -arrestins has distinct biochemical and functional consequences from those mediated by G proteins. Biased ligands have been identified for a number of transmitter systems that preferentially signal through either G protein- or β -arrestin-mediated pathways. These can be detected by comparing the activity of compounds in assays described in the next two chapters.

The majority of GPCRs modulate the function of adenylate cyclase, resulting in altered levels of cAMP and can be used to characterize ligand-receptor interactions. Chapter 8 describes a nonradioactive, chemiluminescent cAMP detection method using enzyme fragment complementation technology to detect a wide range of GPCR modulators which is

also suitable for high-throughput screening. Importantly, antagonists and allosteric modulators can also be characterized. This avoids the limitation of competition binding assays using radioligands which usually cannot distinguish between agonist and antagonist activity.

The recruitment of β -arrestins by activated GPCRs results in quenching of G-protein activation and coupling to clathrin-mediated endocytosis. In this assay (Chapter 9), the reporter enzyme is split between the C-terminus of GPCR and β -arrestin. Agonist binding leads to the formation of GPCR–arrestin complex forcing complementation of the two fragments resulting in active β -galactosidase measured by chemoluminescence (rather than fluorescence as in Chapter 6). This has the merit that ligand binding can be measured in low-cost luminometers, making the technique accessible to a wide range of laboratories. Virtually all GPCRs recruit β -arrestin and the majority in Class 1 and Class 2 are commercially available, including orphan receptors so that the assays can be used in high-throughput screening for the identification of novel ligands.

Positron emission tomography (PET) is a functional imaging technique with the potential to image and quantify receptors in living animals with high sensitivity. Radionuclides such as ^{11}C and ^{18}F are widely used to label radioligands for positron emission tomography (PET). Chapter 10 describes the advantages of using phosphor screens with increased sensitivity and linear dynamic range. This property reduces exposure times compared with film-based autoradiography for the characterization of novel PET radioligands. The recent development of dedicated PET scanners for small animals has facilitated the functional imaging of receptors in rodents at high resolution in vivo (Chapter 11). Both pharmacokinetic and pharmacodynamic information can be obtained at the same time. Longitudinal studies can be performed in the same animal to monitor disease progression or effect of treatment in the same animal to accelerate development and assessment of existing and novel drugs.

Immunocytochemistry (Chapter 12) is used to visualize primary antisera in tissue sections for the precise identification of cell types expressing a specific receptor. The technique is complementary to radioligand binding and quantitative autoradiography, particularly in characterizing mice following the deletion of genes encoding receptors. Receptors can also be identified and distinguished prior to the development of selective radioligands, which is particularly important for mapping orphan receptors, where the identity of the endogenous ligand is not yet known. Finally, measurement of mRNA provides clear evidence that a particular tissue or cell is able to express a particular receptor. Chapter 13 describes methods for the detection of mRNA encoding receptors following extraction from tissue or cells (Northern hybridisation) and the detection of specific mRNA transcripts within their synthesizing cells (in situ hybridization).

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