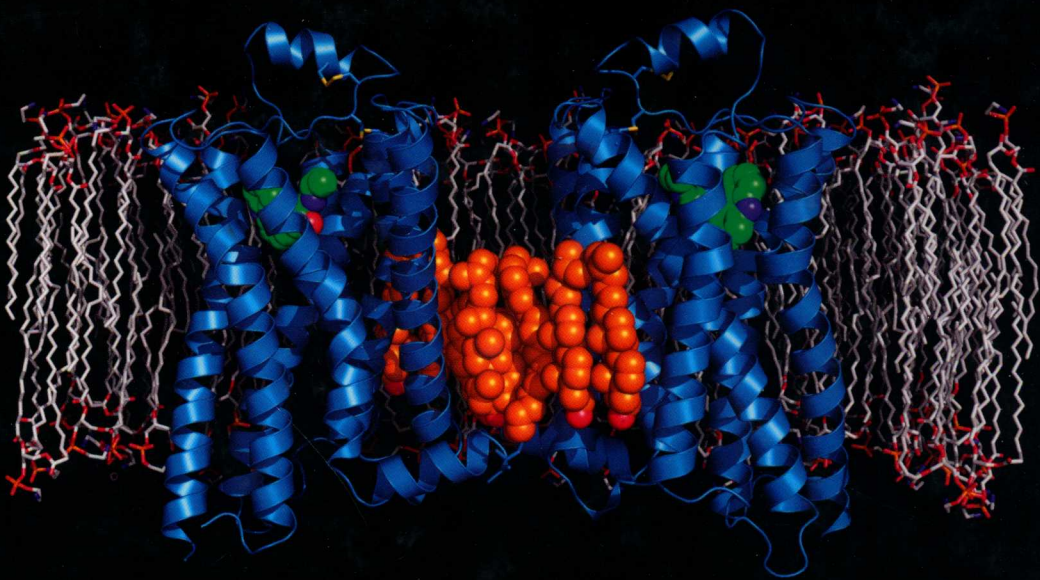
G Protein-Coupled Receptors

Structure, Signaling, and Physiology

edited by
Sandra Siehler and Graeme Milligan



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G PROTEIN-COUPLED RECEPTORS

This text provides a comprehensive overview of recent discoveries and current understandings of G protein-coupled receptors (GPCRs). Recent advances include the first mammalian non-rhodopsin GPCR structures and reconstitution of purified GPCRs into membrane discs for defined studies, novel signaling features including oligomerization, and advances in understanding the complex ligand pharmacology and physiology of GPCRs in new assay technologies and drug targeting.

The first chapters of this book illustrate the history of GPCRs based on distinct species and genomic information. This is followed by discussion of the homo- and hetero-oligomerization features of GPCRs, including receptors for glutamate, $GABA_B$, dopamine, and chemokines. Several chapters are devoted to the key signaling features of GPCRs. The authors take time to detail the importance of the pathophysiological function and drug targeting of GPCRs, specifically β-adrenoceptors in cardiovascular and respiratory diseases, metabotropic glutamate receptors in CNS disorders, S1P receptors in the immune system, and Wnt/Frizzled receptors in osteoporosis.

This book will be invaluable to researchers and graduate students in academia and industry who are interested in the GPCR field.

Dr. Sandra Siehler is a Research Investigator at the Novartis Institutes for BioMedical Research in Basel, Switzerland. Dr. Siehler is a member of the American Society for Pharmacology and Experimental Therapeutics and the British Pharmacological Society.

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Introduction

Sandra Siehler and Graeme Milligan

This book provides a comprehensive overview of recent discoveries and the current understanding in the G protein-coupled receptor (GPCR) field.

A plethora of distinct GPCRs exist on the cell surface of every cell type and generate signals inside cells to regulate key physiological events. The human genome contains between 720 and 800 GPCRs with specific tissue and subcellular expression profiles. Chapter 1 of this volume illustrates the evolutionary history of GPCRs based on genomic information available from distinct species and ancient genomic information. Many GPCRs are involved in olfactory/sensory mechanisms. Three hundred sixty-seven non-sensory human GPCRs are known or predicted to be activated by native ligands; endogenous ligands for 224 human GPCRs are described currently, but remain to be identified for 143 orphan receptors. Three hundred sixty-seven ligand-activated non-sensory GPCRs consist of 284 class A (rhodopsin-like) receptors, 50 class B (secretin-like) receptors, 17 class C (metabotropic receptor-like) receptors, and 11 belong to the atypical class of frizzled-/smoothened receptors. Polymorphisms (e.g., of β adrenoceptors, see Chapter 15) and alternative splicing (e.g., of metabotropic glutamate receptors, see Chapter 16) further increase the variety of GPCR proteins. Posttranslational modifications such as N-linked glycosylation or carboxyterminal palmitoylation can influence their function.

GPCRs are integral membrane proteins containing an extracellular amino terminus of widely varying length, seven transmembrane α-helical stretches, and an intracellular carboxy terminus. The molecular understanding of GPCRs developed with the cloning of the β_2 adrenoceptor in 1986 and appreciation that it was related to the photon receptor rhodopsin. The majority of signaling events originate at the inner face of the plasma membrane and involve transactivation of one or more members of the four G protein families (G_s, $G_{i/o}$, $G_{q/11}$, $G_{12/13}$), which link GPCRs to effector cascades. Chapter 7 explains functions of mammalian G proteins elucidated using subunit- and tissue-specific gene targeting. Besides effector cascades involving G proteins, non-G protein-mediated signaling has been described for various GPCRs. Moreover, the activity of G proteins can be regulated by non-GPCR proteins such as receptor tyrosine kinases. The activity of GPCRs is further modulated by cellular signals in an auto- and transregulatory fashion. GPCRs form intra- and juxtamembrane signaling complexes

comprising not only G proteins, but also other GPCRs, ion channels, membrane and cytosolic kinases and other enzymes, G protein-modulatory proteins, and interact with elements of the cell cytoskeleton. Chapters 3–6 describe homo- and hetero-oligomerization features of GPCRs including receptors for glutamate, $GABA_B$, dopamine, and chemokines. Dopamine receptors can hetero-dimerize not only with other subtypes in the same receptor family, but also with less-related GPCR members and ion channels such as NMDA or $GABA_A$ receptors. For class C receptors, which contain a large extracellular domain, oligomerization is mandatory for receptor function. For other GPCRs, oligomerization may result in altered and/or novel ligand pharmacology. Methods applied to measure GPCR complexes and oligomer signaling comprise GPCR-Gα protein fusion constructs containing either a mutated receptor or Gα mutant, and time-resolved fluorescence resonance energy transfer (TR-FRET).

Downstream of the cellular plasma membrane, the complexity of intracellular communication controlled by GPCRs increases dramatically. Ligand-activated GPCRs often internalize, which mostly causes desensitization of signaling events, although both prolonged signaling and even signaling initiated following receptor internalization have been described. Receptor hetero-oligomers can co-internalize, and activation and internalization of one partner can therefore silence the other interaction partner. Chapters 8–11 describe key signaling features of GPCRs better understood because of significant recent advancements. These include understanding of kinetics of receptor activation and signaling events studied using FRET and bioluminescent RET (BRET). Multiple related proteins control GPCR-mediated cell signaling processes. For example four RhoGTPase nucleotide exchange factors (Rho-GEFs) link $G_{12/13}$ to pathways controlling, for example, contractile complexes of the cytoskeleton, whereas nine mammalian adenylyl cyclases (ACs) are regulated by GPCRs in a receptor- and tissue-specific manner. These enzymes are integral membrane proteins directly regulated by G_s and $G_{i/o}$ proteins, although $G_{q/11}$-coupled GPCRs also influence AC activities via calcium and protein kinase C, and $G_{12/13}$ proteins were recently found to regulate AC activity as well. Arrestins are known to bind to agonist-stimulated phosphorylated GPCRs and promote endocytosis. Novel functions of arrestins include interactions with non-GPCR receptors or direct interaction with signaling proteins including, for example, the ERK MAP kinases. Modern assay technologies to assess GPCR signaling and ligand pharmacology are described in Chapter 12. Multiplexing subcellular readouts using high content screening allows the simultaneous capture of multiple signals, in both temporal and spatial fashion. The pharmacological complexity of orthosteric and allosteric GPCR ligands in the context of both receptor-G protein complexes and activation state models, is illustrated in Chapters 13 and 14. Functional selectivity of GPCR ligands due to receptor allosterism toward intracellular effector pathways contributes to the complex pharmacological nature.

Dysregulated ligand concentration, GPCR protein level, coupling, and/or signaling are implicated in and often causative for many pathophysiological conditions including central nervous system (CNS) disorders, cardiovascular and