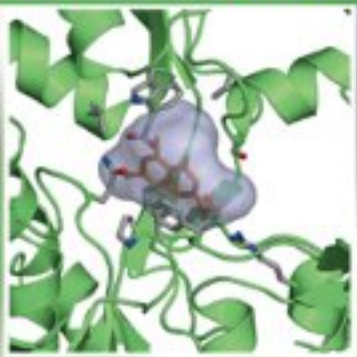
Second Edition

Anesthetic Pharmacology

Basic Principles and Clinical Practice



EDITED BY
Alex S. Evers
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CAMBRIDGE Medicine

Anesthetic Pharmacology

Second Edition

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Preface

Recent years have seen the beginning of a revolution in our understanding of how anesthesia is produced and how the drugs used by perioperative practitioners work at a molecular level. Concomitantly, the clinical practice of anesthesia has become increasingly more complex and demanding. As a result of these developments, there continues to be a growing chasm between clinically sophisticated anesthesiologists who may be inadequately versed in basic and molecular pharmacology, and anesthetic researchers who are well versed in the mechanistic details of anesthetic drug action, but inadequately informed about the clinical context in which these drugs are used. The first edition of *Anesthetic Pharmacology: Physiologic Principles and Clinical Practice* was assembled with the aim of bridging this chasm.

Since then, the understanding of molecular mechanisms of drug action has grown, mechanisms of interindividual variability in drug response are better understood, and the practice of anesthesiology has expanded to the preoperative environment, locations out of the operating room and out of the hospital, and into various intensive care units. Consequently, *Anesthetic Pharmacology* has been significantly revised into a second edition. Significant changes include the addition of a third editor, expansion from three to four sections, and enhanced organization and readability to make the material accessible to a wide range of trainees, practitioners, and pharmacologists.

Anesthetic Pharmacology is designed to be a sophisticated, accessible, reliable, and user-friendly primer of fundamental and applied pharmacology that is targeted for use by the full spectrum of those providing care in the perioperative period.

The book is organized into four fully integrated sections. The first two sections consider the principles and targets of anesthetic drug action, and the last two sections address the pharmacology and therapeutic use of the drugs themselves. Section one, "Principles of drug action," provides detailed theoretical and practical information about anesthetic pharmacokinetics and about cell signaling pathways involved in anesthetic drug action. Section two, "Physiologic substrates of drug action," is conveniently arranged by organ systems and presents the molecular, cellular, and integrated physiology of the organ or functional system, highlighting targets and substrates. Section three, "Essential drugs in anesthetic practice," presents the pharmacology and toxicology of major classes of drugs that are used perioperatively. A fourth section, "Clinical applications: evidence-based anesthesia practice," has been added to this edition to provide integrated and comparative pharmacology, and the practical therapeutic application of drugs for specific perioperative indications.

The layout of the chapters accommodates to the varying needs of the readership. Each chapter contains the fundamental body of knowledge needed by practitioners, as well as more in-depth information, including basic research directions and sophisticated clinical applications. The chapters all conclude with a concise summary of the material deemed to be essential knowledge for trainees and those seeking recertification. Through the judicious use of illustrations, boxes, and tables, information is presented in a comprehensible fashion for all levels of readership.

Section 1
Chapter 1

Principles of drug action

Pharmacodynamic principles of drug action

Stuart A. Forman

Introduction

The effects of drugs on patients in the operating room vary with drug dosage, from patient to patient, and with time. Different doses of drugs result in different concentrations in various tissues, producing a range of therapeutic and sometimes undesirable responses. Responses depend on drug pharmacokinetics (the time course of drug concentration in the body) and drug pharmacodynamics (the relationship between drug concentration and drug effect). These processes may be influenced by factors including pre-existing disease, age, and genetic variability. Patient responses to drugs may also be dynamically altered by factors such as temperature, pH, circulating ion and protein concentrations, levels of endogenous signaling molecules, and coadministration of other drugs in the operating room environment. Pharmacodynamics, the focus of this chapter, is the study of where and how drugs act to produce their effects, encompassing drug actions on biological systems ranging from molecules to organisms and their responses from conformational changes to behavior and emotional states [1,2].

Developments in pharmacology have been greatly affected by the rapid growth in our understanding of biology at the molecular level. Molecular targets for many drugs used in the practice of anesthesia are now known in varying degrees of detail. This knowledge enables development of efficient assays to identify new potential drugs and, in some cases, structure-based design of improved therapeutic drugs. The practice of anesthesiology requires an understanding of human pharmacodynamics and pharmacokinetics, but real expertise, and particularly the ability to innovate, demands deeper understanding of the scientific basis of our practical knowledge. The first and larger part of this chapter focuses on central concepts of molecular drug–receptor interactions. In actuality, most drugs affect more than one molecular target, and the impact of drug actions at the cellular, tissue, and organism levels are the result of integrated effects at these higher system levels. The latter part of the chapter covers pharmacodynamic concepts pertinent to drug responses in animals and humans. Some of the terms used, including *potency, efficacy*, and *selectivity*, have parallel meanings at both the molecular and organism levels.

Throughout this chapter, molecular pharmacodynamics concepts are illustrated both with cartoons and with simple chemical reaction schemes, which lend themselves to quantitative algebraic analyses. This quantitative formalism is provided to encourage a deeper understanding of important pharmacodynamic concepts for those who make the small additional effort.

Drug receptors

Drugs are exogenous chemical substances used to alter a physiological system. A drug may be identical to an endogenous compound, such as a peptide, amino acid, nucleotide, carbohydrate, steroid, fatty acid, or gas. Examples of endogenous factors used in anesthesiology include potassium for diuretic-induced hypokalemia, insulin for diabetes, clotting factor VIII for hemophilia, and nitric oxide for pulmonary hypertension.

Receptors versus drug targets

Pharmacologic receptors are defined as macromolecular proteins on the cell membrane or within the cytoplasm or cell nucleus that bind to specific endogenous factors (drugs), such as neurotransmitters, hormones, or other substances, and initiate cellular responses to these drugs. Protein drug targets also encompass circulating enzymes, non-chemically stimulated (e.g., voltage- or mechanically activated) membrane channels, and membrane transporters. The definition of drug targets can be further broadened to include DNA, RNA, and epigenetic control molecules, components of pathogenic or commensal microbes, toxins, etc. Drug receptor proteins may consist of one or more peptide chains.

Receptor protein structure can be characterized by features at multiple levels:

(1) Primary structure – the amino acid sequence.
(2) Secondary structure – the peptide subdomain folding pattern (e.g., α-helix, β-sheet, random).
(3) Tertiary structure – the entire peptide folding, including domain–domain interactions and disulfide bridges.

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(4) Quaternary structure – assembly of multiple peptides, including peptide–peptide interactions and disulfide bridges.
(5) Post-translational peptide modifications – including phosphorylation, lipidation, biotinylation, glycosylation, etc.

Physicochemical forces that determine receptor structure are intrapeptide, interpeptide, and with surrounding water or lipid. These forces include:

(1) Covalent bonds – sharing of electron pairs between atoms.
(2) Ionic bonds – attraction between oppositely charged ion pairs (repulsion can also affect structure).
(3) Hydrogen bonds – weak dipole–dipole forces between electronegative atoms and hydrogen, usually bonded to oxygen or nitrogen. Solvent water provides many hydrogen bonds for proteins.
(4) Van der Waals interactions – close-range attractive and repulsive forces between atoms.
(5) Hydrophobic interactions – forces arising from the energetically favorable interaction between nonpolar molecular domains that repel (i.e., do not hydrogen-bond with) solvent water.

Enzymes (circulating or intracellular) are in an aqueous environment. Hydrophobic interactions tend to make these proteins have hydrophilic exteriors and hydrophobic interiors.

Transmembrane proteins have at least one hydrophobic domain that crosses the lipid bilayer [3]. They may have multiple hydrophobic domains within the membrane and hydrophilic domains in the extracellular and intracellular spaces.

Receptor nomenclature and categorization

Classically, drug receptors have been categorized based on their sensitivity to various drugs (endogenous or otherwise). For example, **nicotinic** acetylcholine (nACh) receptors in muscle, neurons, and glia are strongly activated (agonized) by acetylcholine and nicotine (an alkaloid from tobacco), and less so by muscarine (an alkaloid from *Amanita muscaria* mushrooms), whereas **muscarinic** acetylcholine (mACh) receptors in smooth and cardiac muscle are strongly activated by acetylcholine and muscarine, but weakly by nicotine. Other receptors named for drugs widely used in anesthesia include opioid receptors and adrenergic receptors (adrenoceptors).

Drug receptor categorization by molecular structure – Analysis of genes and messenger RNA that encode proteins has provided an enormous quantity of data on protein **families** and **superfamilies**, which represent different classes of drug receptors. The *British Journal of Pharmacology*'s "Guide to Receptors and Channels" [4] lists seven classes of pharmacologic protein targets based upon similar structure and function: seven-transmembrane (7TM) receptors, ligand (transmitter)-gated channels, ion channels, catalytic receptors, nuclear receptors, transporters, and enzymes. Nomenclature for this ever-growing list is maintained by the International Union of Basic and Clinical Pharmacology (www.iuphar-db.org). Building upon the example given for classical receptor nomenclature, nicotinic acetylcholine receptors are classified as transmitter-gated channels. More specifically, nicotinic ACh receptors on fetal muscle consist of five homologous polypeptide subunits, $\alpha_1/\alpha_1/\beta_1/\gamma/\delta$, surrounding a transmembrane cation channel. The genes for these subunits were first cloned in the 1980s, providing a complete primary amino acid sequence [5]. Genetic analysis has subsequently identified more than a dozen closely related polypeptides (α_{1-10}, β_{1-4}, γ, δ, and ε) that combine to form a variety of nACh receptors, constituting a **receptor family**. The subunit types and stoichiometry for native pentameric nACh receptors in muscle and neural tissues remains an area of intensive research [6]. In adult muscle nACh receptors, the ε subunit replaces δ, but ε may re-emerge in muscle receptors formed during pathological conditions such as after burn or denervation injury. Neuronal and glial nACh receptors consist mostly of either α_7 subunits or α_4/β_2 combinations, while postsynaptic nACh receptors in autonomic ganglia consist of α_3/β_4 and $\alpha_3/\alpha_4/\beta_2/\beta_4$ combinations.

Muscarinic ACh receptors are distinguished from nicotinic ACh receptors not only by their distinct pharmacology and tissue distribution; they belong to an entirely separate superfamily of receptors, the seven-transmembrane G-protein-coupled receptors. Genetic analysis has revealed five distinct types of muscarinic receptors in a family (M_1 through M_5) [7].

Receptor superfamilies of related cellular receptors have been identified based on structural analyses (mostly peptide sequence homologies from genetic data, but also x-ray crystallography) and functional studies. Receptors within superfamilies are thought to have evolved from common ancestor receptors. This chapter provides a broad overview of several chemoreceptor superfamilies (Fig. 1.1). Following chapters contain detailed discussion of some of these superfamilies.

(1) **The seven-transmembrane receptors**, also known as G-protein-coupled receptors (GPCRs) are the largest superfamily of drug targets, containing over 60 families of proteins [8–11]. Some genes encode seven-transmembrane receptors with yet undefined physiological roles, known as orphan receptors. These are membrane proteins formed by a single peptide containing seven transmembrane helices with an extracellular N-terminal domain and an intracellular C-terminal domain. Endogenous GPCR agonists include neurotransmitters, small peptide hormones, neurotransmitters, prostanoids, and nucleotides. The intracellular domains of these receptors interact with a heterotrimeric G-protein complex that includes a GTPase domain. Activation of GPCRs leads to generation of second messengers such as cAMP, cGMP, and intracellular calcium. Persistent activation leads to a drop-off in activity, termed **desensitization**, via several mechanisms. Intracellular domains may be

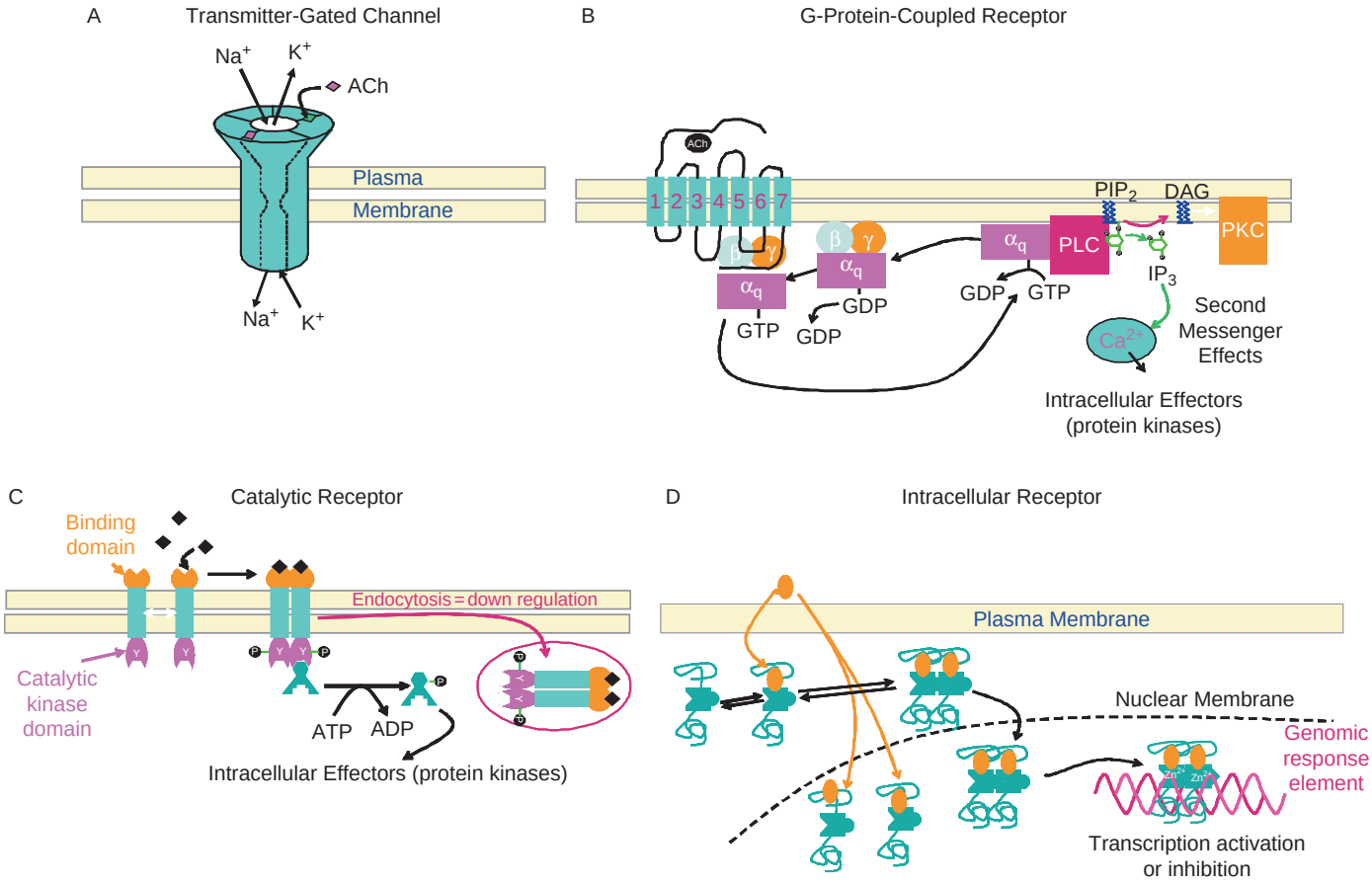

Figure 1.1. Drug receptor superfamilies. Illustrations of different families of receptor proteins, including (A) transmitter-gated ion channels, (B) G-protein-coupled receptors, (C) catalytic receptors, and (D) intracellular receptors.

modified by intracellular enzymes, blocking interactions with G-protein complexes. In addition, these receptors may be removed from the cell surface via endocytosis. This superfamily is described in detail in Chapter 2. Drugs used in anesthesia that target GPCRs include atropine and glycopyrollate (muscarinic ACh receptors), antihistamines (histamine receptors), opioids (opioid receptors), adrenergic drugs (adrenoceptors), adenosine (adenosine receptors), and some antiemetics (dopamine receptors).

(2) **The Cys-loop ligand-gated ion channel** superfamily (LGICs) are transmitter-gated channels. This superfamily includes four families of membrane proteins that are fast neurotransmitter receptors: nicotinic ACh receptors, γ-aminobutyric acid type A ($GABA_A$) receptors, glycine receptors, and serotonin type 3 ($5\text{-}HT_3$) receptors [12,13]. All of these ligand-gated ion channels contain five subunits arranged around a transmembrane ion pore. All subunits in this superfamily have structures that include a large N-terminal extracellular domain containing a Cys-X_{13}-Cys motif (the Cys-loop), four transmembrane (TM) helical domains, and a large intracellular domain between TM3 and TM4. Activating drugs (neurotransmitters) bind to sites formed at the interface between extracellular domains [14]. These binding events are coupled to gating of the ion-conductive pore, and opening of this ion channel leads to altered electrical potential within cells. Persistent activation of these receptors leads to desensitization via a conformational change in the receptor that reduces response to neurotransmitter. This superfamily is described in detail in Chapter 3. Drugs used in anesthesia that target Cys-loop LGICs include neuromuscular blockers (nicotinic ACh receptors), intravenous and volatile general anesthetics ($GABA_A$ and glycine receptors), and antiemetics ($5\text{-}HT_3$ receptors).

(3) **Catalytic receptors** contain an extracellular drug-binding domain, one (typically) or more transmembrane domains, and an intracellular enzyme domain. There are several classes of these receptors: receptor tyrosine kinases (RTKs) [15,16], tyrosine kinase associated receptors (TKARs), receptor serine/threonine kinases (RSTKs),

receptor guanylate cyclases, and receptor tyrosine phosphatases (RTPs). Drugs include growth factors (e.g., insulin), trophic factors, activins, inhibins, cytokines, lymphokines such as tumor necrosis factor [17,18], and natriuretic peptide. Toll-like receptors, which recognize molecular markers on invasive pathogens and activate cellular immune defenses, are also in this class. Drug binding to catalytic receptors usually causes receptor dimerization with accompanying activation. Intracellular enzymatic activity triggers a variety of functional changes. Active dimer forms undergo endocytosis as a mechanism of desensitization.

(4) **Intracellular receptors – Nuclear receptors** are a superfamily of intracellular transcription factors that interact with small hydrophobic molecules such as steroids, vitamin D, thyroid hormones, and retinoid hormones (retinoic acid and vitamin A) [19]. Receptor–drug complexes either form in the nucleus or translocate from cytoplasm to nucleus. Genomic DNA response elements bind to dimeric receptor–drug complexes at 60-amino-acid domains that also coordinate zinc ions. Nuclear receptors regulate gene transcription.

(5) **Endocytotic receptors** are transmembrane receptors that bind extracellular drugs and then translocate into the cell by endocytosis, a process of clathrin-coating, invagination, and vesicle formation. These receptors take up essential cell nutrients such as cholesterol (bound to low-density lipoprotein or LDL) and iron (bound to ferritin). Other cell-surface receptors may undergo endocytosis as a mechanism of receptor downregulation, usually following persistent activation.

(6) **Other protein drug targets**. The above list of receptors is truncated for simplicity. Other drug receptor superfamilies include many ion channels such as transient receptor potential (TRP) ion channels (important in peripheral sensory transduction) and voltage-gated ion channels, including sodium channels, potassium channels, chloride channels, and calcium channels (important in myocardium, skeletal muscle and nerve excitability, and propagation of electrical signals). Other transmitter-gated ion channels include *N*-methyl-D-aspartate (NMDA)-sensitive and kainite-sensitive glutamate receptor ion channels, purinergic receptors, and zinc-activated channels. Drug targets also include a variety of transmembrane pumps and transporters for ions (e.g., the $Na^+/K^+/2Cl^-$ cotransporter target of the diuretic furosemide), neurotransmitters, and other molecules. Intracellular and circulating enzymes represent another large class of drug targets, including cyclooxygenase, lipoxygenases, phosphodiesterases, and hemostatic factors.

There are several **common themes** in the physiology of drug receptor superfamilies. First, receptor–effector coupling is often a multiple-step process, providing these systems with both positive (amplification) and negative feedback. Second, active receptors usually are formed from multiple peptides. Drug-gated ion channels exist as multimers with multiple sites for their endogenous drugs, and in most cases more than a single drug must bind in order to activate these channels. G-protein-coupled receptors are multimeric complexes that dissociate upon activation. Both enzyme-linked receptors and intracellular receptors dimerize as they activate following drug binding. Third, most receptor molecules undergo desensitization following persistent activation.

Drug–receptor interactions

Drug–receptor binding

The first step in the chain of events leading to a drug effect in a physiological system is binding to a site on its receptor. Drug binding sites on receptor molecules are classified as **orthosteric** (the site where endogenous activators bind) or **allosteric**. The term *allosteric* literally means *other place*, and was originally applied to modulatory sites on enzymes that are distinct from active (substrate) sites. When applied to receptors, the term may have multiple meanings. In particular, the orthosteric sites of chemoreceptors "allosterically" alter activity of the "active sites," which may be enzymatic sites where substrates bind, sites where other proteins (e.g. G proteins) bind, or ion pores.

Drug binding studies on receptors are used to characterize their affinities. Measuring binding in tissue, cells, or purified receptor proteins requires the ability to accurately measure receptor-bound drug independently from free (unbound) drug, and correction for nonspecific binding to other components of tissues, cells, and even experimental equipment. Whereas drug binding to receptors will display saturation as all of the receptor sites become occupied, **nonspecific binding** is characterized by low affinity and is therefore usually linear and nonsaturable over the drug concentration range relevant for receptor binding (Fig. 1.2).

Reversible interactions between drugs and their receptor sites are determined by the same noncovalent biophysical forces that affect protein structure: ionic bonds, hydrogen bonds, van der Waals interactions, and the hydrophobic effect. At the molecular level, initial drug–receptor binding is a bimolecular association process, and the drug concentration (in moles/liter, M) is an independent (controllable) variable in in-vitro experiments. The bimolecular association rate is $[D] \times k_{on}$, where k_{on} is the on-rate in units of $M^{-1}\ s^{-1}$. Drug dissociation is a unimolecular process, characterized by an off-rate, k_{off}, with units of s^{-1} (Eq. 1.1). The strength of reversible interactions between a drug and its site(s) on a receptor is reflected in its **equilibrium binding affinity**, which is usually reported as a **dissociation constant**, K_D, with units in moles/liter (M). When the drug concentration $[D] = K_D$, association and dissociation rates are equal. High affinity is associated with a low K_D, and low affinity with a high K_D.

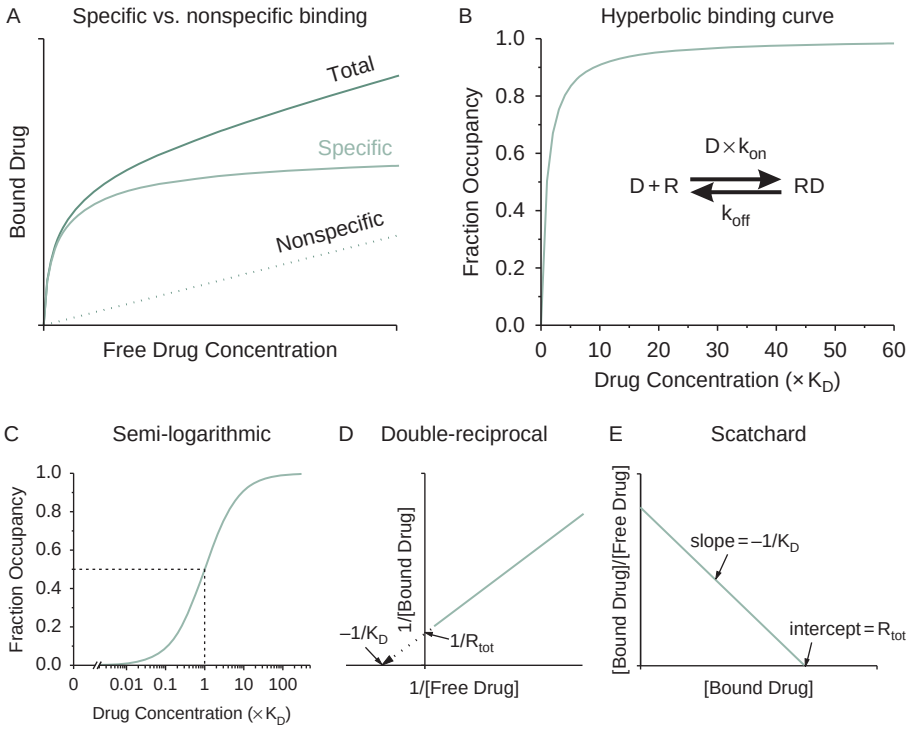

Figure 1.2. Drug binding graphical analysis. (A) Illustration of specific vs. nonspecific binding. (B) Correcting total binding for nonspecific binding produces a saturable hyperbolic binding curve on linear axes (Eq. 1.4). (C) Semilogarithmic plot with logarithmic concentration axes. (D) Lineweaver–Burke double-reciprocal plot. (E) Scatchard plot.

Drug binding

A quantitative treatment of this concept should be familiar from chemical equilibrium theory. In the simplest case with a single drug binding site:

$$D + R \underset{k_{off}}{\overset{[D] \times k_{on}}{\rightleftharpoons}} RD \tag{1.1}$$

$$\text{where } K_D \equiv \frac{k_{off}}{k_{on}} = \frac{[R] \times [D]}{[RD]} \tag{1.2}$$

$$\text{Thus } [RD] = [R] \times \frac{[D]}{K_D} \tag{1.3}$$

Assuming the total number of receptors $R_{tot} = R + RD$ is constant (the law of mass action), then the fraction of bound receptors is:

$$\frac{[RD]}{[R_{tot}]} = \frac{[RD]}{[R] + [RD]} = \frac{[R] \times \frac{[D]}{K_D}}{[R] \times \left(1 + \frac{[D]}{K_D}\right)} = \frac{[D]}{[D] + K_D} \tag{1.4}$$

Equation 1.4 is a **Langmuir isotherm** or a hyperbolic binding curve (Fig. 1.2B). Site occupancy is ~1% at $[D] = 0.01 \times K_D$, 10% at $0.11 \times K_D$, 50% at K_D, 90% at $9 \times K_D$, and 99% at $99 \times K_D$. Because of the wide range (four orders of magnitude) of drug concentrations needed to span from low occupancy to nearly saturated, binding curves are frequently plotted with drug concentration on a logarithmic axis (Fig. 1.2C). The semilog plot displays a sigmoid shape. The midpoint of this curve (50% occupancy) corresponds to K_D.

Linear transformations of Eq. 1.4 are frequently used to provide easier graphical analysis (common before computerized nonlinear regression analysis). The Lineweaver–Burke or double-reciprocal plot (Fig. 1.2D) is readily derived from Eq. 1.4:

$$\frac{[R_{tot}]}{[RD]} = \frac{[D] + K_D}{[D]} = 1 + \frac{K_D}{[D]}, \text{ thus } \frac{1}{[RD]} = \frac{1}{[R_{tot}]} + \frac{1}{[D]} \times \frac{K_D}{[R_{tot}]} \tag{1.5a}$$

Plotting $1/[RD]$ vs. $1/[D]$ (i.e., reciprocal of bound drug vs. reciprocal of free drug) gives a line with slope $= K_D/[R_{tot}]$ and intercept on the y-axis $= 1/[R_{tot}]$. The extrapolated x-axis intercept is $-1/K_D$.

Equation 1.5a can be rearranged to give:

$$\frac{[RD]}{[D]} = \frac{[R_{tot}]}{K_D} - \frac{[RD]}{K_D} \tag{1.5b}$$

Equation 1.5b is the basis for another common linear transformation of binding data, the Scatchard plot (Fig. 1.2E). For Scatchard analysis, the ratio of bound to free drug ($[RD]/[D]$) is plotted against bound drug ($[RD]$), resulting in a line with slope $= -1/K_D$ and x-axis intercept $= R_{tot}$.

Stoichiometry of drug binding may be greater than one site per receptor, especially for multi-subunit receptors. When more than one site is present, there may be different binding affinities associated with different receptor subsites. In addition there may be cooperative interactions between different subsites. **Binding cooperativity** may be positive or negative.

Positive cooperativity is when occupancy of one site enhances binding at another site. Negative cooperativity is when occupancy of one site reduces affinity at another site.

Selectivity – Drug receptor sites display variable degrees of **selectivity** for drugs with slightly different molecular structures [20–22]. An important example of this concept is the selectivity for different adrenoceptor subtypes (α_1, α_2, β_1, and β_2) to various derivatives of the endogenous transmitters epinephrine and norepinephrine (e.g., phenylephrine, dopamine, isoproterenol, terbutaline, etc.) [23]. Another common feature of many drug sites is **stereoselectivity**. Drugs often have one or more chiral centers. A single chiral center means that the drug can exist as a pair of enantiomers (mirror images, R- or S-, *d-* or *l-*), while multiple chiral centers results in diastereomers. Drug enantiomers (and diastereomers) may interact differently with receptor sites and with other sites. If a high-affinity stereoisomer can be isolated, it may act as a more potent, more efficacious, and less toxic drug. Examples used in anesthesia include etomidate, a general anesthetic used as a pure R(+) stereoisomer [24], levobupivacaine, the L-isomer of bupivacaine [25], and cisatracurium, the *cis*-diastereomer of atracurium.

Specificity – Many drugs bind to more than one molecular target at clinically relevant concentrations. One receptor may mediate the desired therapeutic action, while binding to other targets may be associated with side effects or toxicity. Specificity of binding is therefore usually a desirable feature of drugs. High specificity means that the drug interacts with only one or a small number of target sites.

Small hydrophilic drugs can diffuse rapidly and are exploited for rapid cell-to-cell signaling (e.g., neurotransmission). Small drugs have limited ability to form noncovalent binding interactions, so are generally lower affinity and lower specificity than large drugs. In some cases, two or more small drugs are required for effect (e.g., neurotransmitters). Large drugs diffuse more slowly, but can generate more binding affinity and specificity.

Consequences of drug–receptor interactions

The previous section examined drug binding to receptors. This section now examines the consequences of drug binding, that is, drug response or drug effects. Drugs may either increase or decrease various functions of biological systems. **Drug effects** may be studied in molecules, cells, or tissues under conditions of well-defined free drug concentration, resulting in concentration–response relationships [26]. Drug responses are typically **graded** within an experimentally established minimum to maximum range, and may be mediated directly by the drug receptor (e.g., an ionic current due to activation of an ion channel chemoreceptor) or by a second messenger (e.g., cAMP concentration) or other downstream cellular processes (e.g., muscle contraction force).

Most drug effects are **reversible**, ending when drug concentration and occupation of receptor binding sites diminish to zero. Drug effects may also be **irreversible**. Irreversible drugs form covalent bonds with receptors (e.g., aspirin acetylates cyclooxygenase, irreversibly inactivating the enzyme). **Pseudoirreversible** drugs are high-affinity noncovalent drugs that unbind so slowly that they are effectively irreversible. Antibodies and certain toxins that bind with sub-nanomolar affinity behave pseudoirreversibly.

Agonists

Agonists are drugs that bind to and activate receptors, resulting in a biological response. Agonist effects are described by two fundamental characteristics, **efficacy** and **potency** (Fig. 1.3A) [27]. Efficacy reflects the ability of the agonist to activate the receptor, and is the maximal response or effect possible when all receptor sites are fully occupied (sometimes called E_{max}). Agonists may be classified as **full agonists** (high efficacy) or **partial agonists** (low efficacy). Full agonists elicit a maximum possible response from a system, while partial agonists elicit less than a full response, even when all receptors are occupied. At the receptor level, full agonists activate nearly all receptors, while partial agonists activate only a fraction of receptors [28,29]. Partial agonism can be a desirable feature of drugs, particularly when full agonism is associated with toxicity. For example, full opioid receptor agonists can cause profound respiratory depression, whereas partial agonists that cause less respiratory depression may provide a safety advantage, while also limiting antinociceptive efficacy.

Potency refers to the concentration (or amount) of a drug needed to produce a defined effect. The most common measure of agonist potency is the **half-maximal effective concentration (EC_{50})**, the concentration at which a drug produces 50% of its maximal possible response in a molecule, cell, or tissue [27]. Potency and EC_{50} are inversely related: when EC_{50} is low, potency is high, and vice versa. At the molecular level, agonist potency is related to its affinity for the receptor, but is not exactly equal to it, because **receptor activation is not equivalent to agonist binding**. Quantitatively, an agonist's EC_{50} is a function of both its binding affinity, K_A (the subscript A designates an agonist drug) and its efficacy, which depends on the series or network of linked responses that follow binding. A simple example is a two-step model for activation of a receptor-ion channel target, where efficacy is represented by a second monomolecular transition from inactive (nonconductive) to the active (conductive) state (Eq. 1.6). Agonist (A) binding to the inactive receptor (R) is defined by the equilibrium binding site affinity K_A, and channel activation is characterized by the equilibrium between inactive and active drug-bound receptors (RA and RA*, respectively). If the inactive $\leftrightarrow$ active equilibrium strongly favors the RA* state, then the RA state is depopulated, which results in more receptor binding. When this happens, EC_{50} is lower than K_A (Fig. 1.3B).

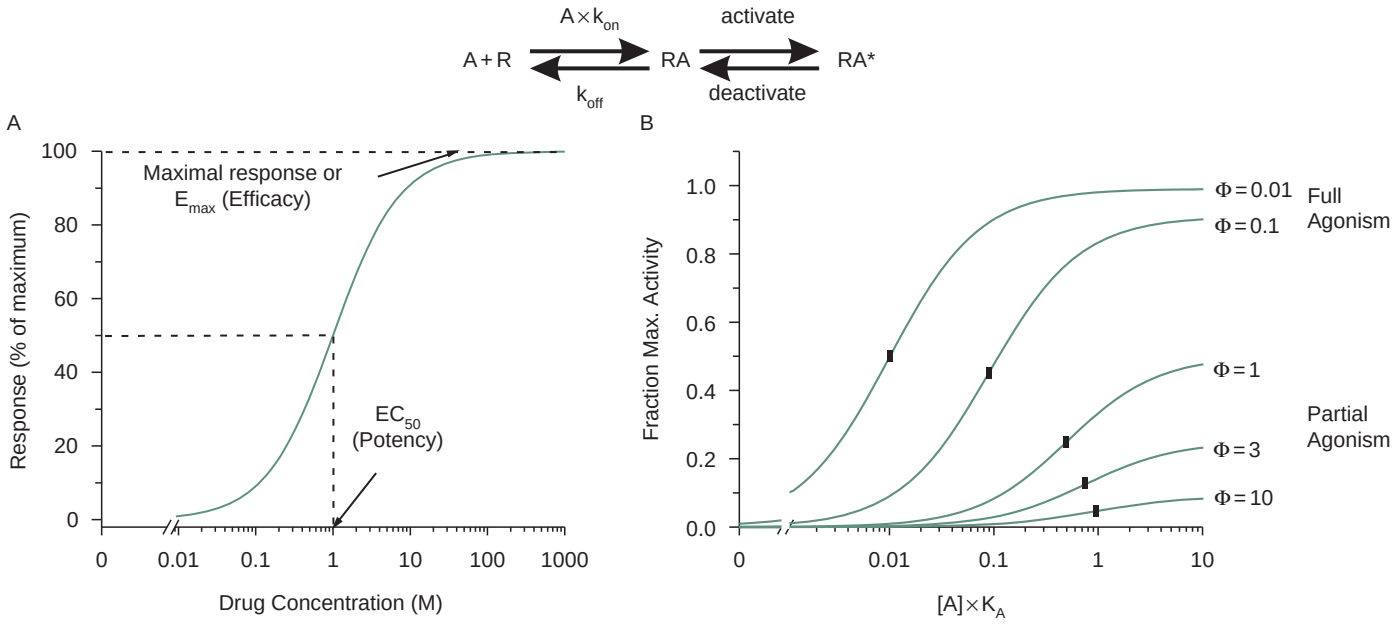

Figure 1.3. Agonist efficacy and apparent potency (EC_{50}). (A) This panel appears similar to Fig. 1.2C, except that the ordinate is a physiological response, rather than binding-site occupancy. The maximal response is drug efficacy. The concentration producing half-maximal response is the EC_{50}. (B) Lines were generated using Eq. 1.7. Affinity for inactive receptors, K_A, was held constant. ϕ is defined as the equilibrium constant for activation: $\phi = [RA]/[RA^*]$. Thus, a low ϕ value is associated with full agonism and a high ϕ is associated with partial agonism. The midpoints of the curves, EC_{50}, are indicated by vertical bars. Note that EC_{50} approximates K_A only when ϕ is much larger than 1.

$$A + R \underset{k_{off}}{\overset{[A]\times k_{on}}{\rightleftharpoons}} RA \underset{k_{deactivate}}{\overset{k_{activate}}{\rightleftharpoons}} RA \tag{1.6}$$

where K_A is the dissociation constant for A binding to R and $\phi \equiv \frac{k_{deactivate}}{k_{activate}} = \frac{[RA]}{[RA^*]}$. The fraction of *active receptors* is:

$$\frac{[RA^*]}{[R_{tot}]} = \frac{[R]\times\frac{[A]}{\phi K_A}}{[R]\times\left(1+\frac{[A]}{K_D}+\frac{[A]}{\phi K_A}\right)} = \frac{[A]}{[A]+\phi[A]+\phi K_A} = \left(\frac{1}{1+\phi}\right)\times\left(\frac{[A]}{[A]+\frac{\phi K_A}{1+\phi}}\right) \tag{1.7}$$

Equation 1.7 has the same form as Eq. 1.4, with a maximum amplitude of $(1+\phi)^{-1}$ and half-maximal concentration (K_A^{app} or EC_{50}) $= \phi K_A/(1+\phi)$. The amplitude factor $(1+\phi)^{-1}$ is agonist **intrinsic efficacy**, often designated as ε [27]. When ϕ is large (inactive state favored), efficacy is low (partial agonism) and when ϕ is small, efficacy is high (full agonism). The EC_{50} is only close to K_A when $\phi >> 1$ (i.e., for weak partial agonists). When efficacy is high (i.e., $\phi < 1$), EC_{50} is less than K_A (Fig. 1.3B).

Note that the serial binding → activation scheme in Eq. 1.6 does not allow nondrugged receptors to activate. The conformational change triggered by agonist binding is presumed to be due to "induced fit," wherein agonist binding to the inactive receptor induces or allows a conformational change that both activates the receptor and tightens agonist binding. Agonist binding to active receptors is characterized by a dissociation constant of ϕK_A.

Multiple agonist sites and the Hill equation – When occupancy of more than one drug-binding site is required to activate a receptor, concentration–response curves often display a steeper dependence on drug concentration. The case with two equivalent sites is:

$$D + R \underset{k_{off}}{\overset{2[A]\times k_{on}}{\rightleftharpoons}} RA \underset{2k_{off}}{\overset{[A]\times k_{on}}{\rightleftharpoons}} RA_2 \tag{1.8}$$

Dissociation constants at each step reflect the different binding and unbinding rates depending on the number of binding sites. Thus:

$$[RA] = [R]\times\frac{2\times[A]}{K_A} \tag{1.9}$$

and

$$[RA_2] = [RA]\times\frac{[A]}{2\times K_A} = [R]\times\frac{[A]^2}{K_A^2} \tag{1.10}$$

The fraction of activatable RA_2 receptors is:

$$\frac{[RA_2]}{[R_{tot}]} = \frac{[RA_2]}{[R]+[RA]+[RA_2]} = \frac{[R]\times\frac{[A]^2}{K_A^2}}{[R]\times\left(1+\frac{2[A]}{K_A}+\frac{[A]^2}{K_A^2}\right)} = \left(\frac{[A]}{[A]+K_A}\right)^2 \tag{1.11}$$

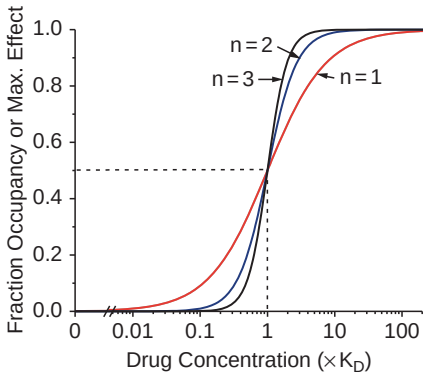

Figure 1.4. Hill analysis for multiple agonists. Semi-logarithmic logistic dose–response curves, generated using Eq. 1.13, with $n = 1$, 2, and 3. Note that the midpoint of the curves (EC_{50}) is not dependent on the Hill-slope, n.

The general form of this equation for n equivalent drug (D) sites is:

$$\frac{RD_n}{R_{tot}} = \left(\frac{D}{D + K_D}\right)^n \tag{1.12}$$

Note that when $D = K_D$, $RD_n/R_{tot} = (0.5)^n$. The half-maximal occupancy/activity concentration, EC_{50}, is no longer proportional to K_D (K_A). A closely related equation that is often used for graphical/parametric analysis of concentration–response data is the **Hill equation** [30], also known as the **logistic equation**:

$$\text{Response} = (E_{max} - E_{min}) \times \frac{[D]^n}{[D]^n + EC_{50}^n} + E_{min} \tag{1.13}$$

E_{max} and E_{min} are respectively, maximum and minimum responses. In Eq. 1.13, the half-maximal effect concentration (EC_{50}) is independent of n (Fig. 1.4). Values of the **Hill coefficient** (n) larger than 1 indicate more than one drug site and possible positive cooperativity. Values of n lower than 1 may also indicate multiple drug sites (heterogeneous binding) with possible negative cooperativity.

Indirect agonists act through mechanisms that do not involve binding to the target receptor. A common example in anesthesia is the use of acetylcholinesterase inhibitors such as neostigmine and pyridostigmine to reverse neuromuscular blockade. By slowing the breakdown of acetylcholine (ACh) in motor synapses, these drugs increase the ACh concentration, increasing the activation of postsynaptic nicotinic ACh receptors.

Antagonists

Antagonists are drugs that inhibit receptor activity [31]. Receptor antagonists can be classified as **competitive** or **noncompetitive** (Fig. 1.5).

Competitive antagonists bind at the orthosteric (agonist) sites, but do not activate receptors. As a result, they prevent agonists from occupying those sites and inhibit receptor activation. In other words, competitive antagonists and agonists display **mutually exclusive binding**. Binding assays with increasing concentrations of competitive antagonists result in reduced agonist binding, and vice versa. Thus, addition of a reversible competitive antagonist results in a rightward shift of the agonist dose–response (toward higher doses), decreasing the apparent potency (increased EC_{50}) of the agonist. Reversible competitive antagonist binding and effects are **surmountable** – increasing the concentration of agonist displaces inhibitor from binding sites and restores full agonist occupancy and response – and therefore agonist efficacy is unchanged (Fig. 1.6B).

$$\begin{array}{ccc} A + I + R & \overset{K_A}{\rightleftharpoons} & RA \\ & k_I \Big\| & \\ & RI & \end{array} \tag{1.14}$$

In Eq. 1.14, A is an agonist, while I is a reversible competitive antagonist with dissociation constant K_I (the subscript I is for inhibitor). We eliminate receptor activation for simplicity. The fraction of activatable RA receptors is:

$$\begin{aligned} \frac{[RA]}{[R_{tot}]} &= \frac{[RA]}{[R] + [RA] + [RI]} = \frac{[R] \times \frac{[A]}{K_A}}{[R] + [R] \times \left(\frac{[A]}{K_A} + \frac{[I]}{K_I}\right)} \\ &= \frac{[A]}{[A] + K_A \times \left(1 + \frac{[I]}{K_I}\right)} \end{aligned} \tag{1.15}$$

This equation again has the general form of a Langmuir isotherm with a constant maximum occupancy of 1.0 and half occupancy at $[A] = K_A \times (1 + [I]/K_I)$. Thus, as [I] increases, agonist concentration–responses shift rightward in a parallel fashion and EC_{50} increases as a linear function of [I] (Fig. 1.6). **Schild analysis** [32] is based on this relationship: the ratio of agonist concentrations needed to evoke an equal response (e.g., 50% of maximum) in the presence vs. absence of a competitive inhibitor is:

$$\frac{[EC_X]_I}{[EC_X]_0} = 1 + \frac{[I]}{K_I} \tag{1.16}$$

A similar relationship exists for the competitive inhibitor when the agonist is varied. The **IC_{50}** for inhibitors is the concentration that inhibits half of the control response with no inhibitor. Thus:

$$\frac{[A]}{[A] + K_A \times \left(1 + \frac{IC_{50}}{K_I}\right)} = \frac{1}{2} \times \frac{[A]}{[A] + K_A} \tag{1.17}$$

Solving for IC_{50}, one obtains:

$$IC_{50} = K_I \times \left(1 + \frac{[A]}{K_A}\right) \tag{1.18}$$

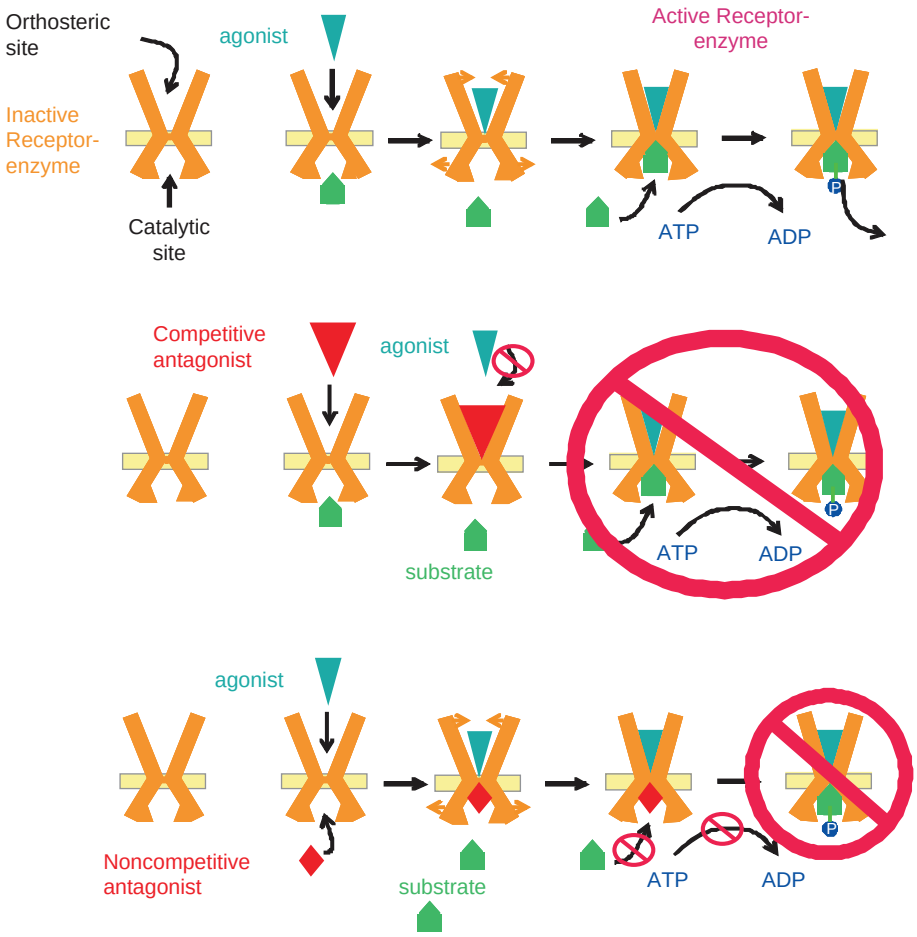

Figure 1.5. Model receptor illustration of agonism and antagonism. Top: A simple catalytic receptor model is illustrated, depicting agonist (blue triangle) binding, which induces a conformational change allowing substrate (green) binding and phosphorylation. Middle: A competitive inhibitor (red triangle) binds to the agonist (orthosteric) site, preventing agonist binding and activation of the receptor. Bottom: A noncompetitive inhibitor (red diamond) does not block agonist binding, but binds at the active site, preventing substrate binding and thereby reducing activity whether or not agonist binds.

Partial agonists as competitive antagonists – In the presence of full agonists, partial agonists appear to inhibit receptors like competitive antagonists. Partial agonists bind at orthosteric sites, preventing occupancy by full agonists, and reducing activation. Partial agonists do not produce full inhibition, because high concentrations activate a fraction of receptors. Their inhibitory effect is surmountable with increased concentrations of full agonist.

Noncompetitive antagonists bind at sites other than the orthosteric site (allosteric sites). Thus, noncompetitive antagonists can bind to receptors whether or not orthosteric sites are occupied by agonist. In the simplest case of noncompetitive inhibition, agonist binding is unaffected, but receptor activation is blocked. Thus, addition of noncompetitive antagonists will not alter agonist binding affinity or the number of agonist sites, but result in a reduced number of activatable receptors. In the presence of noncompetitive antagonism, agonist concentration–responses display reduced agonist efficacy with unaltered EC_{50} (Fig. 1.7). Inhibition by noncompetitive antagonists is not surmountable with high agonist concentrations.

Equation 1.19 and Figure 1.7 illustrate noncompetitive antagonism when the affinities of agonists and antagonists are independent:

$$\begin{array}{ccc} A + I + R & \xrightleftharpoons{K_A} & RA \\ k_I \uparrow\downarrow & & k_I \uparrow\downarrow \\ RI & \xrightleftharpoons{K_A} & RAI \end{array} \qquad (1.19)$$

Thus:

$$\frac{[RA]}{R_{tot}} = \frac{[RA]}{[R] + [RA] + \|RI\| + [RAI]} = \frac{[R] \times \frac{[A]}{K_A}}{[R] \times \left(1 + \frac{[A]}{K_A} + \frac{[I]}{K_I} + \frac{[A] \cdot [I]}{K_I}\right)} = \left(\frac{K_I}{[I] + K_I}\right) \times \frac{[A]}{[A] + K_A} \qquad (1.20)$$

This equation again is a Langmuir isotherm with amplitude = $K_I/([I] + K_I)$ and $EC_{50} = K_A$. (We have again simplified the math by eliminating receptor activation steps.) In this case, $IC_{50} = K_I$. Note that agonist EC_{50} is independent of inhibitor concentration and IC_{50} is independent of agonist concentration.

Irreversible antagonists, whether they act at the orthosteric site (competitive) or not, reduce the number of activatable receptors, while the remaining unbound receptors behave normally. This is another form of insurmountable inhibition,

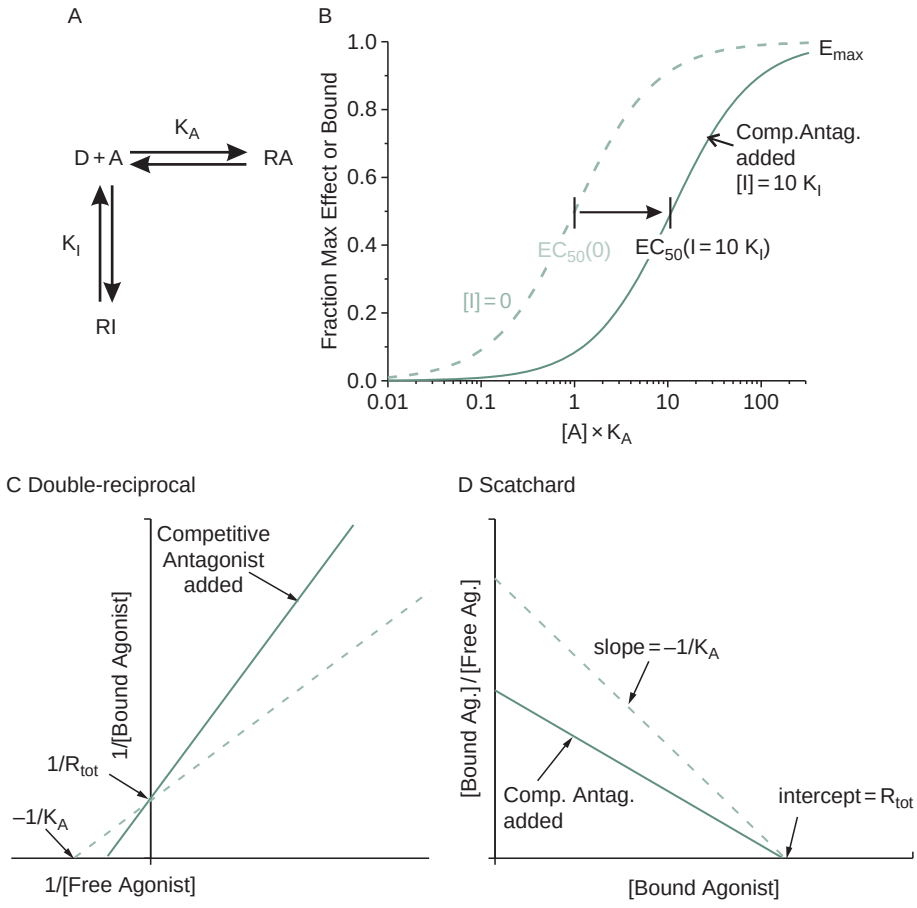

Figure 1.6. Competitive inhibition. (A) Mutually exclusive receptor occupation is depicted schematically. The RA state can activate, but the RI state cannot. (B) Agonist concentration–response curves were generated with Eq. 1.13. Addition of a competitive inhibitor reduces agonist binding and effects at low agonist concentrations, while increasing agonist EC_{50} (shifting agonist concentration–response rightward). The inhibition is surmountable, as E_{max} remains unchanged. (C) A double-reciprocal plot for agonist binding experiments in the presence of a reversible competitive inhibitor shows an altered slope and a change in apparent K_A, but the same number of receptors. (D) A Scatchard plot for agonist binding depicting the change in slope in the presence of a competitive inhibitor.

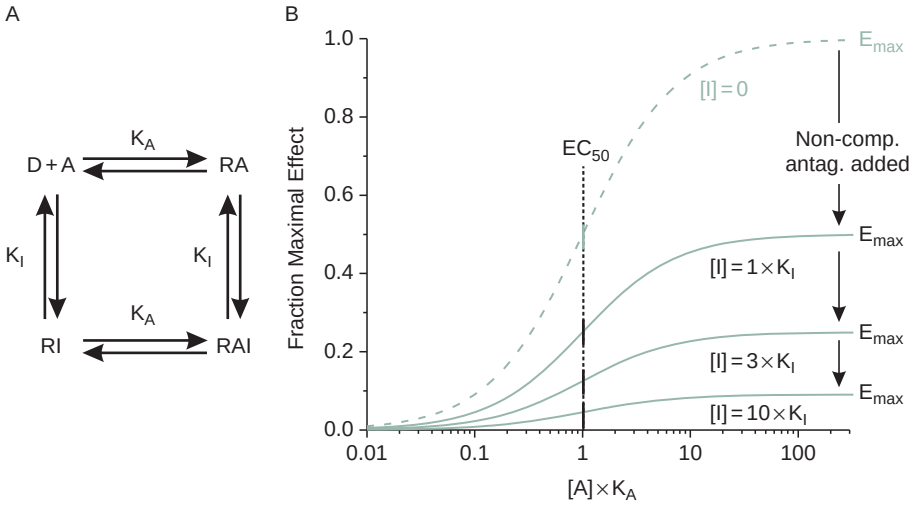

Figure 1.7. Noncompetitive inhibition. Left: A scheme depicting binding of inhibitor (I) to receptors whether or not agonist is bound. Right: The panel shows the effect of non-competitive inhibitor on agonist concentration–response curves. Noncompetitive and irreversible antagonists reduce apparent agonist efficacy (E_{max}) without changing apparent K_A (EC_{50}), indicated by the vertical bars. Note that agonist binding studies in the presence of a noncompetitive inhibitor of this type will not show any change, because the inhibitor does not compete with agonist or alter its affinity.

and concentration–response data in receptors exposed to irreversible antagonists appear similar to those for noncompetitive antagonists. Competitive binding studies can reveal whether an irreversible antagonist binds at the orthosteric site, which would lead to reduced agonist binding, or allosteric sites, which would not reduce agonist binding.

Indirect antagonism occurs without receptor binding. One mechanism of indirect antagonism is direct binding to agonist (or drug), making it unable to bind to its receptor. An example is the use of protamine to bind and inactivate heparin, preventing activation of its molecular target, antithrombin.

Allosteric receptor activation models

A more general treatment of drug–receptor interactions enables formal description of situations that are frequently observed in molecular pharmacology, but which are poorly described by serial binding-activation models. These receptor models, introduced in 1965 by Monod, Wyman, and Changeux, are referred to as allosteric models, based on the fact that agonist binding sites for receptors are distinct from their active sites (ion channels or enzyme domains, etc.) [33]. The major difference between allosteric activation models and the serial binding-activation models described above is that allosteric models allow for receptor activation in the absence of agonists. This adds a fourth state, R*, and results in a cyclic scheme (Eq. 1.20). Many receptors, including many GPCRs, are indeed partially active in the absence of agonists, indicating a pre-existing equilibrium between active and inactive receptors [34]. Agonist binding shifts this equilibrium further toward the active state, and, by implication, agonists bind more tightly to the active state than to the inactive state. The existence of the R* state differs fundamentally from the induced-fit hypothesis implied by serial binding-activation models. In practice, serial binding-activation represents a subset of conditions that can be described by allosteric models, specifically when the fraction of R* is extremely small relative to R.

The simplest allosteric model for agonism is shown in Eq. 1.21.

$$\begin{array}{ccc} A + R & \xrightleftharpoons{K_A} & RA \\ {\scriptstyle L_0}\uparrow\downarrow & & {\scriptstyle L_1}\uparrow\downarrow \\ R^* & \xrightleftharpoons{K^*_A} & RA^* \end{array} \tag{1.21}$$

Note that undrugged R can convert to an active state R* without agonist binding. An equilibrium constant (L_0) characterizes this monomolecular transition: $L_0 = [R]/[R^*]$. L_1 is equivalent to ϕ in Eqs. 1.6 and 1.7. Equation 1.21 also explicitly shows that agonist binding to active receptors is different from binding to inactive receptors. Furthermore, because of the cyclic nature of the scheme, there is a constraint on the system, $K_A \times L_1 = L_0 \times K^*_A$, so:

$$\frac{L_1}{L_0} = \frac{K^*_A}{K_A} \tag{1.22}$$

Thus, this system is defined by only three equilibrium constants.

The ratio $K^*_A/K_A \equiv c$ is the allosteric agonist efficacy. Highly efficacious agonists ($c << 1$) shift the equilibrium strongly toward the active state by binding much more tightly to active than to inactive receptors.

The fraction of active receptors is:

$$\frac{[R^*] + [RA^*]}{R_{tot}} = \frac{1}{1 + L_0 \times \left(\frac{1+[A]/K_A}{1+[A]/K^*_A}\right)} \tag{1.23}$$

When [A] = 0, the minimum fraction of active receptors is $(1+L_0)^{-1}$ and when [A] is very high (occupying all agonist sites), the fraction of active receptors approaches $(1 + cL_0)^{-1}$.

Also implicit in the allosteric gating concept is that there are conformational changes in the agonist binding site that are coupled to activation of the receptor. These conformational changes are associated with tighter binding to agonist at the orthosteric site, but are not necessarily "induced" by agonist binding, because they may occur in the absence of agonists.

Using the formalism of allosteric gating, **full agonists** and **partial agonists** are redefined (Fig. 1.8B) [35]. Agonists shift the equilibrium toward active states, so **for all agonists, $c < 1$**. Full agonists are those for which $c \times L_0 << 1$, and partial agonists are those for which $c \times L_0 > 1$ (i.e., less than 50% activation is induced). An interesting feature of this concept is that agonist efficacy is dependent on L_0, so when L_0 is small, an agonist that only modestly shifts the activation equilibrium can stimulate a large fraction of receptors to activate. Conversely, if L_0 is extremely large (i.e., the receptor has extremely low spontaneous activity), agonists need to shift the activation equilibrium a great deal to activate a significant fraction of receptors (Fig. 1.8C).

The concept of **inverse agonism** can be understood in the context of allosteric activation schemes. Inverse agonists are drugs that bind to the orthosteric site, and where $c > 1$ (Fig. 1.8D) [36]. Thus, inverse agonists stabilize the inactive state relative to the active state by binding to inactive receptors more strongly than to active receptors. Indeed, upon careful study, many drugs that are categorized as competitive antagonists are found to be inverse agonists or extremely weak partial agonists. In the context of allosteric schemes, a truly competitive antagonist binds both active and inactive receptors with equal affinity and has no impact on the activation equilibrium; that is, $c = 1$.

Allosteric gating schemes are useful for modeling multisubunit receptors with multiple agonist sites. For example, with two homologous subunits and only two receptor states (inactive R and active R*), agonist sites are all identical and couple equally to activation:

$$\begin{array}{ccccc} 2A + R & \xrightleftharpoons{1/2K_A} & RA & \xrightleftharpoons{2K_A} & RA_2 \\ {\scriptstyle L_0}\uparrow\downarrow & & {\scriptstyle cL_0}\uparrow\downarrow & & {\scriptstyle c^2L_0}\uparrow\downarrow \\ 2A + R^* & \xrightleftharpoons{1/2K^*_A} & RA^* & \xrightleftharpoons{2K^*_A} & RA_{2^*} \end{array} \tag{1.24}$$

The fraction of active receptors is:

$$\frac{[R^*] + [RA^*] + [RA^*_2]}{R_{tot}} = \frac{1}{1 + L_0 \times \left(\frac{1 + [A]/K_A}{1 + [A]/cK_A}\right)^2} \tag{1.25}$$

Allosteric concepts are also useful for modeling interactions between different drugs on a single receptor. Some drugs are

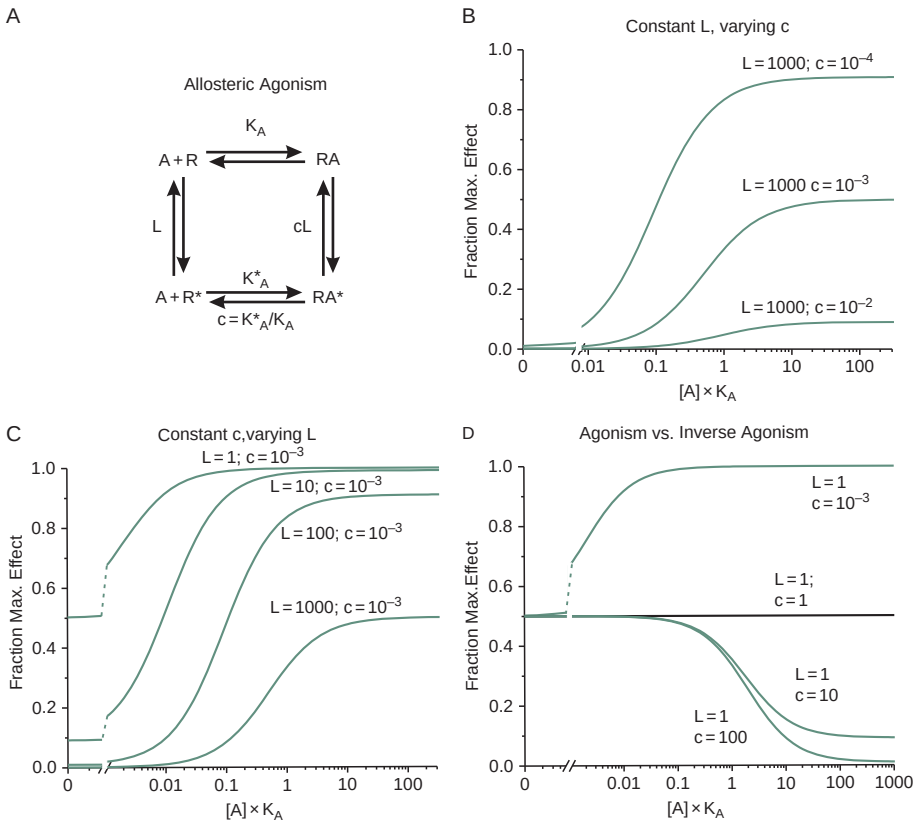

Figure 1.8. Allosteric models: agonism and inverse agonism. (A) A simple allosteric activation scheme is depicted showing inactive (R) and active (R*) receptor forms with different affinities for agonist. Agonist–response curves in panels B–D were generated using Eq. 1.23. (B) L_0 is held constant, c (efficacy) varies. Note how this panel looks just like Fig. 1.3B, because when L_0 is large the allosteric model behaves like a serial binding-activation model. (C) Efficacy (c) is constant, L_0 varies. Note spontaneous activity and higher apparent efficacy and potency of agonist as L_0 decreases. (D) Inverse agonism (reduced activity) is observed when spontaneous activity is present and $c > 1$. Pure competitive antagonism is present when $c = 1$.

allosteric enhancers. Examples include classic benzodiazepines like diazepam and midazolam that sensitize $GABA_A$ receptors to GABA, shifting GABA concentration–responses for $GABA_A$ receptors leftward. This effect could be due to allosteric effects on the GABA binding site (reducing K_A) or due to effects on receptor activation (i.e., reducing L_0). Studies on spontaneously active mutant $GABA_A$ receptors demonstrate that benzodiazepines directly enhance receptor activation in the absence of GABA, indicating that benzodiazepines are in fact weak **allosteric agonists** [37].

Spare receptors

Spare receptors exist if a maximal cellular or tissue response is elicited when receptors are not fully occupied by agonist. Formally, the presence of spare receptors is equivalent to very high drug efficacy for agonists, while reducing the apparent effect of inhibitors (Fig. 1.9). Neuromuscular transmission is characterized by spare receptors. Critical neuromuscular junctions, such as those in the diaphragm and major muscle groups, have an extremely high density of nicotinic ACh receptors. In most cases, activation of a small fraction of nACh receptors is adequate to fully activate postsynaptic muscle fascicles. With administration of nondepolarizing muscle relaxants that competitively block ACh receptor activation, symptoms of weakness typically are first seen in ocular and pharyngeal muscles, which have smaller degrees of spareness for neurotransmission. Weakness in trunk muscles typically occurs when over 80% of receptors are blocked. Agonist concentration–responses in tissues or cells with spare receptors are shifted toward lower concentrations relative to molecular responses (Fig. 1.9A). In contrast, antagonist concentration–responses are shifted toward higher concentrations, because a maximal response may be present until a large fraction of receptors are inhibited (Fig. 1.9B). When noncompetitive inhibitors are studied in experimental systems with spare receptors, they may produce rightward shifts in agonist concentration–response without decreasing apparent efficacy. This occurs because they reduce the degree of spareness. Thus, noncompetitive inhibitors may appear to act competitively in systems exhibiting spare receptors, whereas binding studies can reveal the underlying noncompetitive interaction with agonists.

Signal amplification

Signal amplification is typical of receptors that are coupled to enzymes. Amplification is another mechanism that mimics spare receptors. Drug binding to GPCRs triggers G-protein activation that persists much longer than drug binding at the receptor. Each G protein can catalyze the production of many second-messenger molecules before deactivation. In turn, second messengers can trigger additional cascades of intracellular signal activation.

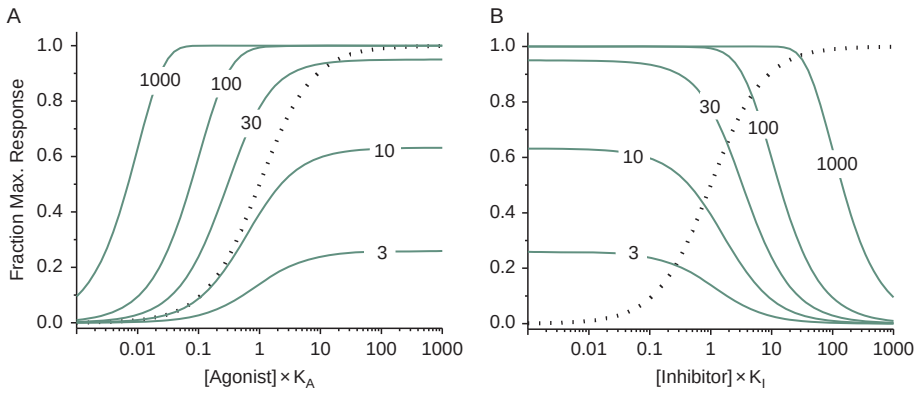

Figure 1.9. Spare receptors. (A) Spare receptors cause an apparent increase in potency and efficacy of agonists. In this model, approximately 35 receptors must be activated for a maximal cellular response and the number of receptors per cell is labeled on each curve. If there are less than 35 receptors in the cell, a sub-maximal response is observed and apparent K_A (EC_{50}) is fairly constant. When there are spare receptors (i.e., more than 35 receptors per cell), low agonist concentrations are needed to achieve the maximum response (whatever concentration activates 35 receptors). EC_{50} is therefore significantly reduced. The dotted line represents fractional agonist occupation (binding) of receptors. (B) Spare receptors cause an apparent decrease in the potency of antagonists. In this case, no inhibition occurs until noncompetitive inhibitor binding has reduced the number of active receptors to less than 35. Thus, higher fractional antagonist occupancy is required as the number of receptors per cell increases. The dotted line represents fractional antagonist occupation (binding).

Thus, GPCR activation is amplified in both space and time and occupation of a small fraction of receptors can result in a maximal cellular or tissue response that outlasts drug binding to the receptor. Similarly, the enzyme-linked surface receptors initiate cascades of phosphorylation or dephosphorylation which amplify the initiating receptor activation.

Signal damping

Signal damping or negative feedback is often present to limit physiological drug responses. This is usually observed as a diminishing response to equal drug doses over time. The term for rapidly (hours) diminishing responses to repeated drug administration is **tachyphylaxis**. Resistance to drug effects that develops over longer periods (days to months) is termed **tolerance**. Tachyphylaxis may be linked to receptor desensitization in some cases, such as the phase II neuromuscular block associated with prolonged succinylcholine administration. Ligand-gated ion channels, when persistently exposed to high agonist concentrations, go through a monomolecular conformational change that reduces channel opening even while agonist is bound. Many voltage-gated ion channels go through a similar process (inactivation). Mechanisms that involve other molecules can also damp responses. Synapses such as the neuromuscular junction show altered structure and activity within hours after physiological changes such as reduced presynaptic motor neuron activity or profound blockade of postsynaptic activity [38]. Some downstream proteins activated following GPCR agonism (receptor kinases) are feedback inhibitors that phosphorylate GPCRs and reduce their activity. Similarly, protein phosphatases (both surface receptor-linked and cytoplasmic) can be mechanisms that oppose various protein kinase enzymes. Other, slower negative feedback mechanisms include depletion of neurotransmitters or metabolites, expression of regulatory factors, receptor downregulation, and transcriptional changes.

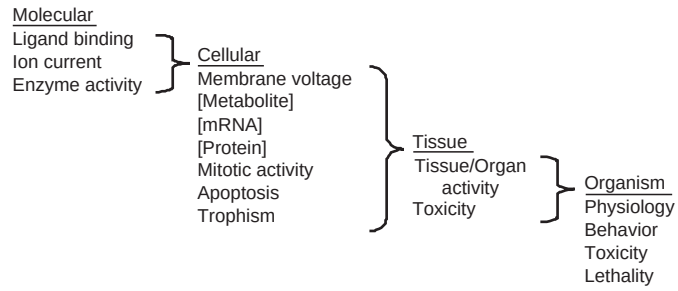

Figure 1.10. Integration of drug responses from molecules to organism.

Drug effects on organisms

Integration of drug effects – Drug responses show different concentration-dependent patterns in molecules vs. cells vs. tissues vs. animals, because the spatial and temporal integration of effects is altered at different system levels (Fig. 1.10). As a result, small changes in occupancy or efficacy at one step in a signal transduction cascade may have large effects on the overall system. Moreover, variability in individual responses at different system levels, due to genetics, environment, drug interactions, and other factors, can lead to significant interindividual differences in response to drugs. Therefore, assessing dose–response relationships in individuals provides different information than studies in populations.

Drug–response analysis in individual organisms is analogous to that in molecular pharmacology, because organism responses are the integration of multiple molecular events, governed by similar underlying relationships, and thus molecular and organism concentration–response curves have similar sigmoidal shapes. However, analysis of drug effects in organisms is usually **parametric** (descriptive), and difficult to relate quantitatively to underlying mechanisms [27]. Clinical concentration–response determinations most commonly

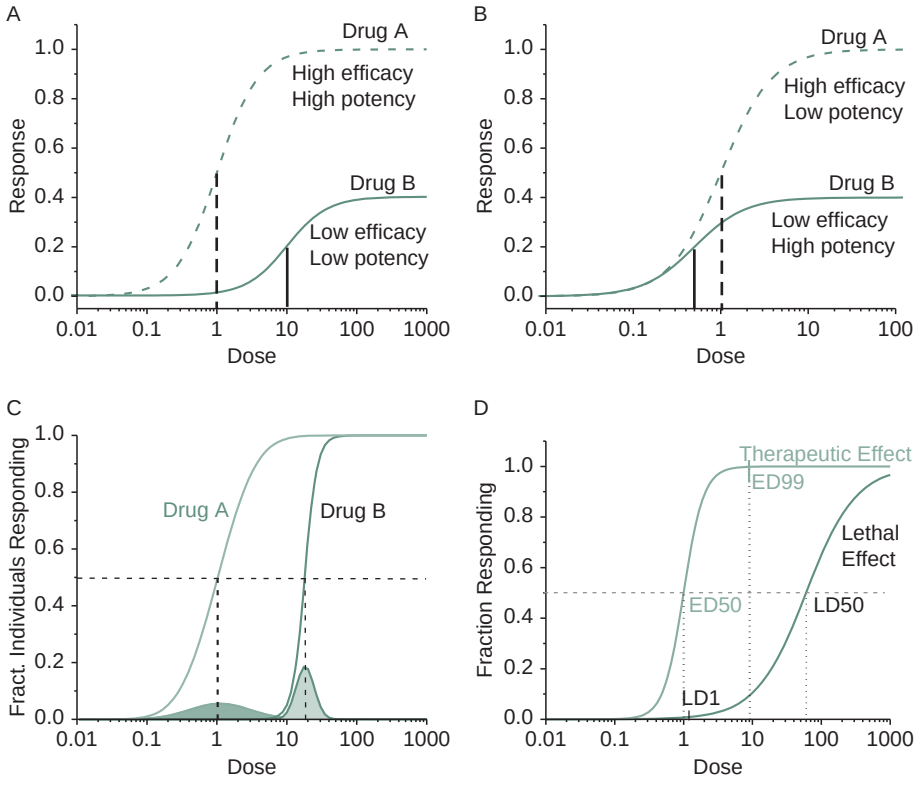

Figure 1.11. Clinical dose–response concepts. (A, B) These panels illustrate the concepts of efficacy and absolute potency in comparing drugs with similar effects. Efficacy is the maximum response. Absolute potency is the inverse of ED_{50}. A drug with low absolute potency but high efficacy may be more effective than an equal dose of a drug with higher absolute potency but lower efficacy. (C) Population dose–response studies are based on quantal (yes/no) outcomes at different drug doses. Quantal responses are often distributed in a classical bell-shaped probability curve on semilogarithmic axes (filled curves). Cumulative response curves (lines) generated from the quantal response data are sigmoidal and can be described using the Hill equation. Note that the slopes of the cumulative curves are determined by the variability (width) of the population response distributions. (D) Toxicity ratios are usually reported as the ratio of the mid-points of toxicity vs. therapeutic dose–responses. Here, the 50% lethal dose in a group of animals (LD50) is 60 times higher than the half-effect dose (ED50). However, the dose that is effective for 99% (ED99) is also lethal for about 10%. The certain safety factor (ED99/LD1) for this drug is low, about 0.1.

measure drug concentrations in blood (plasma or serum) because of its accessibility, although certain exceptions are well-known (e.g., end-tidal volatile anesthetic concentrations). Recognizing that blood is not the real site of drug action, the concept of **effect-site concentration** has been applied in pharmacokinetic/pharmacodynamic (PK/PD) models (see Chapter 5). Frequently, **dosage** rather than concentration is the independent variable used for pharmacological studies in organisms when biophase drug concentrations are not measured.

Pharmacodynamic responses in animals can be therapeutic, toxic, or lethal. **Graded** log drug concentration–response or dose–response curves for an individual subject measure responses on a **continuous scale** from minimum to maximum. Examples of graded responses include pupil size, blood pressure, heart rate, temperature, intracranial pressure, or pain relief. Pharmacodynamic analysis of graded concentration–response curves in individual subjects therefore parallels that in molecular, cellular, and tissue studies. The independent concepts of drug potency and efficacy are applied similarly (Fig. 1.11A–B). **Efficacy** is the maximal response in an individual achievable at the highest drug dose and **absolute drug potency** for graded responses is defined as the reciprocal of the $\mathbf{ED_{50}}$ (the dose producing half-maximal response). Figure 1.11A and B illustrate the comparison of drug pairs with respect to their potency and efficacy for the same effect.

Drug–response analysis in populations – In anesthesiology, it is easy to observe that responses to the same drug dose vary widely among patients. Part of the art of delivering anesthesia is titrating drug doses to provide optimal therapy for a specific patient, particularly when the drug has significant toxicities. At the same time, anesthesiologists must know dosing ranges that are appropriate for broad populations of patients, to provide dosing guidelines. For populations of patients, what matters is determining drug doses that result in important clinical (therapeutic or toxic) endpoints. As a result, characterizing dose–response relationships in populations is based on **quantal responses** and **quantal dose–response curves**. Quantal responses are either/or outcomes in individuals, such as awake/asleep, stroke/no stroke, alive after five years, etc. Graded responses may also be quantized. For example, a 20 mmHg decrease in blood pressure (yes/no), or a 50% decrease in pain (yes/no). Thus the *y*-axis on a quantal dose–response curve is the fraction (or percentage) of the population that exhibits the defined response (Fig. 1.11C–D). The minimum dose required to achieve the specified quantal response in a population of study subjects is usually distributed in a bell-shaped probability curve (Fig. 1.11C). The cumulative fraction of subjects that respond at a given dose (i.e., responding at that dose or lower) appears as a sigmoid curve on semilogarithmic axes. It is important to note that the shape, particularly the slope, of cumulative dose–response relationships derived from quantal data reflects the heterogeneity of the population studied rather than the underlying physiology of drug action (Fig. 1.11C).

Parameters used to describe graded dose–response curves, such as potency and efficacy, have analogs for quantal

dose–response curves. Thus the dose producing a therapeutic effect in 50% of the population studied is the **ED50**, and the maximum fraction of the population displaying the specified response at high drug doses represents **population efficacy**. Note that the nomenclature for quantal (ED50) and graded (ED_{50}) dose–response curves differs, with the subscript numeral used only in the latter case.

In an organism or patient, a single drug may have (in fact usually has) multiple effects, and each effect may have a different dose–response relationship, depending on the mechanisms underlying each effect. Quantal dose–response curves can be used to describe multiple drug effects, such as therapeutic response, drug toxicity, or even drug lethality (more commonly in animals, for toxicity and safety testing). For example, the dose producing a particular toxicity in half the population is the **TD50**, and that causing lethality in half the population is the **LD50**. A more useful measure of drug safety than just the LD50 or TD50 is the distance between the concentration–response curves (Fig. 1.11D). The **therapeutic index** is defined as the LD50/ED50 (or sometimes TD50/ED50). Another, more stringent measure of drug safety is the **certain safety factor**, defined as the LD1/ED99 (or TD1/LD99). Compared with the therapeutic index, certain safety factor is less dependent on assuming similar slopes of the therapeutic and toxic effect curves.

Summary

Pharmacodynamics is the study of how drugs alter physiological functions, which is initiated by drug interactions with molecular targets. Drugs used in anesthesiology interact with a variety of receptors, which are proteins within or on the surface of cells that are activated by endogenous drugs, resulting in altered cell function. Receptors are categorized both by the drugs that activate them and by their structure and function. Families and superfamilies of receptors (e.g., G-protein-coupled receptors and ligand-gated ion channels) are defined by the degree of structural and functional similarity among groups of receptors. Receptors display varying degrees of selectivity for drugs with similar chemical structures. Conversely, drugs show varying degrees of specificity for the plethora of receptors in organisms. Drug–receptor binding can be understood as a simple bimolecular interaction process, which is characterized by an equilibrium dissociation constant, K_D. Saturable drug binding to receptors with one site appears graphically as a hyperbolic Langmuir isotherm. Graphs of bound drug against log[free drug] appears as a sigmoidal (s-shaped) curve. Drug effects are classified as agonism (activation) or antagonism (inhibition of activity). Drug effects in molecules, cells, and tissues are displayed using concentration–response curves, which are characterized by a maximal effect (efficacy), and a half-maximal effect concentration (EC_{50}).

Receptor responses to drugs at the molecular level are not necessarily proportional to drug binding. Intrinsic efficacy describes the fraction of receptors activated when fully bound by an agonist, which is modeled as a second equilibrium between inactive and active agonist-bound receptors. Full agonists and partial agonists activate, respectively, a high fraction or a low fraction of bound receptors, and the EC_{50} of a full agonist will be lower than that of a partial agonist with the same binding affinity for inactive receptors. Cells and tissues may possess spare receptors, implying that activation of a fraction of receptors produces a maximal response, resulting in increased apparent sensitivity to agonists and decreased apparent sensitivity to antagonists. Antagonism may be due to competitive binding at the agonist (orthosteric) site or noncompetitive binding at other (allosteric) sites. The effect of reversible competitive antagonism is surmountable with increasing agonist concentrations, while noncompetitive antagonism and irreversible antagonism are insurmountable. Many receptors display partial activity in the absence of agonists, and these may be described using allosteric activation models that incorporate an undrugged active receptor state. In these models, agonists shift the inactive ↔ active equilibrium toward active states. Inverse agonists are defined in the context of allosteric models as drugs that stabilize inactive more than active receptors, reducing spontaneous activity.

Drug responses in animals and humans are the result of integrated effects, including signal amplification and dampening (negative feedback) mechanisms at cellular, tissue, and physiological systems levels. Tachyphylaxis and tolerance are terms for declining responses to repeated drug dosing over short (hours) or long (days to months) time periods. When drug concentration is not defined, graded dose–response studies in individual animals often display the familiar sigmoid shape and are described by efficacy (maximal effect at high doses) and potency, defined as the inverse of the half-maximal effect dose (ED_{50}). Population studies generally specify quantal (yes/no) drug responses such as a therapeutic endpoint and toxic side effects, including lethality, displaying the fraction of individuals reaching these endpoints at each dose. A single drug may be characterized by different potencies for multiple actions, including ED50 for therapeutic action, TD50 for toxicity, and LD50 for lethality.

References

1. Buxton I. Pharmacokinetics and pharmacodynamics. In: Brunon L, Lazo J, Parker K, eds., *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 11th edn. New York, NY: McGraw Hill, 2006: 1–40.
2. Bourne H, von Zastrow M. Drug receptors and pharmacodynamics. In: Katzung B, ed., *Basic and Clinical Pharmacology*, 10th edn. New York, NY: McGraw Hill, 2007: 11–33.
3. Nyholm TK, Ozdirekcan S, Killian JA. How protein transmembrane segments

sense the lipid environment. *Biochemistry* 2007; **46**: 1457–65.

4. Alexander SPH, Mathie A, Peters JA. Guide to receptors and channels (GRAC), 4th edn. *Br J Pharmacol* 2009; **158** (Suppl. 1): S1–S254.
5. Noda M, Takahashi H, Tanabe T, *et al.* Structural homology of *Torpedo californica* acetylcholine receptor subunits. *Nature* 1983; **302**: 528–32.
6. Arneric SP, Holladay M, Williams M. Neuronal nicotinic receptors: a perspective on two decades of drug discovery research. *Biochem Pharmacol* 2007; **74**: 1092–101.
7. Wess J, Eglen RM, Gautam D. Muscarinic acetylcholine receptors: mutant mice provide new insights for drug development. *Nat Rev Drug Discov* 2007; **6**: 721–33.
8. Pierce KL, Premont RT, Lefkowitz RJ. Seven-transmembrane receptors. *Nat Rev Mol Cell Biol* 2002; **3**: 639–50.
9. Kristiansen K. Molecular mechanisms of drug binding, signaling, and regulation within the superfamily of G-protein-coupled receptors: molecular modeling and mutagenesis approaches to receptor structure and function. *Pharmacol Ther* 2004; **103**: 21–80.
10. Christopoulos A, Kenakin T. G protein-coupled receptor allosterism and complexing. *Pharmacol Rev* 2002; **54**: 323–74.
11. Luttrell LM. Reviews in molecular biology and biotechnology: transmembrane signaling by G protein-coupled receptors. *Mol Biotechnol* 2008; **39**: 239–64.
12. Ortells MO, Lunt GG. Evolutionary history of the drug-gated ion-channel superfamily of receptors. *Trends Neurosci* 1995; **18**: 121–7.
13. Connolly CN, Wafford KA. The Cys-loop superfamily of drug-gated ion channels: the impact of receptor structure on function. *Biochem Soc Trans* 2004; **32**: 529–34.
14. Unwin N. Refined structure of the nicotinic acetylcholine receptor at 4A resolution. *J Mol Biol* 2005; **346**: 967–89.
15. Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell* 2000; **103**: 211–25.
16. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 2001; **414**: 799–806.
17. Locksley RM, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* 2001; **104**: 487–501.
18. Hehlgans T, Pfeffer K. The intriguing biology of the tumour necrosis factor/tumour necrosis factor receptor superfamily: players, rules and the games. *Immunology* 2005; **115**: 1–20.
19. Gronemeyer H, Gustafsson JA, Laudet V. Principles for modulation of the nuclear receptor superfamily. *Nat Rev Drug Discov* 2004; **3**: 950–64.
20. Taylor P, Talley TT, Radic Z, *et al.* Structure-guided drug design: conferring selectivity among neuronal nicotinic receptor and acetylcholine-binding protein subtypes. *Biochem Pharmacol* 2007; **74**: 1164–71.
21. Brancaccio D, Bommer J, Coyne D. Vitamin D receptor activator selectivity in the treatment of secondary hyperparathyroidism: understanding the differences among therapies. *Drugs* 2007; **67**: 1981–98.
22. Boeckler F, Gmeiner P. Dopamine D3 receptor drugs: recent advances in the control of subtype selectivity and intrinsic activity. *Biochim Biophys Acta* 2007; **1768**: 871–87.
23. Muntz KH, Zhao M, Miller JC. Downregulation of myocardial beta-adrenergic receptors. Receptor subtype selectivity. *Circ Res* 1994; **74**: 369–75.
24. Belelli D, Muntoni AL, Merrywest SD, *et al.* The in vitro and in vivo enantioselectivity of etomidate implicates the GABAA receptor in general anaesthesia. *Neuropharmacology* 2003; **45**: 57–71.
25. Foster RH, Markham A. Levobupivacaine: a review of its pharmacology and use as a local anaesthetic. *Drugs* 2000; **59**: 551–79.
26. Colquhoun D. The quantitative analysis of drug-receptor interactions: a short history. *Trends Pharmacol Sci* 2006; **27**: 149–57.
27. Neubig RR, Spedding M, Kenakin T, Christopoulos A. International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. XXXVIII. Update on terms and symbols in quantitative pharmacology. *Pharmacol Rev* 2003; **55**: 597–606.
28. Del Castillo J, Katz B. Interaction at end-plate receptors between different choline derivatives. *Proc R Soc Lond B Biol Sci* 1957; **146**: 369–81.
29. Colquhoun D. Binding, gating, affinity and efficacy: the interpretation of structure–activity relationships for agonists and of the effects of mutating receptors. *Br J Pharmacol* 1998; **125**: 924–47.
30. Hill A. The possible effects of the aggregation of the molecules of hemoglobin on its dissociation curves. *J Physiol* 1910; **40**: iv–vii.
31. Gaddum JH. Theories of drug antagonism. *Pharmacol Rev* 1957; **9**: 211–18.
32. Schild HO. Drug antagonism and pAx. *Pharmacol Rev* 1957; **9**: 242–6.
33. Monod J, Wyman J, Changeux J. On the nature of allosteric transitions: a plausible model. *J Mol Biol* 1965; **12**: 88–118.
34. Milligan G. Constitutive activity and inverse agonists of G protein-coupled receptors: a current perspective. *Mol Pharmacol* 2003; **64**: 1271–6.
35. Ehlert FJ. Analysis of allosterism in functional assays. *J Pharmacol Exp Ther* 2005; **315**: 740–54.
36. Kenakin T. Efficacy as a vector: the relative prevalence and paucity of inverse agonism. *Mol Pharmacol* 2004; **65**: 2–11.
37. Rusch D, Forman SA. Classic benzodiazepines modulate the open-close equilibrium in $\alpha1\beta2\gamma2L$ γ-aminobutyric acid type A receptors. *Anesthesiology* 2005; **102**: 783–92.
38. Akaaboune M, Culican SM, Turney SG, Lichtman JW. Rapid and reversible effects of activity on acetylcholine receptor density at the neuromuscular junction in vivo. *Science* 1999; **286**: 503–7.

Section 1
Chapter
2

Principles of drug action

G-protein-coupled receptors

Marcel E. Durieux

Introduction

G-protein-coupled receptors (GPCRs) are involved in the transduction of signals from a variety of extracellular signaling molecules, including hormones, neurotransmitters, and cytokines. The diversity of the effector pathways which may couple to GPCRs gives rise to considerable signaling flexibility. Furthermore, tissue-specific expression of various downstream targets allows for specificity, and signaling regulation may occur at multiple levels. Consequently, there is a range of potential opportunities for pharmacologic intervention, and GPCRs are extremely important in anesthesiology; for example, α- and β-adrenergic agonists and opiates all act on GPCRs. Tolerance to drugs like opiates poses a significant clinical challenge, and an understanding of the mechanisms underlying desensitization may help identify future targets for intervention. This chapter deals with the general principles of signal transduction and the specifics of GPCR signaling pathways and common second-messenger systems, as well as exploring the mechanisms for receptor desensitization.

General principles

Signal transduction

The terms **signal transduction** and **cell signaling** refer to the mechanisms by which biologic information is transferred between cells. Intercellular and intracellular signaling pathways are essential to the growth, development, metabolism, and behavior of the organism [1,2], which helps explain why the human genome includes at least 3775 genes (or 14.3% of genes) involved in signal transduction [3]. More than 2% of genes encode GPCRs.

The cellular response to an extracellular signaling molecule requires its binding to a specific receptor (Table 2.1), which then transduces this information to changes in the functional properties of the target cell. The particular receptors expressed by the target cell determine its sensitivity to various signaling molecules and determine the specificity involved in cellular responses to various signals. Receptors can be classified by their cellular localization (Fig. 2.1). The majority of hormones and neurotransmitters, including peptides, catecholamines, amino acids, and their derivatives, are water-soluble (hydrophilic) signaling molecules that interact with cell-surface receptors. Prostaglandins are an exception, in that they are lipid-soluble (hydrophobic) signaling molecules that interact with cell-surface receptors. Most hydrophobic signaling molecules diffuse across the plasma membrane and interact with intracellular receptors. Steroid hormones, retinoids, vitamin D, and thyroxine are examples. These molecules are transported in the blood bound to specific transporter proteins, from which they dissociate in order to diffuse across cell membranes to bind to specific receptors in the nucleus or cytosol. The hormone–receptor complex then acts as a transcription factor to modulate gene expression. However, recent evidence suggests that receptors for the steroid estrogen also act at the plasma membrane, modulating intracellular Ca^{2+} and cyclic adenosine 3′-5′-monophosphate (cAMP) levels through G-protein interactions. Nitric oxide (NO), and possibly carbon monoxide (CO), are members of a class of gaseous signaling molecules that readily diffuse across cell membranes to affect neighboring cells. NO, which is unstable and has a short half-life (5–10 seconds), is able to diffuse only a short distance before breaking down, and therefore acts as a paracrine signal only. Cell-surface receptors can also bind to insoluble ligands, such as the extracellular matrix of cell adhesion molecules, interactions which are crucial to cell development and migration.

Properties of signal transduction pathways

Signal transduction pathways have a number of common properties with important functional implications [4]. **Signal amplification** occurs as a result of sequential activation of catalytic signaling molecules. This enables sensitive physiologic responses to small physical (several photons) or chemical (a few molecules of an odorant) stimuli, as well as graded responses to increasingly larger stimuli. **Specificity** is imparted by specific receptor proteins and their association with cell-type-specific signaling pathways and effector mechanisms. Additional specificity is imparted by the existence of distinct

Anesthetic Pharmacology, 2nd edition, ed. Alex S. Evers, Mervyn Maze, Evan D. Kharasch. Published by Cambridge University Press.

Table 2.1. Receptor classification

Cell-surface receptors	
G-protein-coupled	Receptors for hormones, neurotransmitters (biogenic amines, amino acids), and neuropeptides Activate/inhibit adenylate cyclase Activate phospholipase C Modulate ion channels
Ligand-gated ion channels	Receptors for neurotransmitters (biogenic amines, amino acids, peptides) Mediate fast synaptic transmission
Enzyme-linked cell-surface receptors	
Receptor guanylate cyclases	Receptors for atrial natriuretic peptide, Escherichia coli heat-stable enterotoxin
Receptor serine/threonine kinases	Receptors for activin, inhibin, transforming growth factor β (TGFβ)
Receptor tyrosine kinases	Receptors for peptide growth factors
Tyrosine kinase-associated	Receptors for cytokines, growth hormone, prolactin
Receptor tyrosine phosphatases	Ligands unknown in most cases
Intracellular receptors	
Steroid receptor superfamily	Receptors for steroids, sterols, thyroxine (T_3), retinoic acid, and vitamin D

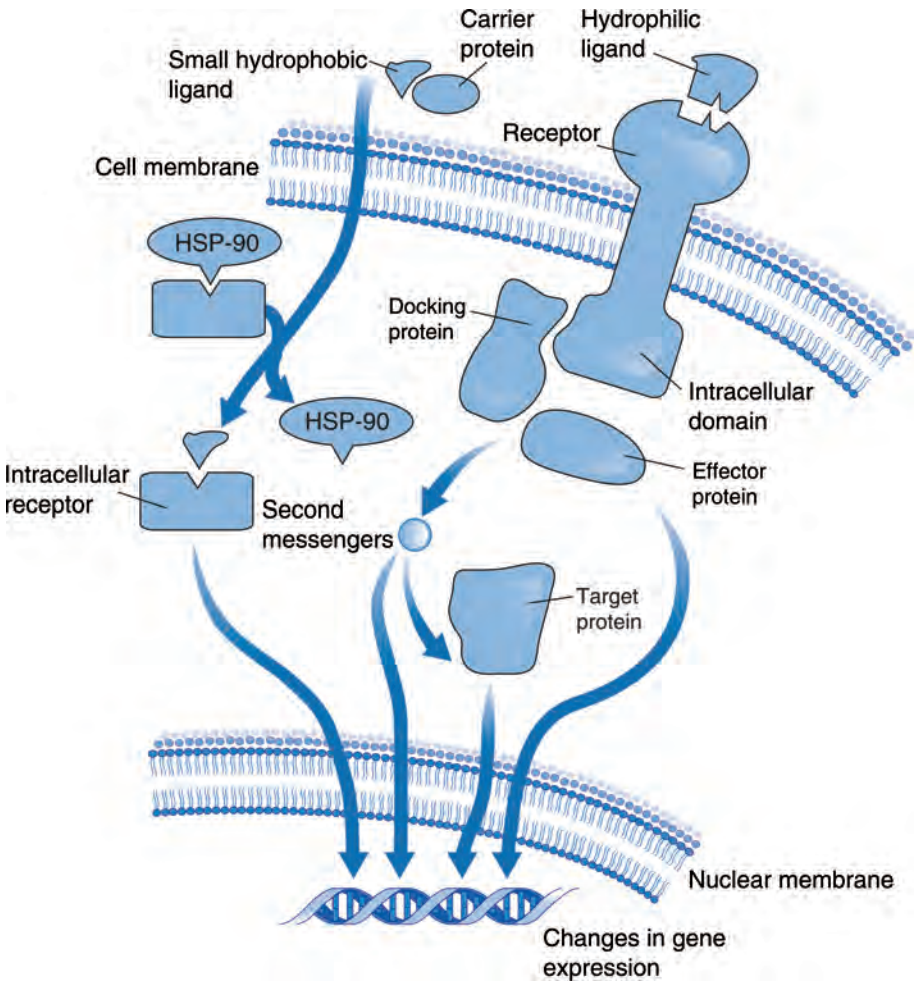

Figure 2.1. Extracellular signaling. Ligands bind to either cell-surface receptors or intracellular receptors. Most signaling molecules are hydrophilic and therefore unable to cross the plasma membrane. They bind to cell-surface receptors, which in turn generate one or more intracellular signals (second messengers) inside the target cell or change the activity of effector proteins (e.g., G proteins, protein kinases, ion channels) through their intracellular effector domains. Receptor activation can result in direct changes in the intrinsic enzymatic activities of the receptor intracellular domain, or it can work indirectly through association of the receptor with intracellular mediators, which in turn regulate the activity of effector proteins. Some effectors translocate to the nucleus to control gene expression (e.g., transcription factors) or to other subcellular compartments. Some small signaling molecules, by contrast, diffuse across the plasma membrane and bind to receptors inside the target cell, either in the cytosol (as shown) or in the nucleus. Many of these small signaling molecules are hydrophobic and nearly insoluble in aqueous solutions; therefore, they are transported in the bloodstream and other extracellular fluids bound to carrier proteins, from which they dissociate before entering the target cell. HSP-90, heat shock protein-90.

receptors coupled to different intracellular signaling pathways that respond to the same extracellular signal. Thus a single extracellular signal can elicit different effects on different target cells depending on the receptor subtype and the signaling mechanisms present. A good example is the neurotransmitter acetylcholine, which stimulates contraction of skeletal muscle, but relaxation of smooth muscle. Differences in the intracellular signaling mechanisms also allow the same receptor to produce different responses in different target cells. **Pleiotropy** results from the ability of a single extracellular signal to generate multiple responses in a target cell: for example, the opening of some ion channels, the closing of others, activation or inhibition of many enzymes, modification of the cytoskeleton, or changes in gene expression.

G-protein-coupled receptors

A variety of signals (hormones, neurotransmitters, cytokines, pheromones, odorants, photons) produce their intracellular actions by a pathway that involves interaction with receptors that activate G proteins [5,6]. G proteins act as molecular switches to relay information from activated receptors to the appropriate effectors [7,8]. An agonist-stimulated receptor can activate several hundred G proteins, which in turn activate a variety of downstream effectors [9]. GPCRs have a particularly important role in pharmacology – more than two-thirds of all nonantibiotic drugs target GPCRs – and are thus critical to anesthesiology [10]. Genetical disruption in their function is involved in a number of disease states [11].

The GPCR signaling pathway

G-protein-coupled signal transduction begins with receptor proteins in the plasma membrane, which sense changes in the extracellular environment. As a result of the interactions between these receptors and their ligands, signals are transduced across the plasma membrane (Fig. 2.1). Ligand binding to a GPCR causes a change in the shape (conformation) of the receptor, which is transmitted to the cell interior. This results in a change in the activity of a coupled intracellular guanine nucleotide (GTP)-binding protein (**G protein**), which subsequently activates or inhibits intracellular enzymes or ion channels. Through this mechanism, the activation of many GPCRs leads to changes in the concentration of intracellular signaling molecules, termed **second messengers**. These changes are usually transient, a result of the tight regulation of the synthesis and degradation (or release and reuptake) of these intracellular signals. Important second messengers include cAMP, cyclic guanosine 3′-5′-monophosphate (cGMP), 1,2-diacylglycerol, inositol 1,4,5-trisphosphate (IP_3), and Ca^{2+}. Changes in the concentrations of these second messengers following receptor activation modulate activities of important regulatory enzymes and effector proteins. The most important second-messenger-regulated enzymes are protein kinases and phosphatases, which catalyze the phosphorylation and dephosphorylation, respectively, of key enzymes and proteins in target cells. Reversible phosphorylation alters the function or localization of specific proteins. It is the predominant effector mechanism involved in mediating cellular responses to almost all extracellular signals.

GPCR structure and function

GPCRs form a large and functionally diverse receptor superfamily; more than 500 (more than 2% of total genes) members have been identified, and a large number of **orphan receptors** (receptors identified as GPCRs by amino acid structure, for which the ligand is not known) brings the total of GPCRs over a thousand.

The G proteins, coupled to by the receptors, are heterotrimeric structures, that is, they consist of three distinct protein subunits: a large α subunit and a smaller βγ subunit dimer. The βγ complex is so tightly bound that it is usually considered a single unit. The binding of extracellular signals to their specific receptors on the cell surface initiates a cycle of reactions to promote guanine nucleotide exchange on the G-protein α subunit. This involves three major steps: (1) the signal (ligand) activates the receptor and induces a conformational change in the receptor; (2) the activated receptor "turns on" a heterotrimeric G protein in the cell membrane by forming a high-affinity ligand–receptor–G-protein complex, which promotes exchange of guanosine triphosphate (GTP) for guanosine diphosphate (GDP) on the α subunit of the G protein, followed by dissociation of the α subunit and the βγ subunit dimer from the receptor and each other; and (3) the appropriate effector protein(s) is then regulated by the dissociated G-protein α or βγ (or both) subunits, which thereby transduces the signal. The dissociation of the G protein from the receptor reduces the affinity of the receptor for the agonist. The system returns to its basal state as the GTP bound to the α subunit is hydrolyzed to GDP by a catalytic activity (or GTPase) inherent in the α subunit, and the trimeric G-protein complex reassociates and turns off the signal.

A number of different isoforms of G-protein α, β, and γ subunits have been identified that mediate the stimulation or inhibition of functionally diverse effector enzymes and ion channels (Table 2.2). Among the effector molecules regulated by G proteins are adenylate cyclase, phospholipase C, phospholipase A_2, cGMP phosphodiesterase, and Ca^{2+} and K^+ channels. These effectors then produce changes in the concentrations of a variety of second-messenger molecules or in the membrane potential of the target cell.

Despite the diversity in the extracellular signals that stimulate the various effector pathways activated by G-protein-coupled receptors, these receptors are structurally homologous, which is consistent with their common mechanism of action. Molecular cloning and sequencing have shown that these receptors are characterized by seven hydrophobic transmembrane α helical segments of 20–25 amino acids connected by alternating intracellular and extracellular loops. Therefore, GPCRs cross the membrane seven times (hence the alternative terms

Table 2.2. Diversity of G-protein-coupled receptor signal transduction pathways: G proteins and their associated receptors and effectors

G protein	Representative receptors	Effectors	Effect
G_s	β_1, β_2, β_3 adrenergic; D_1, D_5 dopamine	Adenylate cyclase Ca^{2+} channels	Increased cAMP; increased Ca^{2+} influx
G_i	α_2 adrenergic; D_2; M_2, M_4 muscarinic; μ, δ, κ opioid	Adenylate cyclase Phospholipase A_2 K^+ channels	Decreased cAMP; eicosanoid release; hyperpolarization
G_k	Atrial muscarinic	K^+ channel	Hyperpolarization
G_q	M_1, M_3 muscarinic; α_1 adrenergic	Phospholipase C β	Increased IP_3, DG, Ca^{2+}
G_{olf}	Odorants	Adenylate cyclase	Increased cAMP (olfactory)
G_t	Photons	cGMP phosphodiesterase	Decreased cGMP (vision)
G_o	?	Phospholipase C Ca^{2+} channels	Increased IP_3, DG, Ca^{2+}; decreased Ca^{2+} influx

cAMP, adenosine 3′-5′-monophosphate; cGMP, guanosine 3′-5′-monophosphate; DG, 1,2,-diacylglycerol; G_s, stimulation; G_i, inhibition; G_k, potassium regulation; G_q, phospholipase C regulation; G_{olf}, olfactory; G_t, transducin; G_o, other; IP_3, inositol trisphosphate.

seven-transmembrane domain, heptahelical, or serpentine receptors; Fig. 2.2). The structural domains of G-protein-coupled receptors involved in ligand binding and in interactions with G proteins have been analyzed by deletion analysis (in which segments of the receptor are sequentially deleted), by site-directed mutagenesis (in which specific single amino acid residues are deleted or mutated), and by constructing chimeric receptor molecules (in which recombinant chimeras are formed by splicing together complementary segments of two related receptors). For example, the agonist isoproterenol binds among the seven transmembrane α helices of the β_2-adrenoceptor near the extracellular surface of the membrane. The intracellular loop between α helices 5 and 6 and the C-terminal segments is important for specific G-protein interactions.

Heterogeneity within the GPCR signaling pathway exists both at the level of the receptors and at the level of the G proteins. A single extracellular signal may activate several closely related receptor subtypes. For example, six genes for α-adrenoceptors and three genes for β-adrenoceptors have been identified, all of which can be activated by the ligand norepinephrine. Likewise, G proteins consist of multiple subtypes. Sixteen homologous α-subunit genes are classified as subtypes (G_s, G_i, G_k, G_q, and so on) based on structural similarities. The different α subunits have distinct functions, coupling with different effector pathways. The different β- and γ-subunit isoforms may also couple with distinct signaling pathways. Heterogeneity in effector pathways makes divergence possible within GPCR-activated pathways. This effector pleiotropy can arise from two distinct mechanisms: (1) a single receptor can activate multiple G-protein types, and/or (2) a single G-protein type can activate more than one second-messenger pathway. Thus a single type of GPCR can activate several different effector pathways within a given cell, whereas the predominant pathway may vary between cell types. All together, this ability of a single agonist to activate multiple receptor subtypes, which in turn can interact with multiple G-protein subtypes and thereby activate various effectors, allows a tremendous amount of flexibility in signaling, as well as many opportunities for regulation.

The structure and function of the α- and β-adrenoceptors for epinephrine and norepinephrine and their associated G proteins exemplify some of these principles (Fig. 2.2). β-adrenoceptors are coupled to the activation of adenylate cyclase, a plasma-membrane-associated enzyme that catalyzes the synthesis of cAMP. cAMP was the first second messenger identified and has been found to exist in all prokaryotes and animals. The G protein that couples β-adrenoceptor stimulation to adenylate cyclase activation is known as G_s, for stimulatory G protein. Epinephrine-stimulated cAMP synthesis can be reconstituted in phospholipid vesicles using purified β-adrenoceptors, G_s, and adenylate cyclase, which demonstrates that no other molecules are required for the initial steps of this signal transduction mechanism. In the resting state, G_s exists as a heterotrimer consisting of α_s and βγ subunits, with GDP bound to α_s. Agonist binding to the β-adrenoceptor alters the conformation of the receptor and exposes a binding site for G_s. The GDP-G_s complex binds to the agonist-activated receptor, thereby reducing the affinity of α_s for GDP, which dissociates, allowing GTP to bind. The α_s subunit bound to GTP then dissociates from the G-protein complex, and binds to and activates adenylate cyclase. The affinity of the receptor for agonist is reduced following dissociation of the G-protein complex, leading to agonist dissociation and a return of the receptor to its inactive state. Activation of adenylate cyclase is rapidly reversed following agonist dissociation from the receptor because the lifetime of active α_s is limited by its intrinsic GTPase activity. The bound GTP is thereby hydrolyzed to GDP, which returns the α subunit to its inactive conformation. The α_s subunit then dissociates from adenylate cyclase, rendering it inactive, and reassociates with βγ to reform G_s.

Nonhydrolyzable analogs of GTP, such as GTPγS or GMPPNP, prolong agonist-induced adenylate cyclase activation

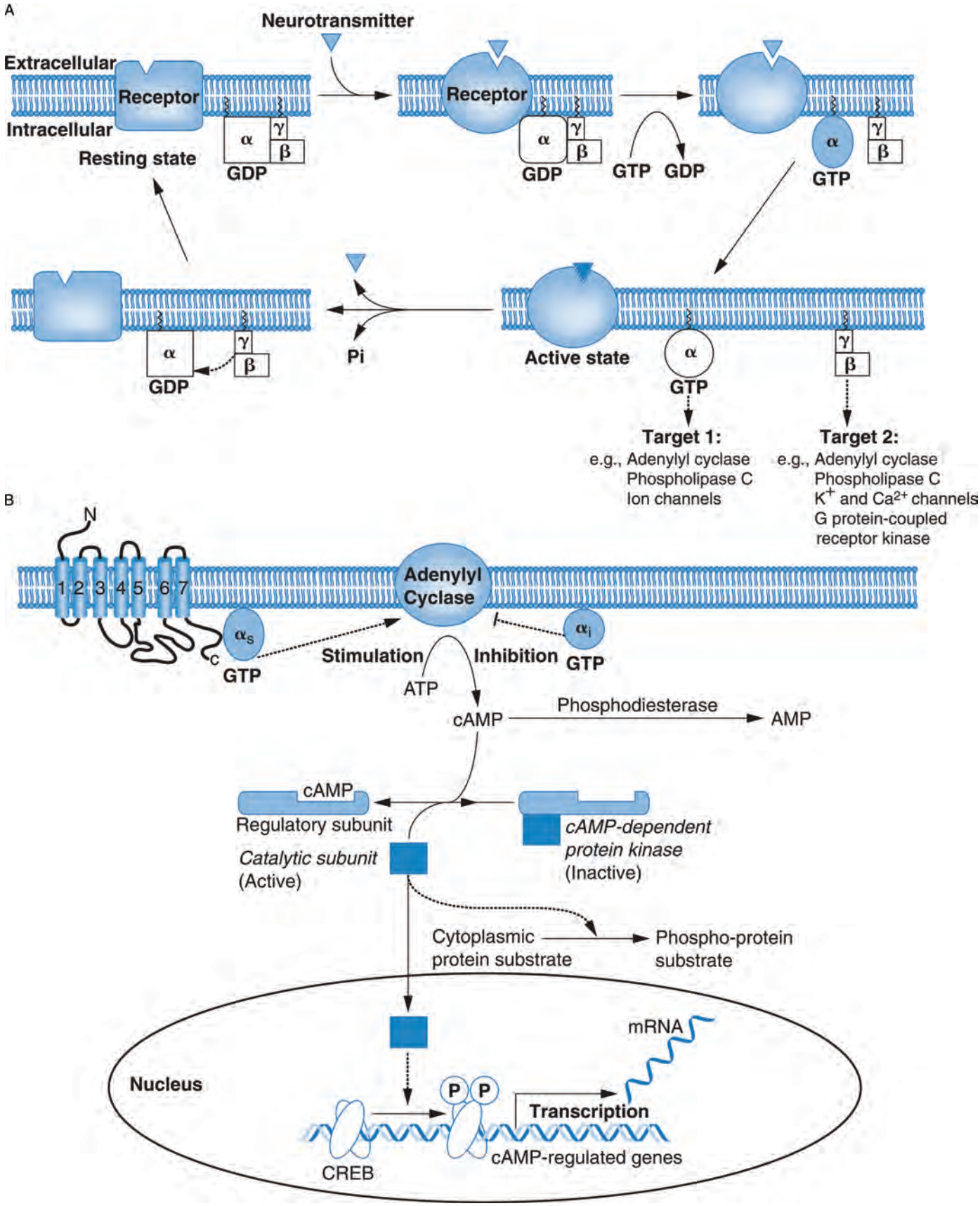

Figure 2.2. G-protein-coupled receptors. (A) General features. Many receptors belong to this class, including those for neurotransmitters, hormones, odorants, light, and Ca^{2+}. These receptors associate with heterotrimeric G proteins composed of three subunits: α, β, and γ. They are not transmembrane proteins but are

by preventing inactivation of active α_s. Such compounds are important research tools, but the mechanism also has clinical implications. Cholera toxin and pertussis toxin are adenosine diphosphate (ADP)-ribosyltransferases, and induce selective ADP ribosylation of α_s or α_i, respectively, which inhibits its GTPase activity and results in prolonged $G_s\alpha$ activation or $G_i\alpha$ inactivation.

The activity of adenylate cyclase can be negatively regulated by receptors coupled to the inhibitory G protein, G_i. An example is the α_2-adrenoceptor, which is coupled to inhibition of adenylate cyclase through G_i. Thus the same extracellular signal, epinephrine in this example, can either stimulate or inhibit the formation of the second messenger cAMP, depending on the particular G protein that couples the receptor to the cyclase. G_i, like G_s, is a heterotrimeric protein consisting of an α_i subunit and a $\beta\gamma$ subunit. Activated α_2 receptors bind to G_i and lead to GDP dissociation, GTP binding, and complex dissociation, as occurs with G_s. Both the released α_i and the $\beta\gamma$ complex are thought to contribute to adenylate cyclase inhibition, α_i by direct inhibition, and $\beta\gamma$ by direct inhibition and indirectly, by binding to and inactivating any free α_s subunits. Activated G_i can also open K^+ channels, an example of how a single G protein can regulate multiple effector molecules.

Receptor desensitization

The number and function of cell-surface receptors are subject to regulation by several mechanisms [9]. Many receptors undergo receptor desensitization in response to prolonged exposure to a high concentration of ligand, a process by which the number or function of receptors is reduced, so that the physiologic response to the ligand is attenuated (tachyphylaxis). This process is often responsible for decreased response to administered drugs (such as adrenergic agonists or opiates). At times, however, it can have beneficial consequences: for example, it appears that the analgesic action of cannabinoids may result in part from their ability to desensitize transient receptor potential vanilloid 1 (TRPV1) receptors [12], and the prolonged effect of the antiemetic palonosetron may be explained in part by its ability to desensitize 5-HT_3 receptors.

Receptor desensitization can occur by several mechanisms, including receptor internalization, downregulation, and modulation (Fig. 2.3). Receptor **internalization** by endocytosis is a common mechanism for desensitization of hormone receptors (e.g., insulin, glucagon, epidermal growth factor), and may be the manner in which palonosetron desensitizes 5-HT_3 receptors. The agonist–receptor complex is sequestered by receptor-mediated endocytosis, which results in translocation of the receptor to intracellular compartments (endosomes) that are inaccessible to ligand. This is a relatively slow process. Cessation of agonist stimulation allows the receptor to recycle to the cell surface by exocytosis. In other cases the internalized receptors are degraded and are no longer available for recycling, a process known as receptor **downregulation**. Receptors must then be replenished by protein synthesis. Receptor downregulation in response to prolonged agonist stimulation can also occur at the level of receptor protein synthesis or of receptor mRNA regulation caused by changes in gene transcription, mRNA stability, or both. These processes are of great importance in modulating the effects of drugs, e.g., opiates [13].

A more rapid and transient form of receptor desensitization involves receptor **modulation** by phosphorylation, which can rapidly change receptor affinity, signaling efficiency, or both. For example, the β-adrenoceptor is desensitized as a result of phosphorylation of a number of sites in its intracellular carboxy-terminal domain by cAMP-dependent protein kinase, protein kinase C (PKC), and β-adrenergic receptor kinase (βARK), a G-protein-coupled receptor kinase (GRK). The former kinase is activated as a result of β-receptor stimulation of adenylate cyclase and results in homologous or heterologous desensitization, whereas the latter kinase is active only on

Caption for Figure 2.2. (*cont.*) associated with the membrane by covalently bound fatty acid molecules. In the resting state, GDP is bound to the α subunit, which is closely attached to the βγ complex. When the neurotransmitter binds to the receptor, the conformation of the receptor changes, inducing a change in the conformation of the α subunit, which expels GDP and replaces it by GTP. The GTP-bound α subunit is no longer capable of interacting with the receptor or γ. GTP-bound α and γ interact with specific targets that differ for each isoform α or γ subunits. After a short time GTP is hydrolyzed to GDP and α-GDP reassociates with γ. At about the same time, the neurotransmitter leaves its receptor, which returns to its resting state. G protein, guanine nucleotide-binding protein; Pi, inorganic phosphate. (B) The adenylate cyclase/protein kinase A (PKA) pathway. cAMP is formed from ATP by a class of transmembrane enzymes, adenylate cyclases. A cytosolic form of adenylate cyclase has also been described recently. Transmembrane adenylate cyclases are activated by two related subtypes of G-protein α subunits, α_s (stimulatory, which is ubiquitous) and α_{off} (olfactory, which is found in olfactory epithelium and a subset of neurons). Adenylate cyclases are inhibited by α_i (inhibitory). In addition, some adenylate cyclases can be stimulated or inhibited by βγ, or Ca^{2+} combined with calmodulin. Cyclic adenosine 3′-5′-monophosphate (cAMP) is inactivated by hydrolysis into AMP by phosphodiesterases, a family of enzymes that is inhibited by theophylline and related methylxanthines. cAMP has only two known targets in vertebrates: one is a cAMP-gated ion channel that is most prominently found in olfactory neurons, and the other is cAMP-dependent protein kinase that is present in all cells. cAMP-dependent protein kinase is a tetramer composed of two catalytic subunits and two regulatory subunits (only one of each is shown). When cAMP binds to the regulatory subunits (two molecules of cAMP bind to each regulatory subunit), they dissociate from the catalytic subunits. The free active catalytic subunit phosphorylates numerous specific substrates including ion channels, receptors, and enzymes. In addition, the catalytic subunit can enter the nucleus, where it phosphorylates transcription factors. One well-characterized transcription factor phosphorylated in response to cAMP is cAMP response element-binding protein (CREB). In the basal state, CREB forms a dimer that binds to a specific DNA sequence in the promoter region of cAMP-responsive genes, called CRE (cAMP-responsive element). CREB is unable to promote transcription when it is not phosphorylated, whereas phospho-CREB strongly stimulates transcription. Genes regulated by CREB include immediate-early genes *c-Fos and* c-Jun. CREB is also activated by Ca^{2+} calmodulin-dependent protein kinase.

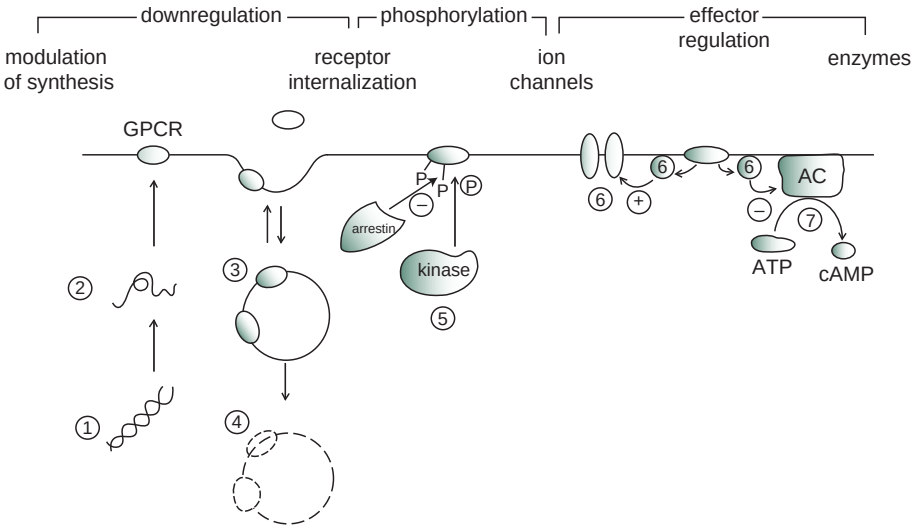

Figure 2.3. Receptor desensitization. A multitude or systems regulates GPCR signaling. Synthesis and expression of receptors can be regulated at the DNA (1) and RNA (2) level, i.e., at the levels of gene transcription as well as translation, post-translational modification, and trafficking to the membrane. Expressed receptors can be removed from the membrane by internalization (3); internalized receptors can either be recycled to the membrane or degraded (4). All the processes are relatively slow, and referred to as receptor downregulation. Faster modulation of receptor functioning often involves phosphorylation of the receptor by one of a variety of kinases (5). Commonly, this phosphorylation allows interaction between the receptor and a member of the arrestin family, thereby blocking receptor functioning. Additional possibilities for regulation exist downstream of the receptor itself. Both ion channels and enzymes such as adenylate cyclase (AC) can be modulated to counteract the effects of GPCR on their signaling. For exampale, whereas opioid receptor signaling normally results in a decrease in cAMP levels because of inhibition of AC, modulating AC into a hyperactive state can induce cellular tolerance to opiate signaling. G, G protein; +, activates; –, inhibits; P, phosphorylates.

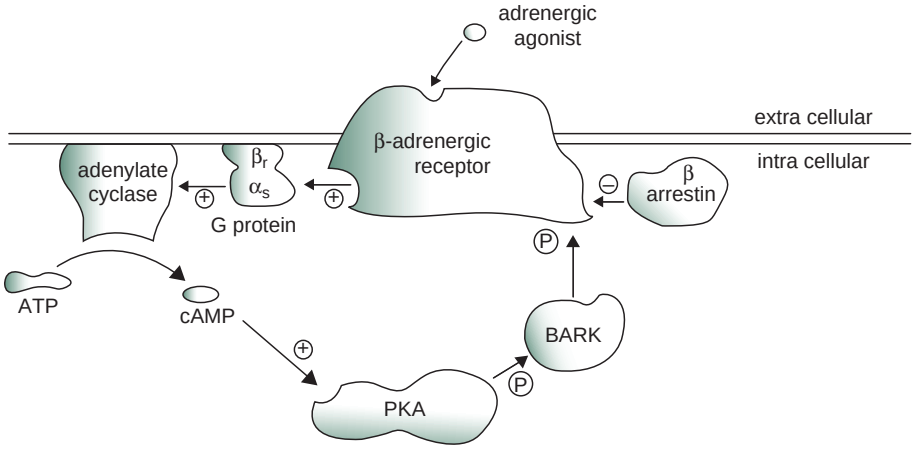

Figure 2.4. Modulation of β-adrenoceptor functioning by β-adrenergic receptor kinase (βARK) and β-arrestin. Ligand binding to the β-adrenoceptor results in activation of its associated G protein. The α_s G-protein subunit in turn activates adenylate cyclase, which converts ATP to cAMP, resulting in increased intracellular levels of this second messenger. One of the enzymes regulated by cAMP is protein kinase A (PKA), and one of its phosphorylation targets is βARK. Once phosphorylated, βARK in turn selectively phosphorylates serine and threonine residues on activated β-adrenoceptor molecules. As a result, these phosphorylated receptors become accessible for interaction with β-arrestin, which binds to the receptor and blocks further signaling. Together, this system forms a feedback loop that prevents over-activated β-adrenoceptor signaling. Similar systems exist for other GPCRs. +, activates; –, inhibits; P, phosphorylates.

β receptors occupied by ligand and therefore results in only homologous desensitization. Phosphorylation by βARK leads to the binding of β-arrestin to the receptor [14]. Arrestins are a group of proteins that sterically hinder the coupling between a GPCR and its associated G protein [15]. These processes both serve to uncouple the active ligand–receptor complex from interacting with the G_s protein, creating a negative feedback loop for modulation of β-receptor activity (Fig. 2.4). In other instances, receptor phosphorylation can affect ligand affinity or associated ion-channel kinetics rather than G-protein coupling.

Signaling regulation is an area of very active research, and a variety of molecular systems involved in this process have been identified. In addition to those mentioned above, these include systems that affect ligand binding specificity and affinity, and coupling between receptors, G proteins, and effectors [9]. These systems all provide potential drug targets, many of which could be of use in anesthesiology. A particularly relevant, yet complex, example is the regulation of opiate signaling. All anesthesiologists are familiar with the profound tolerance that can occur during chronic opiate treatment, at times requiring more than 100 times normal doses for a clinically relevant effect [16]. On the other hand, a degree of tolerance develops to opiates administered during the course of a single anesthetic, and results in increased analgesic requirements postoperatively, an effect most clearly demonstrated with use of remifentanil [17]. The latter primarily results from desensitization and internalization of the μ-opioid receptor itself. Desensitization occurs by a mechanism similar to that described above for adrenoceptors: the opioid receptor is phosphorylated by a G-protein-coupled receptor kinase, increasing the affinity for an arrestin. This subsequently leads

to decreased effector coupling, and induces internalization of the receptor. Chronic tolerance to opiates results from desensitizing effects throughout the various opiate signaling pathways. Opioid receptors couple to G_i and G_o proteins, with the main effectors being an inwardly rectifying potassium channel, voltage-gated calcium channels, and adenylate cyclase (see also Chapters 3 and 4). Each of these effector systems can be regulated to counteract the effects of opiates. Prolonged opiate exposure induces changes in the coupling between the receptor and the coupled potassium channel, resulting in less effect of the drug. However, the magnitude of this effect is insufficient to explain clinically observed increases in tolerance, and indeed other parts of the signaling pathway are affected as well. The intracellular result of opioid receptor signaling is a decrease in cAMP levels (see next section), and cellular tolerance can develop by hyperactivation of adenylate cyclase (possibly induced by G-protein $\beta\gamma$ units) that counteracts this opiate-induced decrease. Yet further desensitization of the system can occur because of changes in feedback from other cells in the network, which functionally counteract the opiate effect. All of these desensitizing actions are in principle amenable to modulation by drugs.

An area of particular interest is the synaptic plasticity induced by opiates, which in essence makes the nervous system "learn" to be less responsive to the drugs. This process, which occurs after both short-term and long-term opiate administration, is highly dependent on changes in glutamate receptor expression, and therefore opiate tolerance can be modulated significantly by drugs that affect glutamate signaling. In the context of anesthesiology, ketamine (an NMDA receptor antagonist) has found a place in preventing or even reversing opiate tolerance. For example, in rats, fentanyl administration reduces the effectiveness of a subsequent morphine bolus. This desensitizing effect can be completely prevented by ketamine pretreatment. In addition, fentanyl induces a long-term hyperalgesia of several days' duration. This hyperalgesia is similarly prevented by ketamine. Hence, it appears the drug has beneficial effects on both short-term and long-term desensitizing processes [18]. However, it is not clear if these findings always translate to the clinical setting, as ketamine did not affect opiate requirements after remifentanil-based anesthesia for major spine surgery [19].

The desensitizing processes mentioned here do not exhaust the list. A number of other systems change their functioning in response to opioid receptor signaling: protein kinase A (PKA), adrenergic systems in the locus coeruleus, γ-aminobutyric acid (GABA) signaling, MAP kinases, and phosphoinositide-3 kinases have all been shown to be affected, although their role in the clinical symptomatology of opiate tolerance remains to be determined. In addition, there are well-described roles of opiates that are not directly associated with pain pathways, but are similarly modulated by desensitization. μ-Opioid receptor activation induces a proinflammatory response, in part by modulating cytokine and chemokine receptors. In contrast, activation of κ-opioid receptors is able to reverse this effect by downregulating these receptor systems [20].

So, even for a single pharmacologic class such as the opiates, we already find a remarkably large number of potential targets for interference with desensitization processes. Only very few of these have been explored outside the cellular laboratory. We may expect, however, that the future will bring us novel classes of drugs, specifically targeted to modulating desensitization of G-protein-coupled receptor systems.

Second messengers

Cyclic adenosine 3′-5′-monophosphate

cAMP, the first intracellular messenger identified, operates as a signaling molecule in all eukaryotic and prokaryotic cells. A variety of hormones and neurotransmitters have been found to regulate the levels of cAMP. Adenylate cyclases form a class of membrane-bound enzymes that catalyze the formation of cAMP, usually under the control of receptor-mediated G-protein-coupled stimulation (by α_s and α_{olf}) and inhibition (by α). The rapid degradation of cAMP to adenosine 5′-monophosphate by one of several isoforms of cAMP phosphodiesterase provides the potential for rapid reversibility and responsiveness of this signaling mechanisms. Most of the actions of cAMP are mediated through the activation of cAMP-dependent protein kinase (PKA) and the concomitant phosphorylation of substrate protein effectors on specific serine or threonine residues.

Substrates for cAMP-dependent protein kinase are characterized by two or more basic amino acid residues on the amino-terminal side of the phosphorylated residue. The various substrates for cAMP-dependent protein kinase present in different cell types explain the diverse tissue-specific effects of cAMP. They include ion channels, receptors, enzymes, cytoskeletal proteins, and transcription factors (e.g., cAMP response element-binding protein [CREB]).

Calcium ion and inositol trisphosphate

Along with cAMP, Ca^{2+} controls a wide variety of intracellular processes [21]. Ca^{2+} entry through Ca^{2+} channels or its release from intracellular stores triggers hormone and neurotransmitter secretion, initiates muscle contraction, and activates many protein kinases and other enzymes. The concentration of free Ca^{2+} is normally maintained at a very low level in the cytosol of most cells ($< 10^{-6}$ M) compared with the extracellular fluid ($\sim 10^{-3}$ M) by a number of homeostatic mechanisms. A Ca^{2+} ATPase in the plasma membrane pumps Ca^{2+} from the cytosol to the cell exterior at the expense of adenosine triphosphate (ATP) hydrolysis, a Ca^{2+} ATPase in the endoplasmic and sarcoplasmic reticulum concentrates Ca^{2+} from the cytosol into intracellular storage organelles, and a Na^+/Ca^{2+} exchanger, which is particularly

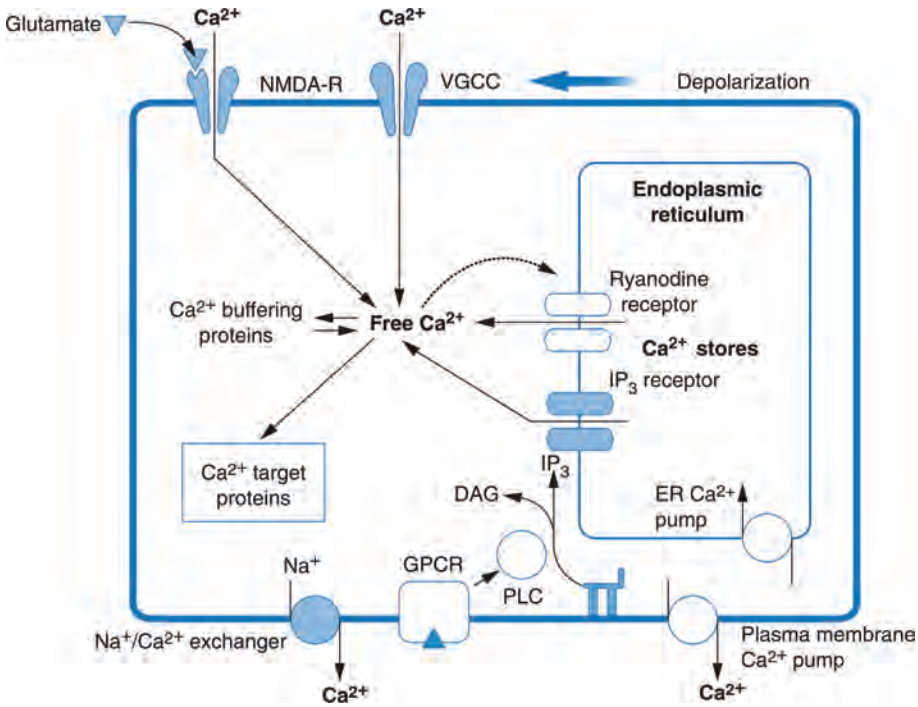

Figure 2.5. Pathways by which Ca^{2+} can enter the cytosol as a second messenger in response to extracellular signals. Ca^{2+} enters a nerve terminal from the extracellular fluid through voltage-gated Ca^{2+} channels when the nerve terminal membrane is depolarized by an action potential. Binding of an extracellular signaling molecule to a cell-surface receptor generates inositol 1,4,5-trisphosphate (IP_3), which stimulates the release of Ca^{2+} from the endoplasmic reticulum. Ca^{2+} is a divalent cation whose concentrations are relatively high in the extracellular space (approximately 1.2 mM) and more than 10 000 times lower within the cytosol (approximately 100 nM). In resting conditions, the plasma membrane is impermeable to Ca^{2+}. In neurons, it can penetrate through specific channels that include voltage-gated Ca^{2+} channels (VGCC) and glutamate receptors of the *N*-methyl-D-aspartate (NMDA) subtype. When these channels are open, in response to depolarization in the case of VGCC or in the presence of glutamate in the case of NMDA receptor, Ca^{2+} flows readily into the cytosol following both its concentration gradient and the electrical potential. Ca^{2+} can also be released into the cytosol from internal stores (the endoplasmic reticulum). Two types of Ca^{2+} channels are responsible for the release of Ca^{2+} from internal stores: one is the IP_3 receptor, the opening of which is triggered by IP_3, a second messenger generated by phospholipase C from phosphatidylinositol 4,5-bisphosphate; and the other is the ryanodine receptor, named after ryanodine, a drug that triggers its opening. Opening of ryanodine receptors is triggered by Ca^{2+} itself by a mechanism called Ca^{2+}-induced Ca^{2+} release, which can give rise to propagation of waves of Ca^{2+} release along the endoplasmic reticulum. In the cytosol, Ca^{2+} is mostly bound to specific binding proteins. Some of them function as buffering proteins, preventing excessive increases in cytosolic free Ca^{2+}. Others are the actual targets of Ca^{2+}, which account for the potent biologic effects of this cation. Among the best-characterized targets are calmodulin and calmodulin-related proteins, which undergo a conformational change enabling them to interact with, and activate, a number of enzymes. Ca^{2+} can also bind to another type of protein domain called C2. Free Ca^{2+} in the cytosol is maintained at very low levels by several highly active processes that include Ca^{2+} pumps and Ca^{2+} exchangers. The Ca^{2+} pumps have a high affinity but a low capacity for Ca^{2+} and are used for fine tuning of Ca^{2+} levels. They are located on the plasma membrane and the membrane of the endoplasmic reticulum, and their energy is provided by adenosine triphosphate (ATP) hydrolysis. Na^+/Ca^{2+} exchangers, whose driving force is provided by the Na^+ gradient, have a large capacity, but a low affinity for Ca^{2+}. DAG, diacylglycerol; ER, endoplasmic reticulum; GPCR, G-protein-coupled receptor; NMDA-R, *N*-methyl-D-aspartate subtype of glutamate receptor; PLC, phospholipase C.

active in excitable plasma membranes, couples the electrochemical potential of Na^+ influx to the efflux of Ca^{2+} (Na^+-driven Ca^{2+} antiport). Although mitochondria have the ability to take up and release Ca^{2+}, they are not widely believed to play a major role in cytosolic Ca^{2+} homeostasis during normal conditions.

Changes in intracellular free Ca^{2+} concentration can be induced directly by depolarization-evoked Ca^{2+} entry down its electrochemical gradient through voltage-gated Ca^{2+} channels (as in neurons and muscle), by extracellular signals that activate Ca^{2+}-permeable ligand-gated ion channels (e.g., the NMDA glutamate receptor), or directly by extracellular signals coupled to the formation of IP_3 (Fig. 2.5). IP_3 is formed in response to a number of extracellular signals that interact with G-protein-coupled cell-surface receptors (G_q, G_{11}) coupled to the activation of phospholipase C [22].

Phospholipase C hydrolyzes phosphatidylinositol 4,5-bisphosphate to IP_3 and diacylglycerol; further degradation of diacylglycerol by phospholipase A_2 can result in the release of arachidonic acid. All three of these receptor-regulated metabolites are important second messengers. IP_3 increases intracellular Ca^{2+} by binding to specific IP_3 receptors on the endoplasmic reticulum, which are coupled to a Ca^{2+} channel that allows Ca^{2+} efflux into the cytosol. IP_3 receptors are similar to the Ca^{2+} release channels (ryanodine receptors) of muscle sarcoplasmic reticulum that release Ca^{2+} in response to excitation. Diacylglycerol remains in the plasma membrane where it activates PKC, whereas arachidonic acid, in addition

to its metabolism to biologically active prostaglandins and leukotrienes, can also activate PKC. The Ca^{2+} signal is terminated by hydrolysis of IP_3 and by the rapid reuptake or extrusion of Ca^{2+}.

Ca^{2+} carries out its second-messenger functions primarily after binding to intracellular Ca^{2+} binding proteins, of which calmodulin is the most important. Calmodulin is a ubiquitous multifunctional Ca^{2+} binding protein, highly conserved between species, which binds four atoms of Ca^{2+} with high affinity. Ca^{2+} can also bind to C2 domains found in several proteins (PKC, phospholipase A_2, synaptotagmin).

PKC is a family of serine/threonine protein kinases consisting of 12 structurally homologous phospholipid-dependent isoforms with conserved catalytic domains, which are distinguished by their variable N-terminal regulatory domains and cofactor dependence [23]. The Ca^{2+}-dependent or conventional isoforms of PKC (cPKC) are components of the phospholipase C/diacylglycerol signaling pathway. They are regulated by the lipid second messenger 1,2-diacylglycerol, by phospholipids such as phosphatidylserine, and by Ca^{2+} through specific interactions with the regulatory region. Binding of diacylglycerol to the C1 domain of cPKC isoforms (α, β_1, β_2, γ) increases their affinity for Ca^{2+} and phosphatidylserine, facilitates PKC translocation and binding to cell membranes, and increases catalytic activity. The novel PKC isoforms (nPKC; δ, ε, η, θ, μ) are similar to cPKCs, but lack the C2 domain and do not require Ca^{2+}. The atypical isoforms (aPKC; ζ, λ) differ considerably in the regulatory region, and do not require Ca^{2+} or diacylglycerol for activity.

Summary

Cell signaling pathways are important for multiple biological functions. The role of the receptor is to bind signaling molecules and transduce this information into a functional response. Receptor expression determines tissue and cell sensitivity to a variety of signaling molecules. Receptors may be expressed at the cell surface, where they typically interact with insoluble ligands or hydrophilic molecules – an exception being prostaglandins, which are hydrophobic. Intracellular receptors interact with hydrophobic signaling molecules which cross the plasma membrane by diffusion. Signal transduction pathway organization can facilitate signal amplification, specificity, and pleiotropy.

The G-protein-coupled receptor (GPCR) superfamily is large and functionally diverse, and constitutes an important pharmacologic target. These receptors have seven transmembrane domains, and are coupled to an intracellular heterotrimeric guanine nucleotide (GTP)-binding protein (G protein). Ligand binding induces a conformational change in the GPCR, and GTP replaces GDP on the G protein. The $\beta\gamma$ subunit and the GTP-bound α subunit of the G protein then dissociate from the receptor. Different isoforms of the G-protein subunits are coupled to diverse effector pathways which alter the concentration of second-messenger molecules or produce changes in the membrane potential of the cell. Effectors activated or inhibited by G-protein subunits include adenylate cyclase, phospholipase C, phospholipase A_2, cyclic GMP (cGMP) phosphodiesterase, and calcium and potassium ion channels. Pleiotropy at multiple stages in GPCR signal transduction gives rise to flexibility in signaling and regulation.

Common second-messenger molecules include cyclic adenosine 3′-5′-monophosphate (cAMP), inositol 1,4,5-trisphosphate (IP_3), 1,2-diacylglycerol (DAG), and calcium ions (Ca^{2+}). Certain G-protein subunits can stimulate or inhibit membrane-bound adenylate cyclase, which catalyzes cAMP formation. cAMP activates cAMP-dependent protein kinase, which in turn phosphorylates a variety of targets such as ion channels, enzymes, and transcription factors, which are present in different cell types. Some G-protein subunits activate phospholipase C to hydrolyze phosphatidylinositol 4,5-bisphosphate to IP_3 and DAG. IP_3 binds receptors on the endoplasmic reticulum, causing release of Ca^{2+} into the cytosol. Calcium signaling pathways are mediated by Ca^{2+} binding proteins such as calmodulin. DAG and its metabolite, arachidonic acid, activate protein kinase C. Second-messenger-regulated enzymes such as kinases and phosphatases modulate the function and localization of downstream proteins to produce a cellular response. Changes in second-messenger concentration are usually transient by virtue of tightly regulated degradation, synthesis, release, and reuptake.

Dissociation of the G protein from the receptor reduces receptor–ligand affinity, and the agonist is released. The α subunit of the G protein has intrinsic GTPase activity, which hydrolyses the bound GTP to GDP. The GDP-bound α subunit rejoins the $\beta\gamma$ subunit, returning the G protein to its resting state.

Receptor desensitization following prolonged exposure to high-concentration agonist is one mechanism of regulation, which can cause decreased responses to drugs, including adrenoceptor agonists and opiates. Receptors may be internalized by endocytosis, their expression may be downregulated by increased degradation or decreased synthesis, or the receptor may be modulated by phosphorylation to achieve rapid, transient desensitization. Receptor modulation can lead to the binding of arrestins, which hinder coupling to the associated G protein, as occurs in the desensitization of the β-adrenoceptor; modulation can also affect ligand affinity or ion channel kinetics. Pharmacologic intervention in the processes underlying desensitization has the potential to reduce tolerance to drugs such as opiates.

References

1. Alberts B, Bray D, Lewis J, *et al.* Cell signaling. In: *Molecular Biology of the Cell*, 3rd edn. New York, NY: Garland, 1994: 721–85.
2. Lodish H, Baltimore D, Berk A, *et al.* Cell-to-cell signaling: hormones and receptors. In: Lodish H, Baltimore D, Berk A, *et al.*, eds., *Molecular Cell Biology*, 3rd edn. New York, NY: Scientific American Books, 1995: 853–924.
3. Hemmings HC. Cell signaling. In: Hemmings HC, Hopkins PM, eds., *Foundations of Anesthesia: Basic and Clinical Sciences*. London: Mosby, 2000: 21–36.
4. Venter JC, Adams MD, Myers EW, *et al.* The sequence of the human genome. *Science* 2001; **291**: 1304–51.
5. Carroll RC, Beattie EC, von Zastrow M, Malenka RC. Role of AMPA receptor endocytosis in synaptic plasticity. *Nat Rev Neurosci* 2001; **2**: 315–24.
6. Hepler JR, Gilman AG. G proteins. *Trends Biochem Sci* 1992; **17**: 383–7.
7. Exton JH. Cell signalling through guanine-nucleotide-binding regulatory proteins (G proteins) and phospholipases. *Eur J Biochem* 1997; **243**: 10–20.
8. Hamm HE, Gilchrist A. Heterotrimeric G proteins. *Curr Opin Cell Biol* 1996; **8**: 189–96.
9. Luttrell LM. Transmembrane signaling by G protein-coupled receptors. *Methods Mol Biol* 2006; **332**: 3–49.
10. Coleman DE, Sprang SR. How G proteins work: a continuing story. *Trends Biochem Sci* 1996; **21**: 41–4.
11. Thompson MD, Percy ME, McIntyre Burnham W, Cole DE. G protein-coupled receptors disrupted in human genetic disease. *Methods Mol Biol* 2008; **448**: 109–37.
12. Patwardhan AM, Jeske NA, Price TJ, *et al.* The cannabinoid WIN 55,212–2 inhibits transient receptor potential vanilloid 1 (TRPV1) and evokes peripheral antihyperalgesia via calcineurin. *Proc Natl Acad Sci U S A* 2006; **103**: 11393–8.
13. Martini L, Whistler JL. The role of mu opioid receptor desensitization and endocytosis in morphine tolerance and dependence. *Curr Opin Neurobiol* 2007; **17**: 556–64.
14. Barki-Harrington L, Rockman HA. Beta-arrestins: multifunctional cellular mediators. *Physiology* 2008; **23**: 17–22.
15. DeWire SM, Ahn S, Lefkowitz RJ, Shenoy SK. Beta-arrestins and cell signaling. *Annu Rev Physiol* 2007; **69**: 483–510.
16. Christie MJ. Cellular neuroadaptations to chronic opioids: tolerance, withdrawal and addiction. *Br J Pharmacol* 2008; **154**: 384–96.
17. Joly V, Richebe P, Guignard B, *et al.* Remifentanil-induced postoperative hyperalgesia and its prevention with small-dose ketamine. *Anesthesiology* 2005; **103**: 147–55.
18. Laulin JP, Maurette P, Corcuff JB, *et al.* The role of ketamine in preventing fentanyl-induced hyperalgesia and subsequent acute morphine tolerance. *Anesth Analg* 2002; **94**: 1263–9.
19. Engelhardt T, Zaarour C, Naser B, *et al.* Intraoperative low-dose ketamine does not prevent a remifentanil-induced increase in morphine requirement after pediatric scoliosis surgery. *Anesth Analg* 2008; **107**: 1170–5.
20. Finley MJ, Happel CM, Kaminsky DE, Rogers TJ. Opioid and nociceptin receptors regulate cytokine and cytokine receptor expression. *Cell Immunol* 2008; **252**: 146–54.
21. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. *N Engl J Med* 1993; **329**: 2002–12.
22. Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. *Nat Rev Mol Cell Biol* 2000; **1**: 11–21.
23. Divecha N, Irvine RF. Phospholipid signaling. *Cell* 1995; **80**: 269–78.

Section 1
Chapter
3

Principles of drug action

Ion channels

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Introduction

Ion channels are integral membrane proteins that form an aqueous channel in the lipid bilayer through which charged particles can pass. There are many different types of ion channels, and they may be classified according to the factors that regulate channel opening and closing (gating), as well as the types of ions allowed to traverse the pore (selectivity). This chapter reviews the structure and function of the major classes of channels, focusing on those that are essential to neuronal and cardiac function and signaling. These include the voltage-gated ion channels, which open and close in response to changes in the voltage across the cell membrane, and the ligand-gated ion channels, which open in the presence of extracellular ligands (e.g., neurotransmitters). Included in the discussion of voltage-gated ion channels are the background or baseline K^+ channels, some of which are activated by anesthetics and thus may contribute to the anesthetic state.

Basic membrane electrophysiology

Membrane potential is determined by ionic conductances

Whether ions go into or out of the cell when a channel opens depends on both the membrane potential and the concentration gradient for that ion at the time the channel is open. Under physiologic conditions, Na^+, Ca^{2+}, and K^+ ions generally flow down their respective concentration gradients. Thus when their respective channels are opened, Na^+ and Ca^{2+} ions flow into the cell, whereas K^+ ions flow out of the cell. However, Na^+ and Ca^{2+} ions will be repelled from entering the cell if the interior of the cell is very positively charged, whereas K^+ ions tend to be retained in the cell if it is very negatively charged. The membrane potential at which net flow for a particular ion through its channel is zero, and beyond which the direction of flow reverses, can be calculated using the Nernst equation [1], which is based on thermodynamic principles and is shown in a simplified form as:

$$E_{\text{ion}} = \frac{60\ \text{mV}}{z_{\text{ion}}} \log \frac{[ion]_{\text{extracellular}}}{[ion]_{\text{intracellular}}} \qquad (3.1)$$

In this equation, E_{ion} is the Nernst potential or reversal potential for the ion of interest, z_{ion} is the charge number for the ion, and the log term is the ratio of extracellular to intracellular concentrations of the ion. For the K^+ ion, for example, the ratio of extracellular to intracellular concentrations is approximately 5 mM/150 mM (= 0.033), making E_K about –90 mV. This means that at membrane potentials more positive than –90 mV, K^+ ions will flow out of the cell, whereas at potentials more negative than –90 mV, K^+ ions will flow into the cell. Conversely, the reversal potentials for Na^+ and Ca^{2+} are about +60 mV and +200 mV, respectively, because the concentrations of these ions are greater outside than inside the cell (especially Ca^{2+}, which has a resting intracellular concentration of about 100 nM).

The Nernst equation is used to determine the membrane potential at which no current will flow when the membrane is permeable to only one ion. Excitable cell membranes, however, are permeable to several different ions, mainly Na^+, Ca^{2+}, K^+, and Cl^-. In cells, the membrane potential at which no current flows is the resting membrane potential, and it can be estimated if the concentration gradients and resting conductances of the major permeant ions are known by using the following equation [2]:

$$E_m = \left[\left(\frac{g_{Na}}{g_{total}} \right) E_{Na} + \left(\frac{g_{Ca}}{g_{total}} \right) E_{Ca} + \left(\frac{g_k}{g_{total}} \right) E_K + \left(\frac{g_{Cl}}{g_{total}} \right) E_{Cl} \right] \qquad (3.2)$$

E_m is the resting potential of the membrane, g stands for conductance (the reciprocal of resistance), g_{total} is the sum of all individual ionic conductances, and E_{Na}, E_{Ca}, and so on are the Nernst potentials for each permeant ion. The resting membrane potential is determined by the weighted sum of the Nernst potentials for all permeant ions, the weighting term being the conductance of each ion relative to the total conductance. Therefore, it is easy to see that the membrane potential will trend toward the Nernst potential for a particular ion

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