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.

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

(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

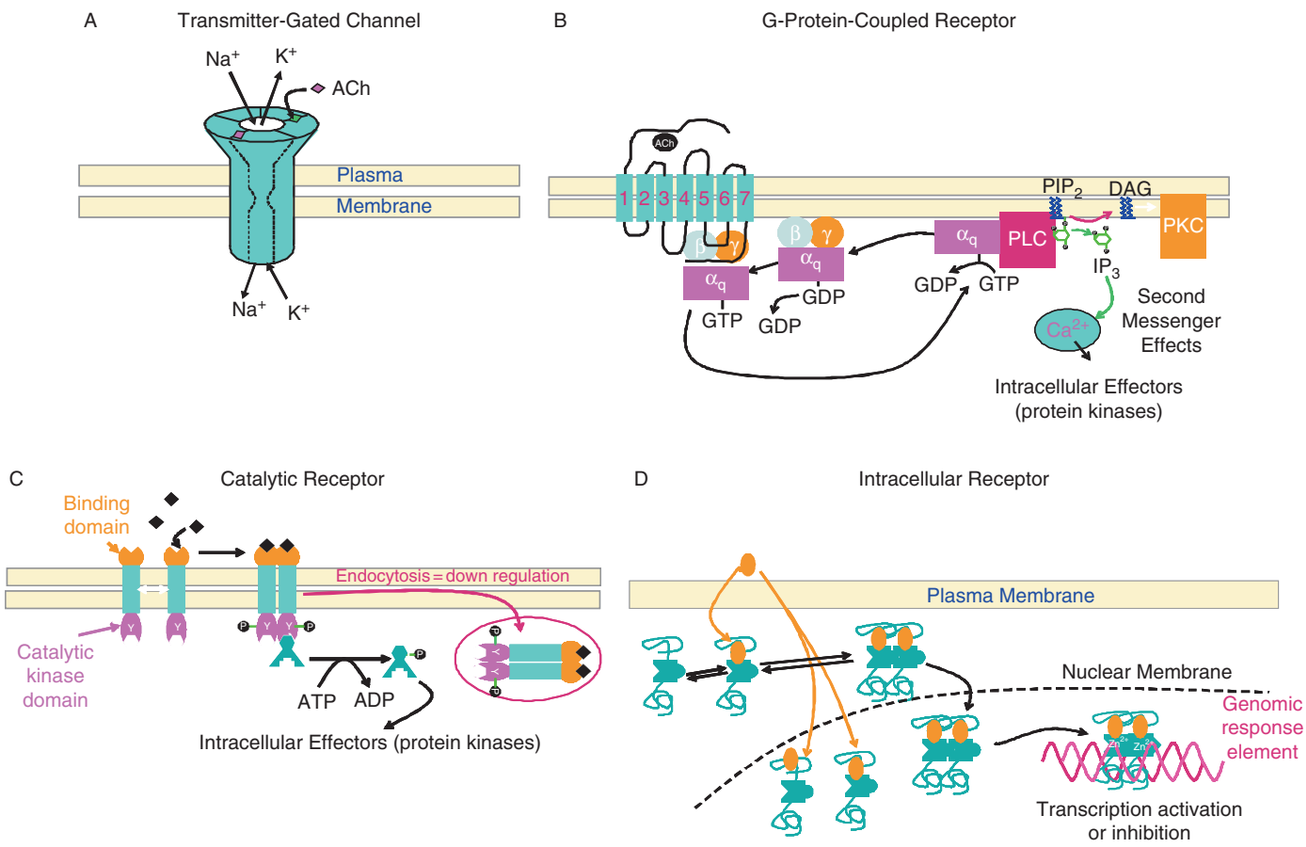

Figure 1.1. Drug receptor superfamilies. Illustrations of different families of receptor proteins, including (A) transmitter-gated ion channels, (B) G-protein-coupled receptors, (C) catalytic receptors, and (D) intracellular receptors.

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.

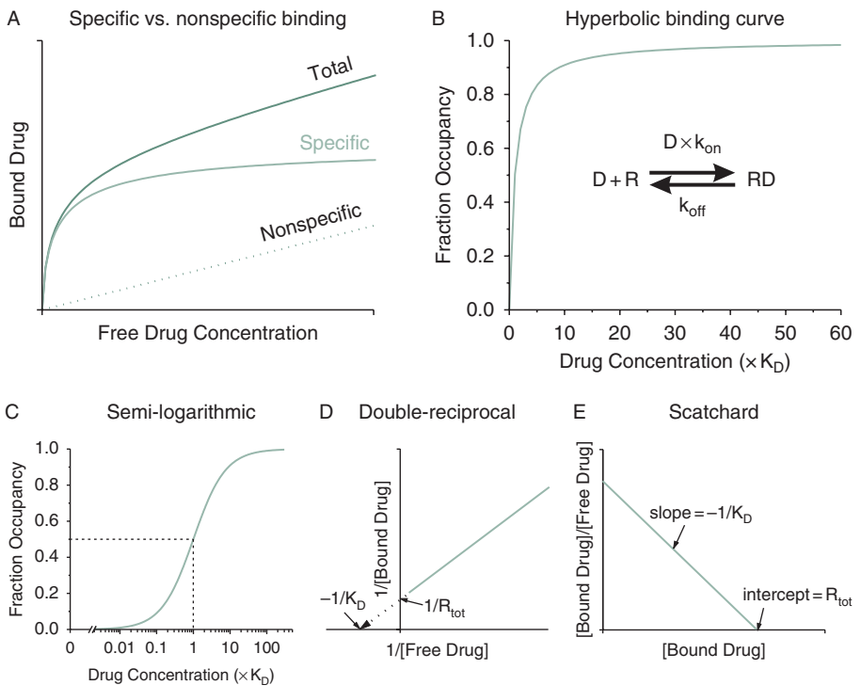

Figure 1.2. Drug binding graphical analysis. (A) Illustration of specific vs. nonspecific binding. (B) Correcting total binding for nonspecific binding produces a saturable hyperbolic binding curve on linear axes (Eq. 1.4). (C) Semilogarithmic plot with logarithmic concentration axes. (D) Lineweaver–Burke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

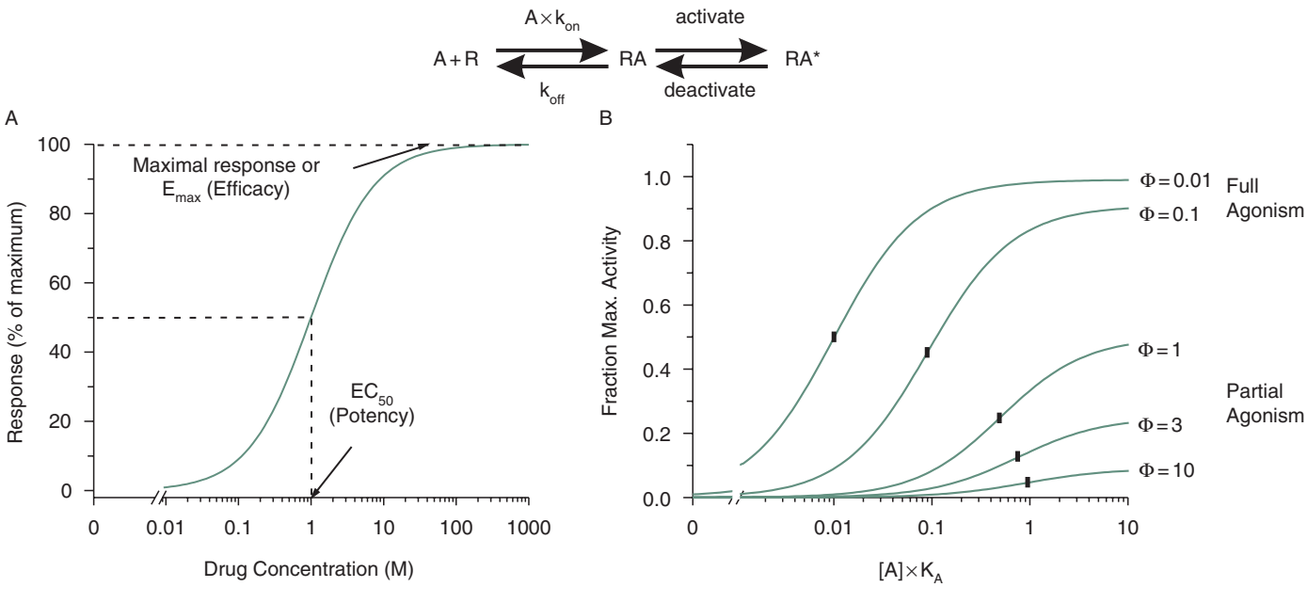

Figure 1.3. Agonist efficacy and apparent potency (EC_{50}). (A) This panel appears similar to Fig. 1.2C, except that the ordinate is a physiological response, rather than binding-site occupancy. The maximal response is drug efficacy. The concentration producing half-maximal response is the EC_{50}. (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:

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

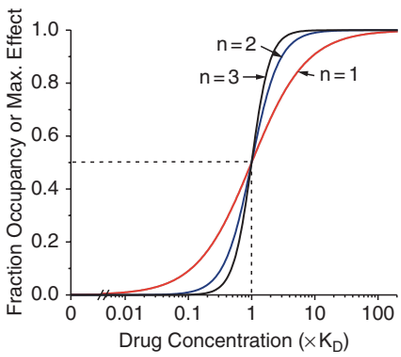

Figure 1.4. Hill analysis for multiple agonists. Semi-logarithmic logistic dose–response curves, generated using Eq. 1.13, with n = 1, 2, and 3. Note that the midpoint of the curves (EC_{50}) is not dependent on the Hill-slope, n.

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).

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

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:

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

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 $\mathbf{IC_{50}}$ 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:

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

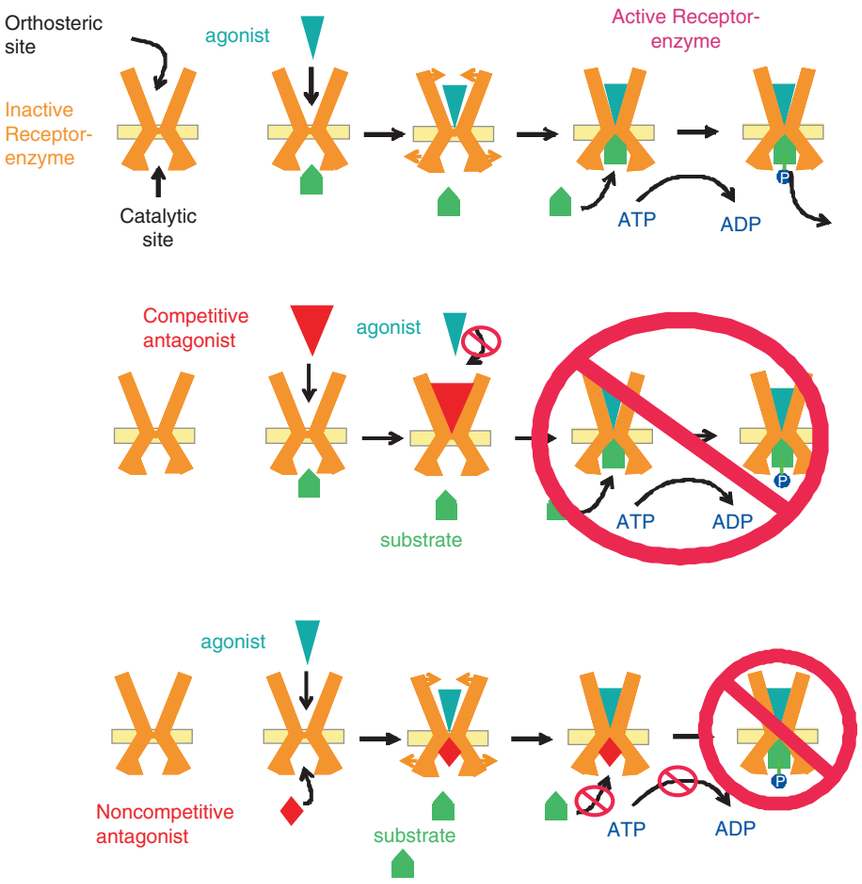

Figure 1.5. Model receptor illustration of agonism and antagonism. Top: A simple catalytic receptor model is illustrated, depicting agonist (blue triangle) binding, which induces a conformational change allowing substrate (green) binding and phosphorylation. Middle: A competitive inhibitor (red triangle) 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:

$$\begin{array}{ccc} A + I + R & \overset{K_A}{\rightleftharpoons} & RA \\ k_I \uparrow\downarrow & & k_I \uparrow\downarrow \\ RI & \overset{K_A}{\rightleftharpoons} & RAI \end{array} \tag{1.19}$$

Thus:

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

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,

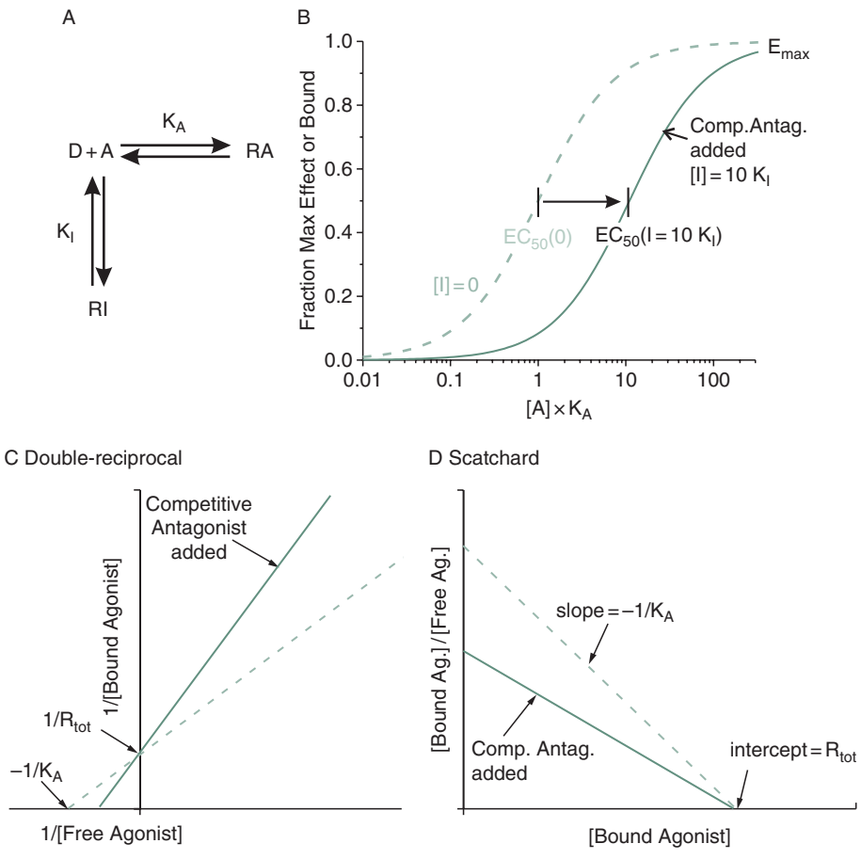

Figure 1.6. Competitive inhibition. (A) Mutually exclusive receptor occupation is depicted schematically. The RA state can activate, but the RI state cannot. (B) Agonist concentration–response curves were generated with Eq. 1.13. Addition of a competitive inhibitor reduces agonist binding and effects 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.

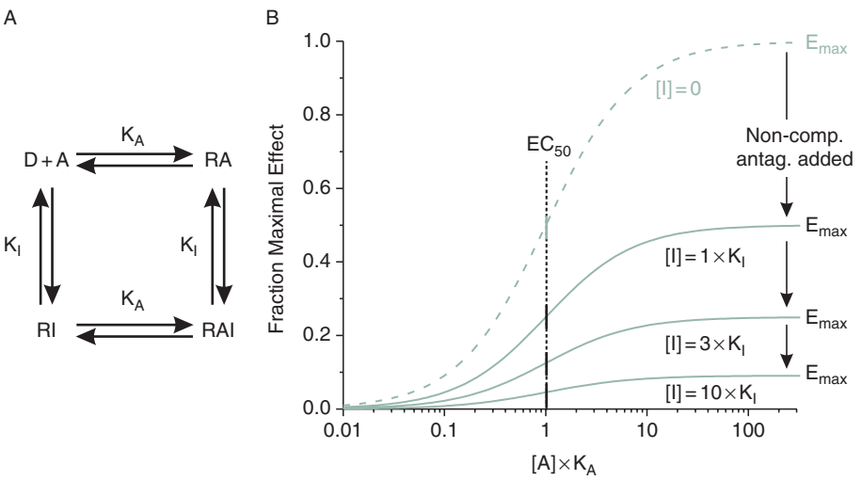

Figure 1.7. Noncompetitive inhibition. Left: A scheme depicting binding of inhibitor (I) to receptors whether or not agonist is bound. Right: The panel shows the effect of non-competitive inhibitor on agonist concentration–response curves. Noncompetitive and irreversible antagonists reduce apparent agonist 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.