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# BASIC & CLINICAL PHARMACOLOGY

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CHAPTER

# 2 Drug Receptors & Pharmacodynamics

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## CASE STUDY

A 51-year-old man presents to the emergency department due to acute difficulty breathing. The patient is afebrile and normotensive but anxious, tachycardic, and markedly tachypneic. Auscultation of the chest reveals diffuse wheezes. The physician provisionally makes the diagnosis of bronchial asthma and administers epinephrine by intramuscular injection, improving the patient's breathing over several minutes. A normal chest X-ray is subsequently obtained, and the medical history is remarkable only for mild hypertension that is being treated with propranolol. The physician instructs the patient to discontinue use of propranolol, and changes the patient's antihypertensive medication to verapamil. Why is the physician correct to discontinue propranolol? Why is verapamil a better choice for managing hypertension in this patient? What alternative treatment change might the physician consider?

Therapeutic and toxic effects of drugs result from their interactions with molecules in the patient. Most drugs act by associating with specific macromolecules in ways that alter the macromolecules' biochemical or biophysical activities. This idea, more than a century old, is embodied in the term **receptor:** the component of a cell or organism that interacts with a drug and initiates the chain of events leading to the drug's observed effects.

Receptors have become the central focus of investigation of drug effects and their mechanisms of action (pharmacodynamics). The receptor concept, extended to endocrinology, immunology, and molecular biology, has proved essential for explaining many aspects of biologic regulation. Many drug receptors have been isolated and characterized in detail, thus opening the way to precise understanding of the molecular basis of drug action.

The receptor concept has important practical consequences for the development of drugs and for arriving at therapeutic decisions in clinical practice. These consequences form the basis for understanding the actions and clinical uses of drugs described in almost every chapter of this book. They may be briefly summarized as follows:

1. **Receptors largely determine the quantitative relations between dose or concentration of drug and pharmacologic effects.** The receptor's affinity for binding a drug determines the concentration of drug required to form a significant number of drug-receptor complexes, and the total number of receptors may limit the maximal effect a drug may produce.
2. **Receptors are responsible for selectivity of drug action.** The molecular size, shape, and electrical charge of a drug determine whether—and with what affinity—it will bind to a particular receptor among the vast array of chemically different binding sites available in a cell, tissue, or patient. Accordingly, changes in the chemical structure of a drug can dramatically increase or decrease a new drug's affinities for different classes of receptors, with resulting alterations in therapeutic and toxic effects.
3. **Receptors mediate the actions of pharmacologic agonists and antagonists.** Some drugs and many natural ligands, such as hormones and neurotransmitters, regulate the function of receptor macromolecules as **agonists;** this means that they activate the receptor to signal as a direct result of binding to it. Some agonists activate a single kind of receptor to produce all their biologic functions, whereas others selectively promote one receptor function more than another.

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Other drugs act as pharmacologic **antagonists;** that is, they bind to receptors but do not activate generation of a signal; consequently, they interfere with the ability of an agonist to activate the receptor. Some of the most useful drugs in clinical medicine are pharmacologic antagonists. Still other drugs bind to a different site on the receptor than that bound by endogenous ligands; such drugs can produce useful and quite different clinical effects by acting as so-called **allosteric modulators** of the receptor.

## MACROMOLECULAR NATURE OF DRUG RECEPTORS

Most receptors for clinically relevant drugs, and almost all of the receptors that we discuss in this chapter, are proteins. Traditionally, drug binding was used to identify or purify receptor proteins from tissue extracts; consequently, receptors were discovered after the drugs that bind to them. Advances in molecular biology and genome sequencing made it possible to identify receptors by predicted structural homology to other (previously known) receptors. This effort revealed that many known drugs bind to a larger diversity of receptors than previously anticipated and motivated efforts to develop increasingly selective drugs. It also identified a number of **orphan receptors,** so-called because their natural ligands are presently unknown; these may prove to be useful targets for future drug development.

The best-characterized drug receptors are **regulatory proteins,** which mediate the actions of endogenous chemical signals such as neurotransmitters, autacoids, and hormones. This class of receptors mediates the effects of many of the most useful therapeutic agents. The molecular structures and biochemical mechanisms of these regulatory receptors are described in a later section entitled Signaling Mechanisms & Drug Action.

Other classes of proteins have been clearly identified as drug receptors. **Enzymes** may be inhibited (or, less commonly, activated) by binding a drug. Examples include dihydrofolate reductase, the receptor for the antineoplastic drug methotrexate; 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase, the receptor for statins; and various protein and lipid kinases. **Transport proteins** can be useful drug targets. Examples include $Na^+/K^+$-ATPase, the membrane receptor for cardioactive digitalis glycosides; norepinephrine and serotonin transporter proteins that are membrane receptors for antidepressant drugs; and dopamine transporters that are membrane receptors for cocaine and a number of other psychostimulants. **Structural proteins** are also important drug targets, such as tubulin, the receptor for the anti-inflammatory agent colchicine.

This chapter deals with three aspects of drug receptor function, presented in increasing order of complexity: (1) receptors as determinants of the quantitative relation between the concentration of a drug and the pharmacologic response, (2) receptors as regulatory proteins and components of chemical signaling mechanisms that provide targets for important drugs, and (3) receptors as key determinants of the therapeutic and toxic effects of drugs in patients.

## RELATION BETWEEN DRUG CONCENTRATION & RESPONSE

The relation between dose of a drug and the clinically observed response may be complex. In carefully controlled in vitro systems, however, the relation between concentration of a drug and its effect is often simple and can be described with mathematical precision. It is important to understand this idealized relation in some detail because it underlies the more complex relations between dose and effect that occur when drugs are given to patients.

### Concentration-Effect Curves & Receptor Binding of Agonists

Even in intact animals or patients, responses to low doses of a drug usually increase in direct proportion to dose. As doses increase, however, the response increment diminishes; finally, doses may be reached at which no further increase in response can be achieved. This relation between drug concentration and effect is traditionally described by a hyperbolic curve (Figure 2–1A) according to the following equation:

$$E = \frac{E_{max} \times C}{C + EC_{50}}$$

where E is the effect observed at concentration C, $E_{max}$ is the maximal response that can be produced by the drug, and $EC_{50}$ is the concentration of drug that produces 50% of maximal effect.

This hyperbolic relation resembles the mass action law that describes the association between two molecules of a given affinity. This resemblance suggests that drug agonists act by binding to ("occupying") a distinct class of biologic molecules with a characteristic affinity for the drug. Radioactive receptor ligands have been used to confirm this occupancy assumption in many drug-receptor systems. In these systems, drug bound to receptors (B) relates to the concentration of free (unbound) drug (C) as depicted in Figure 2–1B and as described by an analogous equation:

$$B = \frac{B_{max} \times C}{C + K_d}$$

in which $B_{max}$ indicates the total concentration of receptor sites (ie, sites bound to the drug at infinitely high concentrations of free drug) and $K_d$ (the equilibrium dissociation constant) represents the concentration of free drug at which half-maximal binding is observed. This constant characterizes the receptor's affinity for binding the drug in a reciprocal fashion: If the $K_d$ is low, binding affinity is high, and vice versa. The $EC_{50}$ and $K_d$ may be identical but need not be, as discussed below. Dose-response data are often presented as a plot of the drug effect (ordinate) against the *logarithm* of the dose or concentration (abscissa), transforming the hyperbolic curve of Figure 2–1 into a sigmoid curve with a linear midportion (eg, Figure 2–2). This

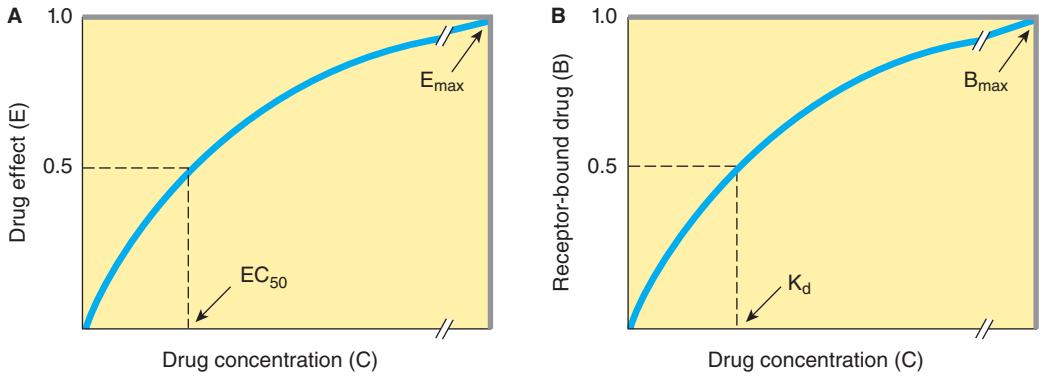


**FIGURE 2–1** Relations between drug concentration and drug effect (**A**) or receptor-bound drug (**B**). The drug concentrations at which effect or receptor occupancy is half-maximal are denoted by $EC_{50}$ and $K_d$, respectively.

transformation is convenient because it expands the scale of the concentration axis at low concentrations (where the effect is changing rapidly) and compresses it at high concentrations (where the effect is changing slowly), but otherwise has no biologic or pharmacologic significance.

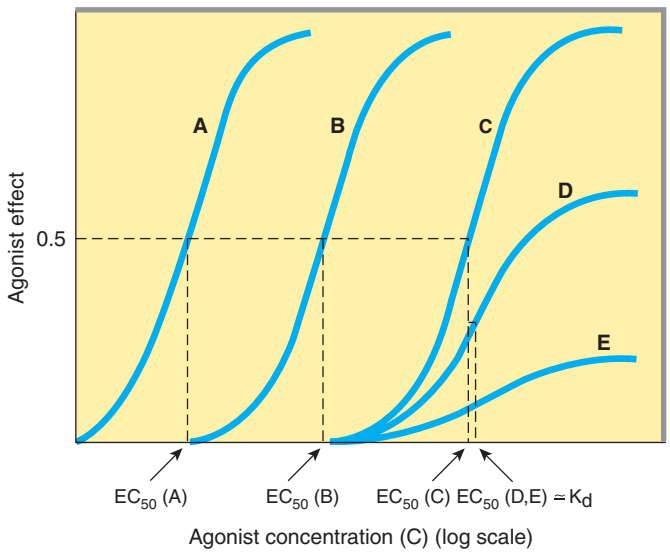


**FIGURE 2–2** Logarithmic transformation of the dose axis and experimental demonstration of spare receptors, using different concentrations of an irreversible antagonist. Curve **A** shows agonist response in the absence of antagonist. After treatment with a low concentration of antagonist (curve **B**), the curve is shifted to the right. Maximal responsiveness is preserved, however, because the remaining available receptors are still in excess of the number required. In curve **C,** produced after treatment with a larger concentration of antagonist, the available receptors are no longer "spare"; instead, they are just sufficient to mediate an undiminished maximal response. Still higher concentrations of antagonist (curves **D** and **E**) reduce the number of available receptors to the point that maximal response is diminished. The apparent $EC_{50}$ of the agonist in curves **D** and **E** may approximate the $K_d$ that characterizes the binding affinity of the agonist for the receptor.

## Receptor-Effector Coupling & Spare Receptors

When an agonist occupies a receptor, conformational changes occur in the receptor protein that represent the fundamental basis of receptor activation and the first of often many steps required to produce a pharmacologic response. The overall transduction process that links drug occupancy of receptors and pharmacologic response is called **coupling.** The relative efficiency of occupancy-response coupling is determined, in part, at the receptor itself; full agonists tend to shift the conformational equilibrium of receptors more strongly than partial agonists (described in the text that follows). Coupling is also determined by "downstream" biochemical events that transduce receptor occupancy into cellular response. For some receptors, such as ligand-gated ion channels, the relationship between drug occupancy and response can be simple because the ion current produced by a drug is often directly proportional to the number of receptors (ion channels) bound. For other receptors, such as those linked to enzymatic signal transduction cascades, the occupancy-response relationship is often more complex because the biologic response reaches a maximum before full receptor occupancy is achieved.

Many factors can contribute to nonlinear occupancy-response coupling, and often these factors are only partially understood. A useful concept for thinking about this is that of **receptor reserve** or **spare receptors**. Receptors are said to be "spare" for a given pharmacologic response if it is possible to elicit a maximal biologic response at a concentration of agonist that does not result in occupancy of all of the available receptors. Experimentally, spare receptors may be demonstrated by using irreversible antagonists to prevent binding of agonist to a proportion of available receptors and showing that high concentrations of agonist can still produce an undiminished maximal response (Figure 2–2). For example, the same maximal inotropic response of heart muscle to catecholamines can be elicited even when 90% of β adrenoceptors to which they bind are occupied by a quasi-irreversible antagonist. Accordingly, myocardial cells are said to contain a large proportion of spare β adrenoceptors.

What accounts for the phenomenon of spare receptors? In some cases, receptors may be simply *spare in number* relative to

the total number of downstream signaling mediators present in the cell, so that a maximal response occurs without occupancy of all receptors. In other cases, "spareness" of receptors appears to be *temporal*. For example, β-adrenoceptor activation by an agonist promotes binding of guanosine triphosphate (GTP) to a trimeric G protein, producing an activated signaling intermediate whose lifetime may greatly outlast the agonist-receptor interaction (see also the following section on G Proteins & Second Messengers). Here, maximal response is elicited by activation of relatively few receptors because the response initiated by an individual ligand-receptor-binding event persists longer than the binding event itself. Irrespective of the biochemical basis of receptor reserve, the sensitivity of a cell or tissue to a particular concentration of agonist depends not only on the *affinity* of the receptor for binding the agonist (characterized by the $K_d$) but also on the *degree of spareness*—the total number of receptors present compared with the number actually needed to elicit a maximal biologic response.

The concept of spare receptors is very useful clinically because it allows one to think precisely about the effects of drug dosage without having to consider (or even fully understand) biochemical details of the signaling response. The $K_d$ of the agonist-receptor interaction determines what fraction ($B/B_{max}$) of total receptors will be occupied at a given free concentration (C) of agonist regardless of the receptor concentration:

$$\frac{B}{B_{max}} = \frac{C}{C + K_d}$$

Imagine a responding cell with four receptors and four effectors. Here the number of effectors does not limit the maximal response, and the receptors are *not* spare in number. Consequently, an agonist present at a concentration equal to the $K_d$ will occupy 50% of the receptors, and half of the effectors will be activated, producing a half-maximal response (ie, two receptors stimulate two effectors). Now imagine that the number of receptors increases tenfold to 40 receptors but that the total number of effectors remains constant. Most of the receptors are now spare in number. As a result, a much lower concentration of agonist suffices to occupy 2 of the 40 receptors (5% of the receptors), and this same low concentration of agonist is able to elicit a half-maximal response (two of four effectors activated). Thus, it is possible to change the sensitivity of tissues with spare receptors by changing receptor number.

## Competitive & Irreversible Antagonists

Receptor antagonists bind to receptors but do not activate them; the primary action of antagonists is to reduce the effects of agonists (other drugs or endogenous regulatory molecules) that normally activate receptors. While antagonists are traditionally thought to have no functional effect in the absence of an agonist, some antagonists exhibit "inverse agonist" activity (see Chapter 1) because they also reduce receptor activity below basal levels observed in the absence of any agonist at all. Antagonist drugs are further divided into two classes depending on whether or not they act *competitively* or *noncompetitively* relative to an agonist present at the same time.

In the presence of a fixed concentration of agonist, increasing concentrations of a **competitive antagonist** progressively inhibit the agonist response; high antagonist concentrations prevent the response almost completely. Conversely, sufficiently high concentrations of agonist can surmount the effect of a given concentration of the antagonist; that is, the $E_{max}$ for the agonist remains the same for any fixed concentration of antagonist (Figure 2–3A). Because

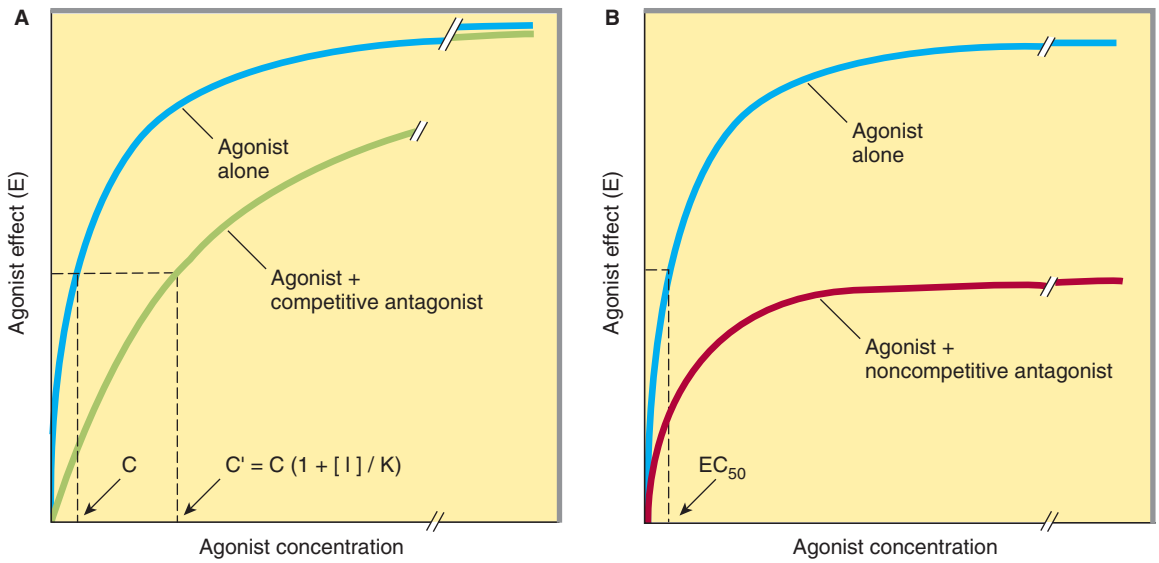


**FIGURE 2–3** Changes in agonist concentration-effect curves produced by a competitive antagonist (**A**) or by an irreversible antagonist (**B**). In the presence of a competitive antagonist, higher concentrations of agonist are required to produce a given effect; thus the agonist concentration (C′) required for a given effect in the presence of concentration [I] of an antagonist is shifted to the right, as shown. High agonist concentrations can overcome inhibition by a competitive antagonist. This is not the case with an irreversible (or noncompetitive) antagonist, which reduces the maximal effect the agonist can achieve, although it may not change its $EC_{50}$.

the antagonism is competitive, the presence of antagonist increases the agonist concentration required for a given degree of response, and so the agonist concentration-effect curve is shifted to the right.

The concentration (C′) of an agonist required to produce a given effect in the presence of a fixed concentration ([I]) of competitive antagonist is greater than the agonist concentration (C) required to produce the same effect in the absence of the antagonist. The ratio of these two agonist concentrations (called the dose ratio) is related to the dissociation constant ($K_i$) of the antagonist by the **Schild equation**:

$$\frac{C'}{C} = 1 + \frac{[I]}{K_i}$$

Pharmacologists often use this relation to determine the $K_i$ of a competitive antagonist. Even without knowledge of the relation between agonist occupancy of the receptor and response, the $K_i$ can be determined simply and accurately. As shown in Figure 2–3, concentration-response curves are obtained in the presence and in the absence of a fixed concentration of competitive antagonist; comparison of the agonist concentrations required to produce identical degrees of pharmacologic effect in the two situations reveals the antagonist's $K_i$. If C′ is twice C, for example, then [I] = $K_i$.

For the clinician, this mathematical relation has two important therapeutic implications:

1. The degree of inhibition produced by a competitive antagonist depends on the concentration of antagonist. The competitive β-adrenoceptor antagonist propranolol provides a useful example. Patients receiving a fixed dose of this drug exhibit a wide range of plasma concentrations, owing to differences among individuals in the clearance of propranolol. As a result, inhibitory effects on physiologic responses to norepinephrine and epinephrine (endogenous adrenergic receptor agonists) may vary widely, and the dose of propranolol must be adjusted accordingly.
2. Clinical response to a competitive antagonist also depends on the concentration of agonist that is competing for binding to receptors. Again, propranolol provides a useful example: When this drug is administered at moderate doses sufficient to block the effect of basal levels of the neurotransmitter norepinephrine, resting heart rate is decreased. However, the increase in the release of norepinephrine and epinephrine that occurs with exercise, postural changes, or emotional stress may suffice to overcome this competitive antagonism. Accordingly, the same dose of propranolol may have little effect under these conditions, thereby altering therapeutic response. Conversely, the same dose of propranolol that is useful for treatment of hypertension in one patient may be excessive and toxic to another, based on differences between the patients in the amount of endogenous norepinephrine and epinephrine that they produce.

The actions of a **noncompetitive antagonist** are different because, once a receptor is bound by such a drug, agonists cannot surmount the inhibitory effect irrespective of their concentration. In many cases, noncompetitive antagonists bind to the receptor in an **irreversible** or nearly irreversible fashion, sometimes by forming a covalent bond with the receptor. After occupancy of some proportion of receptors by such an antagonist, the number of remaining unoccupied receptors may be too low for the agonist (even at high concentrations) to elicit a response comparable to the previous maximal response (Figure 2–3B). If spare receptors are present, however, a lower dose of an irreversible antagonist may leave enough receptors unoccupied to allow achievement of maximum response to agonist, although a higher agonist concentration will be required (Figure 2–2B and C; see Receptor-Effector Coupling & Spare Receptors).

Therapeutically, such irreversible antagonists present distinct advantages and disadvantages. Once the irreversible antagonist has occupied the receptor, it need not be present in unbound form to inhibit agonist responses. Consequently, the duration of action of such an irreversible antagonist is relatively independent of its own rate of elimination and more dependent on the rate of turnover of receptor molecules.

Phenoxybenzamine, an irreversible α-adrenoceptor antagonist, is used to control the hypertension caused by catecholamines released from pheochromocytoma, a tumor of the adrenal medulla. If administration of phenoxybenzamine lowers blood pressure, blockade will be maintained even when the tumor episodically releases very large amounts of catecholamine. In this case, the ability to prevent responses to varying and high concentrations of agonist is a therapeutic advantage. If overdose occurs, however, a real problem may arise. If the α-adrenoceptor blockade cannot be overcome, excess effects of the drug must be antagonized "physiologically," ie, by using a pressor agent that does not act via α adrenoceptors.

Antagonists can function noncompetitively in a different way; that is, by binding to a site on the receptor protein separate from the agonist binding site; in this way, the drug can modify receptor activity without blocking agonist binding (see Chapter 1, Figure 1–2C and D). Although these drugs act noncompetitively, their actions are often reversible. Such drugs are called *negative allosteric modulators* because they act through binding to a different (ie, "allosteric") site on the receptor relative to the classical (ie, "orthosteric") site bound by the agonist and reduce activity of the receptor. Not all allosteric modulators act as antagonists; some potentiate rather than reduce receptor activity. For example, benzodiazepines are considered *positive allosteric modulators* because they bind to an allosteric site on the ion channels activated by the neurotransmitter γ-aminobutyric acid (GABA) and potentiate the net activating effect of GABA on channel conductance. Benzodiazepines have little activating effect on their own, and this property is one reason that benzodiazepines are relatively safe in overdose; even at high doses, their ability to increase ion conductance is limited by the release of endogenous neurotransmitter. Allosteric modulation can also occur at targets lacking a known orthosteric binding site. For example, ivacaftor binds to the **cystic fibrosis transmembrane regulator (CFTR)** ion channel that is mutated in cystic fibrosis. Certain mutations that render the channel hypoactive can be partially rescued by ivacaftor, representing positive allosteric modulation of a channel for which there is no presently known endogenous ligand.

## Partial Agonists

Based on the maximal pharmacologic response that occurs when all receptors are occupied, agonists can be divided into two

classes: **partial agonists** produce a lower response, at full receptor occupancy, than do **full agonists.** Partial agonists produce concentration-effect curves that resemble those observed with full agonists in the presence of an antagonist that irreversibly blocks some of the receptor sites (compare Figures 2–2 [curve D] and 2–4B). It is important to emphasize that the failure of partial agonists to produce a maximal response is not due to decreased affinity for binding to receptors. Indeed, a partial agonist's inability to cause a maximal pharmacologic response, even when present at high concentrations that effectively saturate binding to all receptors, is indicated by the fact that partial agonists competitively inhibit the responses produced by full agonists (Figure 2–4). This mixed "agonist-antagonist" property of partial agonists can have both beneficial and deleterious effects in the clinic. For example, buprenorphine, a partial agonist of μ-opioid receptors, is a generally safer analgesic drug than morphine because it produces less respiratory depression in overdose. However, buprenorphine is effectively antianalgesic when administered in combination with more efficacious opioid drugs, and it may precipitate a drug withdrawal syndrome in opioid-dependent patients.

## Other Mechanisms of Drug Antagonism

Not all mechanisms of antagonism involve interactions of drugs or endogenous ligands at a single type of receptor, and some types of antagonism do not involve a receptor at all. For example, protamine, a protein that is positively charged at physiologic pH, can be used clinically to counteract the effects of heparin, an anticoagulant that is negatively charged. In this case, one drug acts as a **chemical antagonist** of the other simply by ionic binding that makes the other drug unavailable for interactions with proteins involved in blood clotting.

Another type of antagonism is **physiologic antagonism** between endogenous regulatory pathways mediated by different receptors. For example, several catabolic actions of the glucocorticoid hormones lead to increased blood sugar, an effect that is physiologically opposed by insulin. Although glucocorticoids and

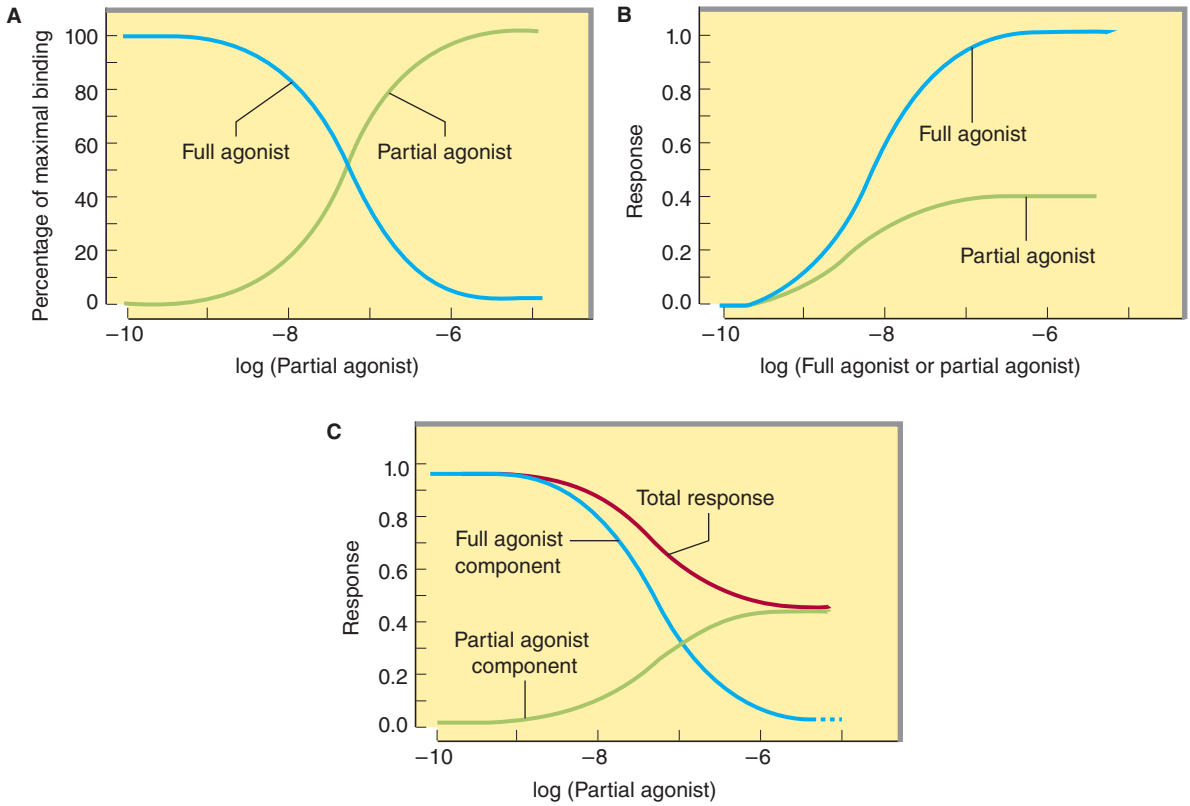


**FIGURE 2–4** **A:** The percentage of receptor occupancy resulting from full agonist (present at a single concentration) binding to receptors in the presence of increasing concentrations of a partial agonist. Because the full agonist (blue line) and the partial agonist (green line) compete to bind to the same receptor sites, when occupancy by the partial agonist increases, binding of the full agonist decreases. **B:** When each of the two drugs is used alone and response is measured, occupancy of all the receptors by the partial agonist produces a lower maximal response than does similar occupancy by the full agonist. **C:** Simultaneous treatment with a single concentration of full agonist and increasing concentrations of the partial agonist produces the response patterns shown in the bottom panel. The fractional response caused by a single high concentration of the full agonist decreases as increasing concentrations of the partial agonist compete to bind to the receptor with increasing success; at the same time, the portion of the response caused by the partial agonist increases, while the total response—ie, the sum of responses to the two drugs (red line)—gradually decreases, eventually reaching the value produced by partial agonist alone (compare with B).

insulin act on quite distinct receptor-effector systems, the clinician must sometimes administer insulin to oppose the hyperglycemic effects of a glucocorticoid hormone, whether the latter is elevated by endogenous synthesis (eg, a tumor of the adrenal cortex) or as a result of glucocorticoid therapy.

In general, use of a drug as a physiologic antagonist produces effects that are less specific and less easy to control than are the effects of a receptor-specific antagonist. Thus, for example, to treat bradycardia caused by increased release of acetylcholine from vagus nerve endings, the physician could use isoproterenol, a β-adrenoceptor agonist that increases heart rate by mimicking sympathetic stimulation of the heart. However, use of this physiologic antagonist would be less rational—and potentially more dangerous—than use of a receptor-specific antagonist such as atropine (a competitive antagonist of acetylcholine receptors that slow heart rate as the direct targets of acetylcholine released from vagus nerve endings).

## SIGNALING MECHANISMS & DRUG ACTION

Until now we have considered receptor interactions and drug effects in terms of equations and concentration-effect curves. We must also understand the molecular mechanisms by which a drug acts. We should also consider different structural families of receptor protein, and this allows us to ask basic questions with important clinical implications:

- Why do some drugs produce effects that persist for minutes, hours, or even days after the drug is no longer present?
- Why do responses to other drugs diminish rapidly with prolonged or repeated administration?
- How do cellular mechanisms for amplifying external chemical signals explain the phenomenon of spare receptors?
- Why do chemically similar drugs often exhibit extraordinary selectivity in their actions?
- Do these mechanisms provide targets for developing new drugs?

Most transmembrane signaling is accomplished by a small number of different molecular mechanisms. Each type of mechanism has been adapted, through the evolution of distinctive protein families, to transduce many different signals. These protein families include receptors on the cell surface and within the cell, as well as enzymes and other components that generate, amplify, coordinate, and terminate postreceptor signaling by chemical second messengers in the cytoplasm. This section first discusses the mechanisms for carrying chemical information across the plasma membrane and then outlines key features of cytoplasmic second messengers.

Five basic mechanisms of transmembrane signaling are well understood (Figure 2–5). Each represents a different family of receptor protein and uses a different strategy to circumvent the barrier posed by the lipid bilayer of the plasma membrane. These strategies use (1) a lipid-soluble ligand that crosses the membrane and acts on an intracellular receptor; (2) a transmembrane receptor protein whose intracellular enzymatic activity is allosterically regulated by a ligand that binds to a site on the protein's extracellular domain; (3) a transmembrane receptor that binds and stimulates an intracellular protein tyrosine kinase; (4) a ligand-gated transmembrane ion channel that can be induced to open or close by the binding of a ligand; or (5) a transmembrane receptor protein that stimulates a GTP-binding signal transducer protein (G protein), which in turn modulates production of an intracellular second messenger.

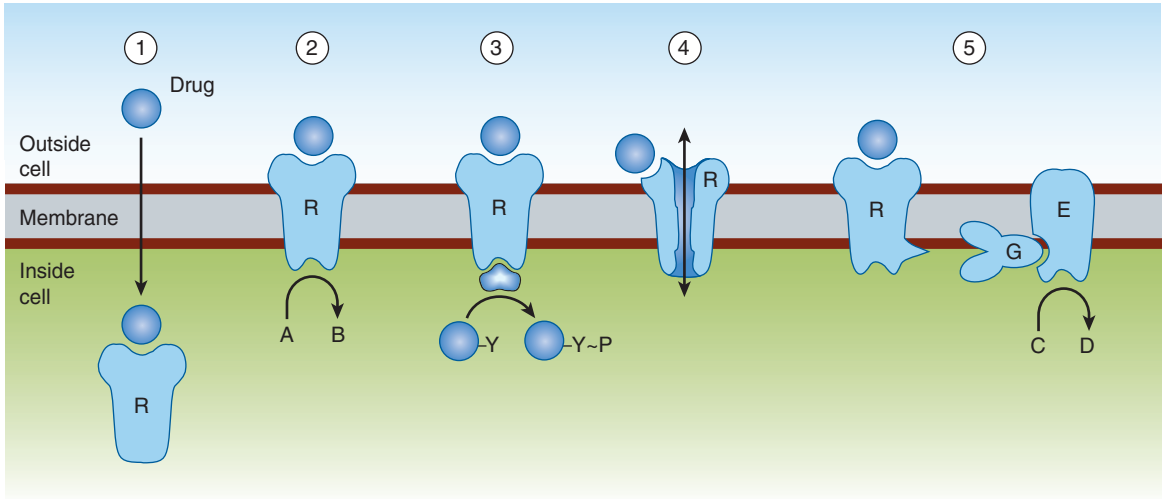


**FIGURE 2–5** Known transmembrane signaling mechanisms: **1:** A lipid-soluble chemical signal crosses the plasma membrane and acts on an intracellular receptor (which may be an enzyme or a regulator of gene transcription); **2:** the signal binds to the extracellular domain of a transmembrane protein, thereby activating an enzymatic activity of its cytoplasmic domain; **3:** the signal binds to the extracellular domain of a transmembrane receptor bound to a separate protein tyrosine kinase, which it activates; **4:** the signal binds to and directly regulates the opening of an ion channel; **5:** the signal binds to a cell-surface receptor linked to an effector enzyme by a G protein. (A, C, substrates; B, D, products; R, receptor; G, G protein; E, effector [enzyme or ion channel]; Y, tyrosine; P, phosphate.)

Although the five established mechanisms do not account for all the chemical signals conveyed across cell membranes, they do transduce many of the most important signals exploited in pharmacotherapy.

## Intracellular Receptors for Lipid-Soluble Agents

Several biologic ligands are sufficiently lipid-soluble to cross the plasma membrane and act on intracellular receptors. One class of such ligands includes steroids (corticosteroids, mineralocorticoids, sex steroids, vitamin D) and thyroid hormone, whose receptors stimulate the transcription of genes by binding to specific DNA sequences (often called **response elements**) near the gene whose expression is to be regulated.

These "gene-active" receptors belong to a protein family that evolved from a common precursor. Dissection of the receptors by recombinant DNA techniques has provided insights into their molecular mechanism. For example, binding of glucocorticoid hormone to its normal receptor protein relieves an inhibitory constraint on the transcription-stimulating activity of the protein. Figure 2–6 schematically depicts the molecular mechanism of glucocorticoid action: In the absence of hormone, the receptor is bound to hsp90, a protein that prevents normal folding of several structural domains of the receptor. Binding of hormone to the ligand-binding domain triggers release of hsp90. This allows the DNA-binding and transcription-activating domains of the receptor to fold into their functionally active conformations, so that the activated receptor can initiate transcription of target genes.

The mechanism used by hormones that act by regulating gene expression has two therapeutically important consequences:

1. All of these hormones produce their effects after a characteristic lag period of 30 minutes to several hours—the time required for the synthesis of new proteins. This means that the gene-active hormones cannot be expected to alter a pathologic state within minutes (eg, glucocorticoids will not immediately relieve the symptoms of bronchial asthma).
2. The effects of these agents can persist for hours or days after the agonist concentration has been reduced to zero. The persistence of effect is primarily due to the relatively slow turnover of most enzymes and proteins, which can remain active in cells for hours or days after they have been synthesized. Consequently, it means that the beneficial (or toxic) effects of a gene-active hormone usually decrease slowly when administration of the hormone is stopped.

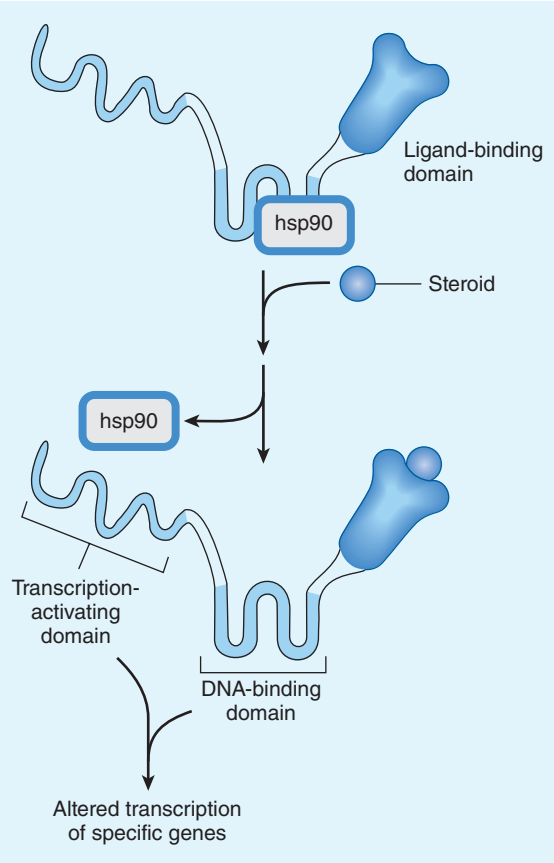


**FIGURE 2–6** Mechanism of glucocorticoid action. The glucocorticoid receptor polypeptide is schematically depicted as a protein with three distinct domains. A heat-shock protein, hsp90, binds to the receptor in the absence of hormone and prevents folding into the active conformation of the receptor. Binding of a hormone ligand (steroid) causes dissociation of the hsp90 stabilizer and permits conversion to the active configuration.

## Ligand-Regulated Transmembrane Enzymes Including Receptor Tyrosine Kinases

This class of receptor molecules mediates the first steps in signaling by insulin, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), atrial natriuretic peptide (ANP), transforming growth factor-β (TGF-β), and many other trophic hormones. These receptors are polypeptides consisting of an extracellular hormone-binding domain and a cytoplasmic enzyme domain, which may be a protein tyrosine kinase, a serine kinase, or a guanylyl cyclase (Figure 2–7). In all these receptors, the two domains are connected by a hydrophobic segment of the polypeptide that resides in the lipid bilayer of the plasma membrane.

The receptor tyrosine kinase signaling function begins with binding of ligand, typically a polypeptide hormone or growth factor, to the receptor's extracellular domain. The resulting change in receptor conformation causes two receptor molecules to bind to one another (*dimerize*). This activates the tyrosine kinase enzyme activity present in the cytoplasmic domain of the dimer, leading to phosphorylation of the receptor as well as additional downstream signaling proteins. Activated receptors catalyze phosphorylation of tyrosine residues on different target signaling proteins, thereby allowing a single type of activated receptor to modulate a number of biochemical processes. (Some receptor tyrosine kinases form oligomeric complexes larger than dimers upon activation by ligand, but the pharmacologic significance of such higher-order complexes is presently unclear.)

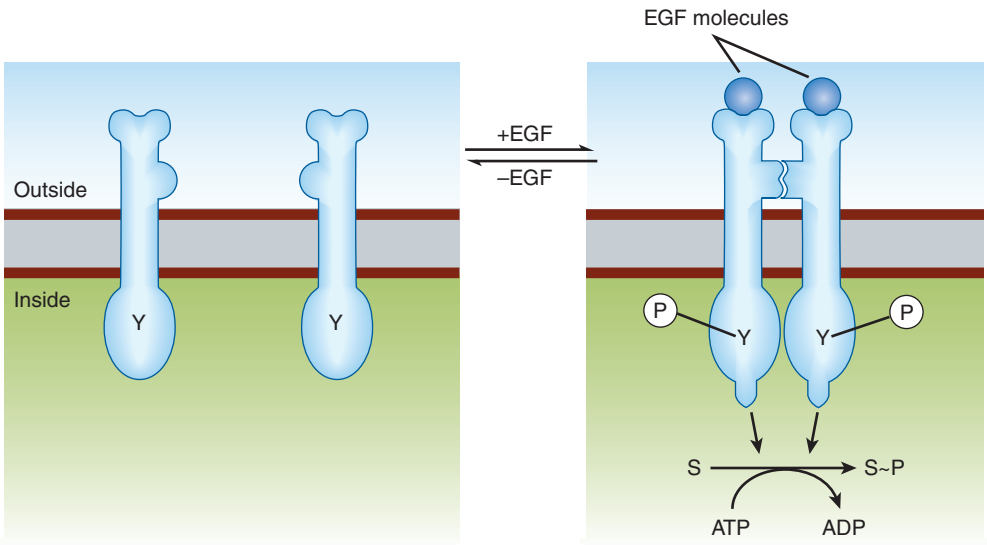


**FIGURE 2–7** Mechanism of activation of the epidermal growth factor (EGF) receptor, a representative receptor tyrosine kinase. The receptor polypeptide has extracellular and cytoplasmic domains, depicted above and below the plasma membrane. Upon binding of EGF (circle), the receptor converts from its inactive monomeric state (*left*) to an active dimeric state (*right*), in which two receptor polypeptides bind noncovalently. The cytoplasmic domains become phosphorylated (P) on specific tyrosine residues (Y), and their enzymatic activities are activated, catalyzing phosphorylation of substrate proteins (S).

Insulin, for example, uses a single class of tyrosine kinase receptors to trigger increased uptake of glucose and amino acids and to regulate metabolism of glycogen and triglycerides in the cell. Activation of the receptor in specific target cells drives a complex program of cellular events ranging from altered membrane transport of ions and metabolites to changes in the expression of many genes.

Inhibitors of particular receptor tyrosine kinases are finding increased use in neoplastic disorders in which excessive growth factor signaling is often involved. Some of these inhibitors are monoclonal antibodies (eg, trastuzumab, cetuximab), which bind to the extracellular domain of a particular receptor and interfere with binding of growth factor. Other inhibitors are membrane-permeant small molecule chemicals (eg, gefitinib, erlotinib), which inhibit the receptor's kinase activity in the cytoplasm.

The intensity and duration of action of EGF, PDGF, and other agents that act via receptor tyrosine kinases are often limited by a process called receptor **down-regulation.** Ligand binding often induces accelerated endocytosis of receptors from the cell surface, followed by the degradation of those receptors (and their bound ligands). When this process occurs at a rate faster than de novo synthesis of receptors, the total number of cell-surface receptors is reduced (down-regulated), and the cell's responsiveness to ligand is correspondingly diminished. A well-understood example is the EGF receptor tyrosine kinase, which internalizes from the plasma membrane at a greatly accelerated rate after activation by EGF and then is delivered to lysosomes and proteolyzed. This down-regulation process is essential physiologically to limit the strength and duration of the growth factor signal; genetic mutations that interfere with the down-regulation process cause excessive and prolonged responses that underlie or contribute to many forms of cancer. Endocytosis of other receptor tyrosine kinases, most notably receptors for nerve growth factor, serves a very different function. Internalized nerve growth factor receptors are not rapidly degraded but are translocated in endocytic vesicles from the distal axon, where receptors are activated by nerve growth factor released from the innervated tissue, to the cell body. In the cell body, the growth factor signal is transduced to transcription factors regulating the expression of genes controlling cell survival. This process, effectively opposite to down-regulation, transports a critical survival signal from its site of agonist release to the site of a critical downstream signaling effect and can do so over a remarkably long distance—up to a meter in some neurons.

A number of regulators of growth and differentiation, including TGF-β, act on another class of transmembrane receptor enzymes that phosphorylate serine and threonine residues. Atrial natriuretic peptide (ANP), an important regulator of blood volume and vascular tone, acts on a transmembrane receptor whose intracellular domain, a guanylyl cyclase, generates cGMP (see below). Receptors in both groups, like the receptor tyrosine kinases, are active in their dimeric forms.

## Cytokine Receptors

Cytokine receptors respond to a heterogeneous group of peptide ligands, which include growth hormone, erythropoietin, several kinds of interferon, and other regulators of growth and differentiation. These receptors use a mechanism (Figure 2–8) closely resembling that of receptor tyrosine kinases, except that in this case, the protein tyrosine kinase activity is not intrinsic to the receptor molecule. Instead, a separate protein tyrosine kinase, from the Janus-kinase (JAK) family, binds noncovalently to the receptor. As in the case of the EGF receptor, cytokine receptors

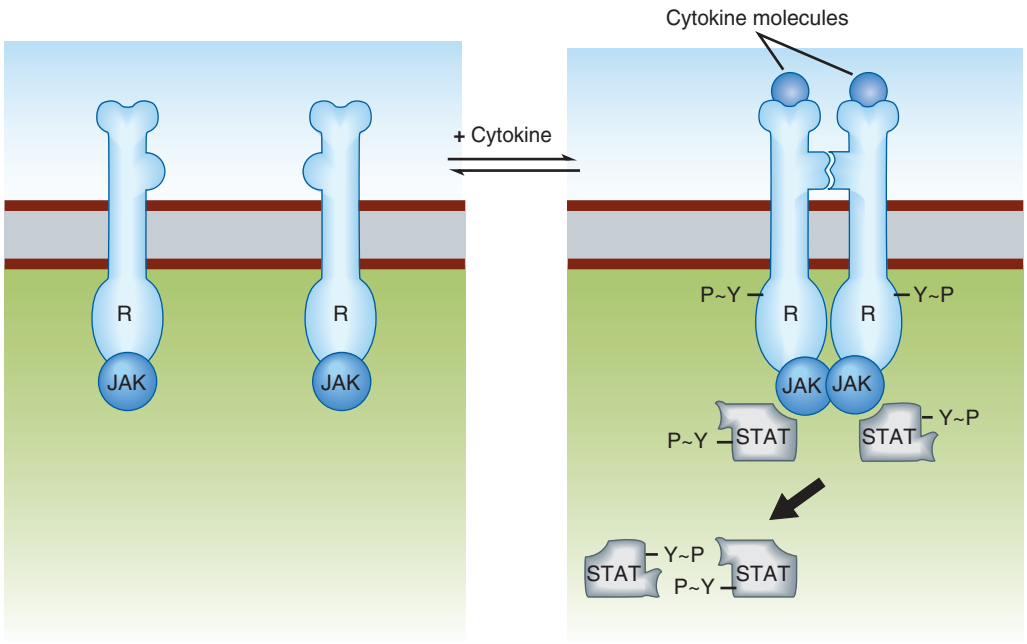


**FIGURE 2–8** Cytokine receptors, like receptor tyrosine kinases, have extracellular and intracellular domains and form dimers. However, after activation by an appropriate ligand, separate mobile protein tyrosine kinase molecules (JAK) are activated, resulting in phosphorylation of signal transducers and activation of transcription (STAT) molecules. STAT dimers then travel to the nucleus, where they regulate transcription.

dimerize after they bind the activating ligand, allowing the bound JAKs to become activated and to phosphorylate tyrosine residues on the receptor. Phosphorylated tyrosine residues on the receptor's cytoplasmic surface then set in motion a complex signaling dance by binding another set of proteins, called STATs (signal transducers and activators of transcription). The bound STATs are themselves phosphorylated by the JAKs, two STAT molecules dimerize (attaching to one another's tyrosine phosphates), and finally the STAT/STAT dimer dissociates from the receptor and travels to the nucleus, where it regulates transcription of specific genes.

## Ion Channels

Many of the most useful drugs in clinical medicine act on ion channels. For ligand-gated ion channels, drugs often mimic or block the actions of natural agonists. Natural ligands of such receptors include acetylcholine, serotonin, GABA, and glutamate; all are synaptic transmitters.

Each of their receptors transmits its signal across the plasma membrane by increasing transmembrane conductance of the relevant ion and thereby altering the electrical potential across the membrane. For example, acetylcholine causes the opening of the ion channel in the nicotinic acetylcholine receptor (nAChR), which allows $Na^+$ to flow down its concentration gradient into cells, producing a localized excitatory postsynaptic potential—a depolarization.

The nAChR is one of the best characterized of all cell-surface receptors for hormones or neurotransmitters (Figure 2–9). One form of this receptor is a pentamer made up of four different polypeptide subunits (eg, two α chains plus one β, one γ, and one δ chain, all with molecular weights ranging from 43,000–50,000). These polypeptides, each of which crosses the lipid bilayer four times, form a cylindrical structure that is approximately 10 nm in diameter but is impermeable to ions. When acetylcholine binds to sites on the α subunits, a conformational change occurs that

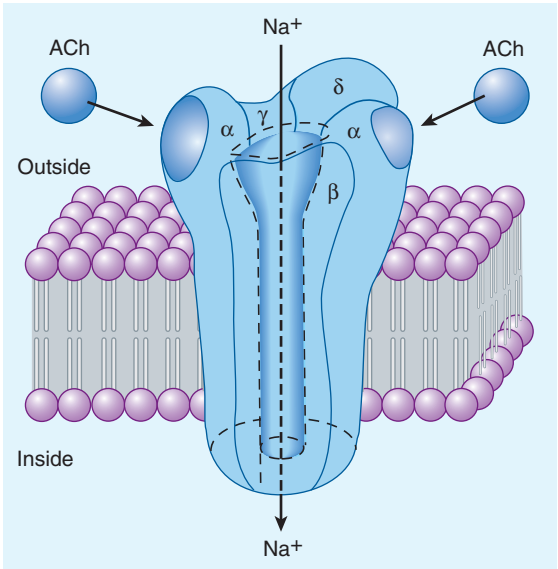


**FIGURE 2–9** The nicotinic acetylcholine (ACh) receptor, a ligand-gated ion channel. The receptor molecule is depicted as embedded in a rectangular piece of plasma membrane, with extracellular fluid above and cytoplasm below. Composed of five subunits (two α, one β, one γ, and one δ), the receptor opens a central transmembrane ion channel when ACh binds to sites on the extracellular domain of its α subunits.

results in the transient opening of a central aqueous channel, approximately 0.5 nm in diameter, through which sodium ions penetrate from the extracellular fluid to cause electrical depolarization of the cell. The structural basis for activating other ligand-gated ion channels has been determined recently, and similar general principles apply, but there are differences in key details that may open new opportunities for drug action. For example, receptors that mediate excitatory neurotransmission at central nervous system synapses bind glutamate, a major excitatory neurotransmitter, through a large appendage domain that protrudes from the receptor and has been called a "flytrap" because it physically closes around the glutamate molecule; the glutamate-loaded flytrap domain then moves as a unit to control pore opening. Drugs can regulate the activity of such glutamate receptors by binding to the flytrap domain, to surfaces on the membrane-embedded portion around the pore, or within the pore itself.

The time elapsed between the binding of the agonist to a ligand-gated channel and the cellular response can often be measured in milliseconds. The rapidity of this signaling mechanism is crucially important for moment-to-moment transfer of information across synapses. Ligand-gated ion channels can be regulated by multiple mechanisms, including phosphorylation and endocytosis. In the central nervous system, these mechanisms contribute to synaptic plasticity involved in learning and memory.

Voltage-gated ion channels do not bind neurotransmitters directly but are controlled by membrane potential; such channels are also important drug targets. Drugs that regulate voltage-gated channels typically bind to a site of the receptor different from the charged amino acids that constitute the "voltage sensor" domain of the protein used for channel opening by membrane potential. For example, verapamil binds to a region in the pore of voltage-gated calcium channels that are present in the heart and in vascular smooth muscle, inhibiting the ion conductance separately from the voltage sensor, producing antiarrhythmic effects, and reducing blood pressure without mimicking or antagonizing any known endogenous transmitter. Other channels, such as the CFTR, although not strongly sensitive to either a known natural ligand or voltage, are still important drug targets. Lumacaftor binds CFTR and promotes its delivery to the plasma membrane after biosynthesis. Ivacaftor binds to a different site and enhances channel conductance. Both drugs act as allosteric modulators of the CFTR and were recently approved for treatment of cystic fibrosis, but each has a different effect.

## G Proteins & Second Messengers

Many extracellular ligands act by increasing the intracellular concentrations of second messengers such as **cyclic adenosine-3′,5′-monophosphate (cAMP), calcium ion,** or the **phosphoinositides** (described below). In most cases, they use a transmembrane signaling system with three separate components. First, the extracellular ligand is selectively detected by a cell-surface receptor. The receptor in turn triggers the activation of a GTP-binding protein **(G protein)** located on the cytoplasmic face of the plasma membrane. The activated G protein then changes the activity of an effector element, usually an enzyme or ion channel. This element then changes the concentration of the intracellular second messenger. For cAMP, the effector enzyme is adenylyl cyclase, a membrane protein that converts intracellular adenosine triphosphate (ATP) to cAMP. The corresponding G protein, $G_s$, stimulates adenylyl cyclase after being activated by hormones and neurotransmitters that act via specific $G_s$-coupled receptors. There are many examples of such receptors, including α and β adrenoceptors, glucagon receptors, thyrotropin receptors, and certain subtypes of dopamine and serotonin receptors.

$G_s$ and other G proteins activate their downstream effectors when bound by GTP and also have the ability to hydrolyze GTP (Figure 2–10); this hydrolysis reaction inactivates the G protein but can occur at a relatively slow rate, effectively amplifying the transduced signal by allowing the activated (GTP-bound) G protein to have a longer lifetime in the cell than the activated receptor itself. For example, a neurotransmitter such as norepinephrine may encounter its membrane receptor for only a few milliseconds. When the encounter generates a GTP-bound $G_s$ molecule, however, the duration of activation of adenylyl cyclase depends on the longevity of GTP binding to $G_s$ rather than on the duration of norepinephrine's binding to the receptor. Indeed, like other G proteins, GTP-bound $G_s$ may remain active for tens of seconds, enormously amplifying the original signal. This mechanism also helps explain how signaling by G proteins produces the phenomenon of spare receptors. The family of G proteins contains several functionally diverse subfamilies (Table 2–1), each of which mediates effects of a particular set of receptors to a distinctive group of effectors. Note that an endogenous ligand (eg, norepinephrine, acetylcholine, serotonin, many others not listed in Table 2–1) may bind and stimulate receptors that couple to different subsets

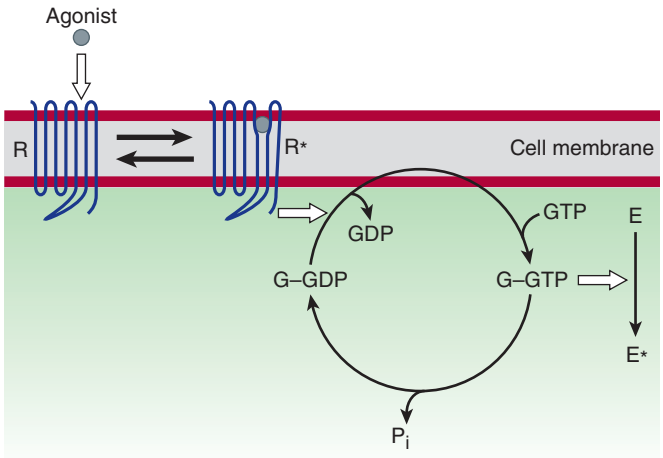


**FIGURE 2–10** The guanine nucleotide-dependent activation-inactivation cycle of G proteins. The agonist activates the receptor (R→R*), which promotes release of GDP from the G protein (G), allowing entry of GTP into the nucleotide binding site. In its GTP-bound state (G-GTP), the G protein regulates activity of an effector enzyme or ion channel (E→E*). The signal is terminated by hydrolysis of GTP, followed by return of the system to the basal unstimulated state. Open arrows denote regulatory effects. ($P_i$, inorganic phosphate.)

**TABLE 2–1 G proteins and their receptors and effectors.**

| G Protein | Receptors for | Effector/Signaling Pathway |
|---|---|---|
| $G_s$ | β-Adrenergic amines, histamine, serotonin, glucagon, and many other hormones | ↑ Adenylyl cyclase →↑ cAMP |
| $G_{i1}$, $G_{i2}$, $G_{i3}$ | $\alpha_2$-Adrenergic amines, acetylcholine (muscarinic), opioids, serotonin, and many others | Several, including:<br>↓ Adenylyl cyclase →↓ cAMP<br>Open cardiac $K^+$ channels →↓ heart rate |
| $G_{olf}$ | Odorants (olfactory epithelium) | ↑ Adenylyl cyclase →↑ cAMP |
| $G_o$ | Neurotransmitters in brain (not yet specifically identified) | Not yet clear |
| $G_q$ | Acetylcholine (muscarinic), bombesin, serotonin (5-$HT_2$), and many others | ↑ Phospholipase C →↑ $IP_3$, diacylglycerol, cytoplasmic $Ca^{2+}$ |
| $G_{t1}$, $G_{t2}$ | Photons (rhodopsin and color opsins in retinal rod and cone cells) | ↑ cGMP phosphodiesterase →↓ cGMP (phototransduction) |

cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; IP3, inositol-1,4,5-trisphosphate.

of G proteins. The apparent promiscuity of such a ligand allows it to elicit different G protein-dependent responses in different cells. For instance, the body responds to danger by using catecholamines (norepinephrine and epinephrine) both to increase heart rate and to induce constriction of blood vessels in the skin, by acting on $G_s$-coupled β adrenoceptors and $G_q$-coupled $\alpha_1$ adrenoceptors, respectively. Ligand promiscuity also offers opportunities in drug development (see Receptor Classes & Drug Development in the following text).

Receptors that signal via G proteins are often called "G protein-coupled receptors" **(GPCRs)**. GPCRs make up the largest receptor family and are also called "seven-transmembrane" (7TM) or "serpentine" receptors because the receptor polypeptide chain "snakes" across the plasma membrane seven times (Figure 2–11). Receptors for adrenergic amines, serotonin, acetylcholine (muscarinic but not nicotinic), many peptide hormones, odorants, and even visual receptors (in retinal rod and cone cells) all belong to the GPCR family. All were derived from a common evolutionary precursor. A few GPCRs (eg, $GABA_B$ and metabotropic glutamate receptors) require stable assembly into *homodimers* (complexes of two identical receptor polypeptides) or *heterodimers* (complexes of different isoforms) for functional activity. However, in contrast to tyrosine kinase and cytokine receptors, dimerization is not universally required for GPCR activation, and many GPCRs are thought to function as monomers.

GPCRs can bind agonists in a variety of ways, but they all appear to transduce signals across the plasma membrane in a similar way. Agonist binding (eg, a catecholamine or acetylcholine) stabilizes a conformational state of the receptor in which the cytoplasmic ends of the transmembrane helices spread apart by about 1 nm, opening a cavity in the receptor's cytoplasmic surface that binds a critical regulatory surface of the G protein. This reduces nucleotide affinity for the G protein, allowing GDP to dissociate and GTP to replace it (this occurs because GTP is normally present in the cytoplasm at much higher concentration than GDP). The GTP-bound form of G protein then dissociates from the receptor and can engage downstream mediators. Thus GPCR–G protein coupling involves coordinated conformational change in both proteins, allowing agonist binding to the receptor to effectively "drive" a nucleotide exchange reaction that "switches" the G protein from its inactive (GDP-bound) to active (GTP-bound) form. Figure 2–11 shows the main components schematically.

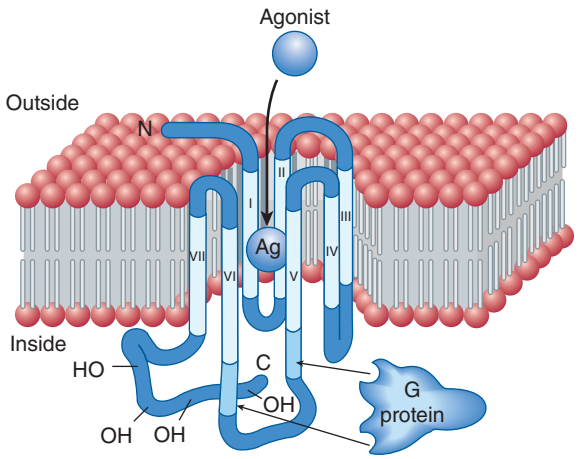


**FIGURE 2–11** Transmembrane topology of a typical "serpentine" GPCR. The receptor's amino (N) terminal is extracellular (above the plane of the membrane), and its carboxyl (C) terminal intracellular, with the polypeptide chain "snaking" across the membrane seven times. The hydrophobic transmembrane segments (light color) are designated by Roman numerals (I–VII). Agonist (Ag) approaches the receptor from the extracellular fluid and binds to a site surrounded by the transmembrane regions of the receptor protein. G protein interacts with cytoplasmic regions of the receptor, especially around the third cytoplasmic loop connecting transmembrane regions V and VI. Lateral movement of these helices during activation exposes an otherwise buried cytoplasmic surface of the receptor that promotes guanine nucleotide exchange on the G protein and thereby activates the G protein, as discussed in the text. The receptor's cytoplasmic terminal tail contains numerous serine and threonine residues whose hydroxyl (-OH) groups can be phosphorylated. This phosphorylation is associated with diminished receptor-G protein coupling and can promote receptor endocytosis.

Many high-resolution structures of GPCRs are available from the Protein Data Bank (www.rcsb.org). An animated model depicting the conformational change associated with activation is available from the Protein Data Bank in Europe (http://www.ebi.ac.uk/pdbe/quips?story=B2AR).

## Receptor Regulation

G protein-mediated responses to drugs and hormonal agonists often attenuate with time (Figure 2–12A). After reaching an initial high level, the response (eg, cellular cAMP accumulation, $Na^+$ influx, contractility, etc) diminishes over seconds or minutes, even in the continued presence of the agonist. In some cases, this **desensitization** phenomenon is rapidly reversible; a second exposure to agonist, if provided a few minutes after termination of the first exposure, results in a response similar to the initial response.

Multiple mechanisms contribute to desensitization of GPCRs. One well-understood mechanism involves phosphorylation of the receptor. The agonist-induced change in conformation of the β-adrenoceptor causes it not only to activate G protein, but also to recruit and activate a family of protein kinases called G protein-coupled receptor kinases (GRKs). GRKs phosphorylate serine and threonine residues in the receptor's cytoplasmic tail (Figure 2–12B), diminishing the ability of activated β adrenoceptors to activate $G_s$ and also increasing the receptor's affinity for binding a third protein, β-arrestin. Binding of β-arrestin to the receptor further diminishes the receptor's ability to interact with $G_s$, attenuating the cellular response (ie, stimulation of adenylyl cyclase as discussed below). Upon removal of agonist, phosphorylation by the GRK is terminated, β-arrestin can dissociate, and cellular phosphatases remove the phosphorylations, reversing the desensitized state and allowing activation to occur again upon another encounter with agonist.

For β adrenoceptors, and for many other GPCRs, β-arrestin can produce other effects. One effect is to accelerate endocytosis of β adrenoceptors from the plasma membrane. This can down-regulate β adrenoceptors if receptors subsequently travel to lysosomes, similar to down-regulation of EGF receptors, but it can also help reverse the desensitized state for those receptors returned to the plasma membrane by exposing receptors to phosphatase enzymes in endosomes (Figure 2–12B). In some cases, β-arrestin can itself act as a positive signal transducer, analogous to G proteins but through a different mechanism, by serving as a molecular scaffold to bind other signaling proteins (rather than through binding GTP). In this way, β-arrestin can confer on GPCRs a great deal of flexibility in signaling and regulation. This flexibility is still poorly understood but is presently thought to underlie the ability of some drugs to produce a different spectrum of downstream effects from other drugs, despite binding to the same GPCR. Current drug development efforts are exploring the potential of this phenomenon, called **functional selectivity** or **agonist bias**, as a means to achieve specificity in drug action beyond that presently possible using conventional agonists and antagonists. Functionally selective agonists are thought to occupy the orthosteric ligand-binding site, making their binding competitive with conventional orthosteric agonists, but differ from conventional agonists in effects on receptor conformation after binding. Allosteric ligands may also stabilize different conformational states of the receptor, but differ from functionally selective ligands by binding noncompetitively to a different site.

## Well-Established Second Messengers

### A. Cyclic Adenosine Monophosphate (cAMP)

Acting as an intracellular second messenger, cAMP mediates such hormonal responses as the mobilization of stored energy (the breakdown of carbohydrates in liver or triglycerides in fat cells stimulated by β-adrenomimetic catecholamines), conservation of water by the kidney (mediated by vasopressin), $Ca^{2+}$ homeostasis (regulated by parathyroid hormone), and increased rate and contractile force of heart muscle (β-adrenomimetic catecholamines). It also regulates the production of adrenal and sex steroids (in response to corticotropin or follicle-stimulating hormone), relaxation of smooth muscle, and many other endocrine and neural processes.

cAMP exerts most of its effects by stimulating cAMP-dependent protein kinases (Figure 2–13). These kinases are composed of a cAMP-binding regulatory (R) dimer and two catalytic (C) chains. When cAMP binds to the R dimer, active C chains are released to diffuse through the cytoplasm and nucleus, where they transfer phosphate from ATP to appropriate substrate proteins, often enzymes. The specificity of the regulatory effects of cAMP resides in the distinct protein substrates of the kinases that are expressed in different cells. For example, the liver is rich in phosphorylase kinase and glycogen synthase, enzymes whose reciprocal regulation by cAMP-dependent phosphorylation governs carbohydrate storage and release.

When the hormonal stimulus stops, the intracellular actions of cAMP are terminated by an elaborate series of enzymes. cAMP-stimulated phosphorylation of enzyme substrates is rapidly reversed by a diverse group of specific and nonspecific phosphatases. cAMP itself is degraded to 5′-AMP by several cyclic nucleotide phosphodiesterases (PDEs; Figure 2–13). Milrinone, a selective inhibitor of type 3 phosphodiesterases that are expressed in cardiac muscle cells, has been used as an adjunctive agent in treating acute heart failure. Competitive inhibition of cAMP degradation is one way that caffeine, theophylline, and other methylxanthines produce their effects (see Chapter 20).

### B. Phosphoinositides and Calcium

Another well-studied second messenger system involves hormonal stimulation of phosphoinositide hydrolysis (Figure 2–14). Some of the hormones, neurotransmitters, and growth factors that trigger this pathway bind to receptors linked to G proteins, whereas others bind to receptor tyrosine kinases. In all cases, the crucial step is stimulation of a membrane enzyme, phospholipase C (PLC), which splits a minor phospholipid component of the plasma membrane, phosphatidylinositol-4,5-bisphosphate ($PIP_2$), into two second messengers, **diacylglycerol (DAG)** and **inositol-1,4,5-trisphosphate** (**$IP_3$** or **$InsP_3$**). Diacylglycerol is confined to the membrane, where it activates a phospholipid- and

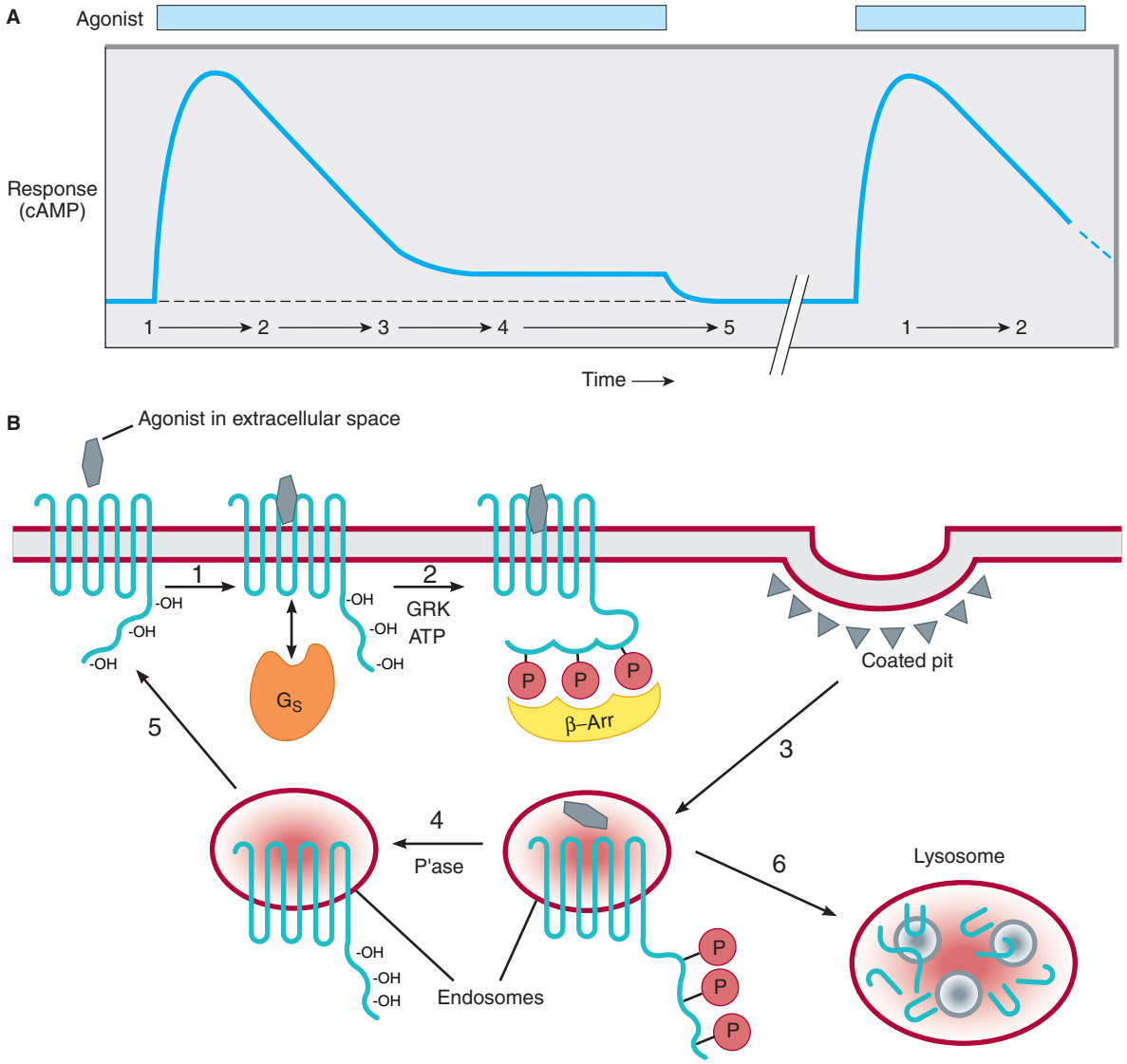


**FIGURE 2–12** Rapid desensitization, resensitization, and down-regulation of β adrenoceptors. **A:** Response to a β-adrenoceptor agonist (ordinate) versus time (abscissa). (Numbers refer to the phases of receptor function in B.) Exposure of cells to agonist (indicated by the light-colored bar) produces a cyclic AMP (cAMP) response. A reduced cAMP response is observed in the continued presence of agonist; this "desensitization" typically occurs within a few minutes. If agonist is removed after a short time (typically several to tens of minutes, indicated by broken line on abscissa), cells recover full responsiveness to a subsequent addition of agonist (second light-colored bar). This "resensitization" fails to occur, or occurs incompletely, if cells are exposed to agonist repeatedly or over a more prolonged time period. **B:** Agonist binding to receptors initiates signaling by promoting receptor interaction with G proteins ($G_s$) located in the cytoplasm (step 1 in the diagram). Agonist-activated receptors are phosphorylated by a G protein-coupled receptor kinase (GRK), preventing receptor interaction with $G_s$ and promoting binding of a different protein, β-arrestin (β-Arr), to the receptor (step 2). The receptor-arrestin complex binds to coated pits, promoting receptor internalization (step 3). Dissociation of agonist from internalized receptors reduces β-Arr binding affinity, allowing dephosphorylation of receptors by a phosphatase (P'ase, step 4) and return of receptors to the plasma membrane (step 5); together, these events result in the efficient resensitization of cellular responsiveness. Repeated or prolonged exposure of cells to agonist favors the delivery of internalized receptors to lysosomes (step 6), promoting receptor down-regulation rather than resensitization.

calcium-sensitive protein kinase called protein kinase C. $IP_3$ is water-soluble and diffuses through the cytoplasm to trigger release of $Ca^{2+}$ by binding to ligand-gated calcium channels in the limiting membranes of internal storage vesicles. Elevated cytoplasmic $Ca^{2+}$ concentration resulting from $IP_3$-promoted opening of these channels promotes the binding of $Ca^{2+}$ to the calcium-binding protein calmodulin, which regulates activities of other enzymes, including calcium-dependent protein kinases.

With its multiple second messengers and protein kinases, the phosphoinositide signaling pathway is much more complex than

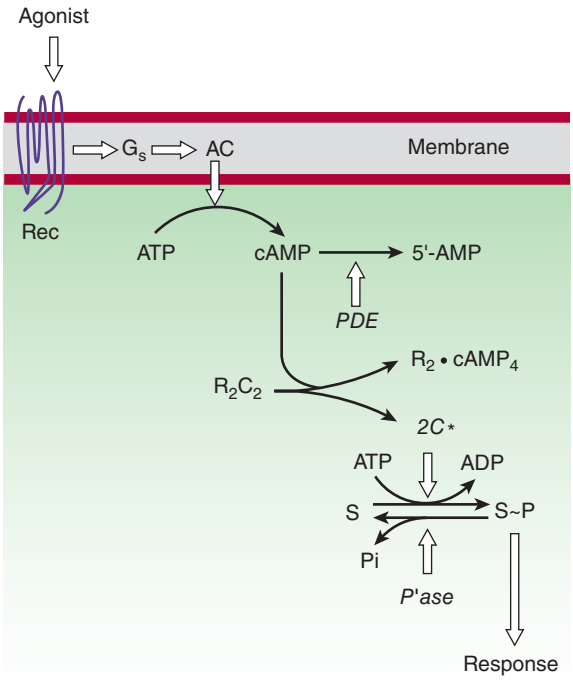


**FIGURE 2–13** The cAMP second messenger pathway. Key proteins include hormone receptors (Rec), a stimulatory G protein ($G_s$), catalytic adenylyl cyclase (AC), phosphodiesterases (PDE) that hydrolyze cAMP, cAMP-dependent kinases, with regulatory (R) and catalytic (C) subunits, protein substrates (S) of the kinases, and phosphatases (P'ase), which remove phosphates from substrate proteins. Open arrows denote regulatory effects.

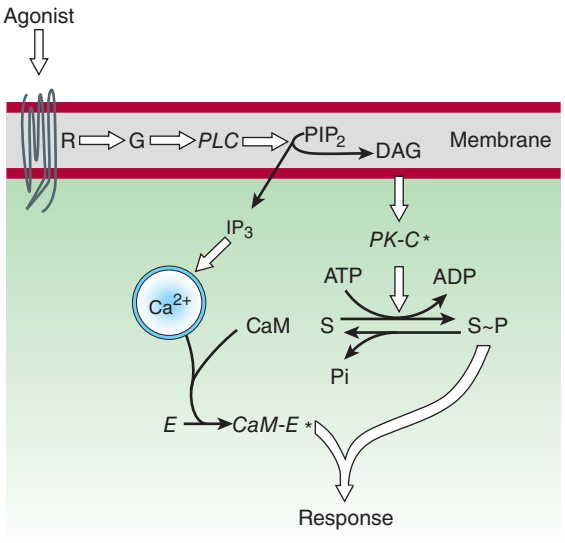


**FIGURE 2–14** The $Ca^{2+}$-phosphoinositide signaling pathway. Key proteins include hormone receptors (R), a G protein (G), a phosphoinositide-specific phospholipase C (PLC), protein kinase C substrates of the kinase (S), calmodulin (CaM), and calmodulin-binding enzymes (E), including kinases, phosphodiesterases, etc. ($PIP_2$, phosphatidylinositol-4,5-bisphosphate; DAG, diacylglycerol; $IP_3$, inositol trisphosphate. Asterisk denotes activated state. Open arrows denote regulatory effects.)

the cAMP pathway. For example, different cell types may contain one or more specialized calcium- and calmodulin-dependent kinases with limited substrate specificity (eg, myosin light-chain kinase) in addition to a general calcium- and calmodulin-dependent kinase that can phosphorylate a wide variety of protein substrates. Furthermore, at least nine structurally distinct types of protein kinase C have been identified.

As in the cAMP system, multiple mechanisms damp or terminate signaling by this pathway. $IP_3$ is inactivated by dephosphorylation; diacylglycerol is either phosphorylated to yield phosphatidic acid, which is then converted back into phospholipids, or it is deacylated to yield arachidonic acid; $Ca^{2+}$ is actively removed from the cytoplasm by $Ca^{2+}$ pumps.

These and other nonreceptor elements of the calcium-phosphoinositide signaling pathway are of considerable importance in pharmacotherapy. For example, lithium ion, used in treatment of bipolar (manic-depressive) disorder, affects the cellular metabolism of phosphoinositides (see Chapter 29).

### C. Cyclic Guanosine Monophosphate (cGMP)

Unlike cAMP, the ubiquitous and versatile carrier of diverse messages, cGMP has established signaling roles in only a few cell types. In intestinal mucosa and vascular smooth muscle, the cGMP-based signal transduction mechanism closely parallels the cAMP-mediated signaling mechanism. Ligands detected by cell-surface receptors stimulate membrane-bound guanylyl cyclase to produce cGMP, and cGMP acts by stimulating a cGMP-dependent protein kinase. The actions of cGMP in these cells are terminated by enzymatic degradation of the cyclic nucleotide and by dephosphorylation of kinase substrates.

Increased cGMP concentration causes relaxation of vascular smooth muscle by a kinase-mediated mechanism that results in dephosphorylation of myosin light chains (see Figure 12–2). In these smooth muscle cells, cGMP synthesis can be elevated by two transmembrane signaling mechanisms utilizing two different guanylyl cyclases. Atrial natriuretic peptide, a blood-borne peptide hormone, stimulates a transmembrane receptor by binding to its extracellular domain, thereby activating the guanylyl cyclase activity that resides in the receptor's intracellular domain. The other mechanism mediates responses to nitric oxide (NO; see Chapter 19), which is generated in vascular endothelial cells in response to natural vasodilator agents such as acetylcholine and histamine. After entering the target cell, nitric oxide binds to and activates a cytoplasmic guanylyl cyclase (see Figure 19–2). A number of useful vasodilating drugs, such as nitroglycerin and sodium nitroprusside used in treating cardiac ischemia and acute hypertension, act by generating or mimicking nitric oxide. Other drugs produce vasodilation by inhibiting specific phosphodiesterases, thereby interfering with the metabolic breakdown of cGMP. One such drug is sildenafil, used in treating erectile dysfunction and pulmonary hypertension (see Chapter 12).

## Interplay among Signaling Mechanisms

The calcium-phosphoinositide and cAMP signaling pathways oppose one another in some cells and are complementary in others. For example, vasopressor agents that contract smooth muscle

act by $IP_3$-mediated mobilization of $Ca^{2+}$, whereas agents that relax smooth muscle often act by elevation of cAMP. In contrast, cAMP and phosphoinositide second messengers act together to stimulate glucose release from the liver.

## Isolation of Signaling Mechanisms

The opposite of signal interplay is seen in some situations—an effective isolation of signaling according to location in the cell. For example, calcium signaling in the heart is highly localized because calcium released into the cytoplasm is rapidly sequestered by nearby calcium-binding proteins and is locally pumped from the cytoplasm into the sarcoplasmic reticulum. Even the second messenger cAMP can have surprisingly local effects, with signals mediated by the same messenger effectively isolated according to location. Here, it appears that signal isolation occurs by local hydrolysis of the second messenger by phosphodiesterase enzymes and by physical scaffolding of signaling pathway components into organized complexes that allow cAMP to transduce its local effects before hydrolysis. One mechanism by which phosphodiesterase inhibitor drugs produce toxic effects may be through "scrambling" local cAMP signals within the cell.

## Phosphorylation: A Common Theme

Almost all second messenger signaling involves reversible phosphorylation, which performs two principal functions in signaling: amplification and flexible regulation. In **amplification,** rather like GTP bound to a G protein, the attachment of a phosphoryl group to a serine, threonine, or tyrosine residue powerfully amplifies the initial regulatory signal by recording a molecular memory that the pathway has been activated; dephosphorylation erases the memory, taking a longer time to do so than is required for dissociation of an allosteric ligand. In **flexible regulation,** differing substrate specificities of the multiple protein kinases regulated by second messengers provide branch points in signaling pathways that may be independently regulated. In this way, cAMP, $Ca^{2+}$, or other second messengers can use the presence or absence of particular kinases or kinase substrates to produce quite different effects in different cell types. Inhibitors of protein kinases have great potential as therapeutic agents, particularly in neoplastic diseases. Trastuzumab, an antibody that antagonizes growth factor receptor signaling (discussed earlier), is a useful therapeutic agent for breast cancer. Another example of this general approach is imatinib, a small molecule inhibitor of the cytoplasmic tyrosine kinase Abl, which is activated by growth factor signaling pathways. Imatinib is effective for treating chronic myelogenous leukemia, which is caused by a chromosomal translocation event that produces an active Bcr/Abl fusion protein in hematopoietic cells.

# RECEPTOR CLASSES & DRUG DEVELOPMENT

The existence of a specific drug receptor is usually inferred from studying the **structure-activity relationship** of a group of structurally similar congeners of the drug that mimic or antagonize its effects. Thus, if a series of related agonists exhibits identical relative potencies in producing two distinct effects, it is likely that the two effects are mediated by similar or identical receptor molecules. In addition, if identical receptors mediate both effects, a competitive antagonist will inhibit both responses with the same $K_i$; a second competitive antagonist will inhibit both responses with its own characteristic $K_i$. Thus, studies of the relation between structure and activity of a series of agonists and antagonists can identify a species of receptor that mediates a set of pharmacologic responses.

Exactly the same experimental procedure can show that observed effects of a drug are mediated by *different* receptors. In this case, effects mediated by different receptors may exhibit different orders of potency among agonists and different $K_i$ values for each competitive antagonist.

Wherever we look, evolution has created many different receptors that function to mediate responses to any individual chemical signal. In some cases, the same chemical acts on completely different structural receptor classes. For example, acetylcholine uses ligand-gated ion channels (nicotinic AChRs) to initiate a fast (in milliseconds) excitatory postsynaptic potential (EPSP) in postganglionic neurons. Acetylcholine also activates a separate class of G protein-coupled receptors (muscarinic AChRs), which mediate slower (seconds to minutes) modulatory effects on the same neurons. In addition, each structural class usually includes multiple subtypes of receptor, often with significantly different signaling or regulatory properties. For example, many biogenic amines (eg, norepinephrine, acetylcholine, histamine, and serotonin) activate more than one receptor, each of which may activate a different G protein, as previously described (see also Table 2–1). The existence of many receptor classes and subtypes for the same endogenous ligand has created important opportunities for drug development. For example, propranolol, a selective antagonist of β adrenoceptors, can reduce an accelerated heart rate without preventing the sympathetic nervous system from causing vasoconstriction, an effect mediated by $\alpha_1$ adrenoceptors.

The principle of drug selectivity may even apply to structurally identical receptors expressed in different cells, eg, receptors for steroids (Figure 2–6). Different cell types express different accessory proteins, which interact with steroid receptors and change the functional effects of drug-receptor interaction. For example, tamoxifen is a drug that binds to steroid receptors naturally activated by estrogen. Tamoxifen acts as an *antagonist* on estrogen receptors expressed in mammary tissue but as an *agonist* on estrogen receptors in bone. Consequently, tamoxifen may be useful not only in the treatment of breast cancer but also in the prevention of osteoporosis by increasing bone density (see Chapters 40 and 42). Tamoxifen may create complications in postmenopausal women, however, by exerting an agonist action in the uterus, stimulating endometrial cell proliferation.

New drug development is not confined to agents that act on receptors for extracellular chemical signals. Increasingly, pharmaceutical chemists are determining whether elements of signaling pathways distal to the receptors may also serve as targets of selective and useful drugs. We have already discussed drugs that act on phosphodiesterase and some intracellular kinases. Several new kinase inhibitors and modulators are presently in therapeutic

trials, and there are preclinical efforts under way directed at developing inhibitors of specific G proteins.

## RELATION BETWEEN DRUG DOSE & CLINICAL RESPONSE

In this chapter, we have dealt with receptors as molecules and shown how receptors can quantitatively account for the relation between dose or concentration of a drug and pharmacologic responses, at least in an idealized system. When faced with a patient who needs treatment, the prescriber must make a choice among a variety of possible drugs and devise a dosage regimen that is likely to produce maximal benefit and minimal toxicity. To make rational therapeutic decisions, the prescriber must understand how drug-receptor interactions underlie the relations between dose and response in patients, the nature and causes of variation in pharmacologic responsiveness, and the clinical implications of selectivity of drug action.

### Dose & Response in Patients

#### A. Graded Dose-Response Relations

To choose among drugs and to determine appropriate doses of a drug, the prescriber must know the relative **pharmacologic potency** and **maximal efficacy** of the drugs in relation to the desired therapeutic effect. These two important terms, often confusing to students and clinicians, can be explained by referring to Figure 2–15, which depicts graded dose-response curves that relate the dose of four different drugs to the magnitude of a particular therapeutic effect.

**1. *Potency*—**Drugs A and B are said to be more potent than drugs C and D because of the relative positions of their dose-response curves along the **dose axis** of Figure 2–15. Potency refers to the concentration ($EC_{50}$) or dose ($ED_{50}$) of a drug required to produce 50% of that drug's maximal effect. Thus, the pharmacologic potency of drug A in Figure 2–15 is less than that of drug B, a partial agonist because the $EC_{50}$ of A is greater than the $EC_{50}$ of B. Potency of a drug depends in part on the affinity ($K_d$) of receptors for binding the drug and in part on the efficiency with which drug-receptor interaction is coupled to response. Note that some doses of drug A can produce larger effects than any dose of drug B, despite the fact that we describe drug B as pharmacologically more potent. The reason for this is that drug A has a larger maximal efficacy (as described below).

For therapeutic purposes, the potency of a drug should be stated in dosage units, usually in terms of a particular therapeutic end point (eg, 50 mg for mild sedation, 1 mcg/kg/min for an increase in heart rate of 25 bpm). Relative potency, the ratio of equi-effective doses (0.2, 10, etc), may be used in comparing one drug with another.

**2. *Maximal efficacy*—**This parameter reflects the limit of the dose-response relation on the **response axis.** Drugs A, C, and D in Figure 2–15 have equal maximal efficacy, and all have greater maximal efficacy than drug B. The maximal efficacy (sometimes referred to simply as efficacy) of a drug is obviously crucial for making clinical decisions when a large response is needed. It may be determined by the drug's mode of interactions with receptors (as with partial agonists)* or by characteristics of the receptor-effector system involved.

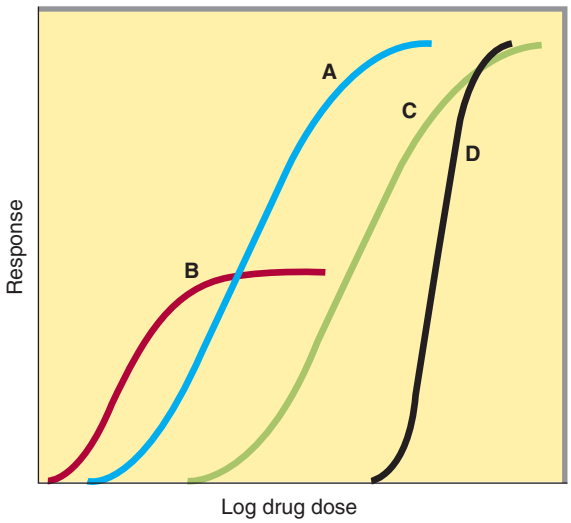


**FIGURE 2–15** Graded dose-response curves for four drugs, illustrating different pharmacologic potencies and different maximal efficacies. (See text.)

Thus, diuretics that act on one portion of the nephron may produce much greater excretion of fluid and electrolytes than diuretics that act elsewhere. In addition, the *practical* efficacy of a drug for achieving a therapeutic end point (eg, increased cardiac contractility) may be limited by the drug's propensity to cause a toxic effect (eg, fatal cardiac arrhythmia) even if the drug could otherwise produce a greater therapeutic effect.

#### B. Shape of Dose-Response Curves

Although the responses depicted in curves A, B, and C of Figure 2–15 approximate the shape of a simple Michaelis-Menten relation (transformed to a logarithmic plot), some clinical responses do not. Extremely steep dose-response curves (eg, curve D) may have important clinical consequences if the upper portion of the curve represents an undesirable extent of response (eg, coma caused by a sedative-hypnotic). Steep dose-response curves in patients can result from cooperative interactions of several different actions of a drug (eg, effects on brain, heart, and peripheral vessels, all contributing to lowering of blood pressure).

*Note that "maximal efficacy," used in a therapeutic context, does not have exactly the same meaning that the term denotes in the more specialized context of drug-receptor interactions described earlier in this chapter. In an idealized in vitro system, efficacy denotes the relative maximal efficacy of agonists and partial agonists that act via the same receptor. In therapeutics, efficacy denotes the extent or degree of an effect that can be achieved in the intact patient. Thus, therapeutic efficacy may be affected by the characteristics of a particular drug-receptor interaction, but it also depends on a host of other factors as noted in the text.

### C. Quantal Dose-Effect Curves

Graded dose-response curves of the sort described above have certain limitations in their application to clinical decision making. For example, such curves may be impossible to construct if the pharmacologic response is an either-or (quantal) event, such as prevention of convulsions, arrhythmia, or death. Furthermore, the clinical relevance of a quantitative dose-response relation in a single patient, no matter how precisely defined, may be limited in application to other patients, owing to the great potential variability among patients in severity of disease and responsiveness to drugs.

Some of these difficulties may be avoided by determining the dose of drug required to produce a specified magnitude of effect in a large number of individual patients or experimental animals and plotting the cumulative frequency distribution of responders versus the log dose (Figure 2–16). The specified quantal effect may be chosen on the basis of clinical relevance (eg, relief of headache) or for preservation of safety of experimental subjects (eg, using low doses of a cardiac stimulant and specifying an increase in heart rate of 20 bpm as the quantal effect), or it may be an inherently quantal event (eg, death of an experimental animal). For most drugs, the doses required to produce a specified quantal effect in individuals are lognormally distributed; that is, a frequency distribution of such responses plotted against the log of the dose produces a gaussian normal curve of variation (colored areas, Figure 2–16). When these responses are summated, the resulting cumulative frequency distribution constitutes a quantal dose-effect curve (or dose-percent curve) of the proportion or percentage of individuals who exhibit the effect plotted as a function of log dose.

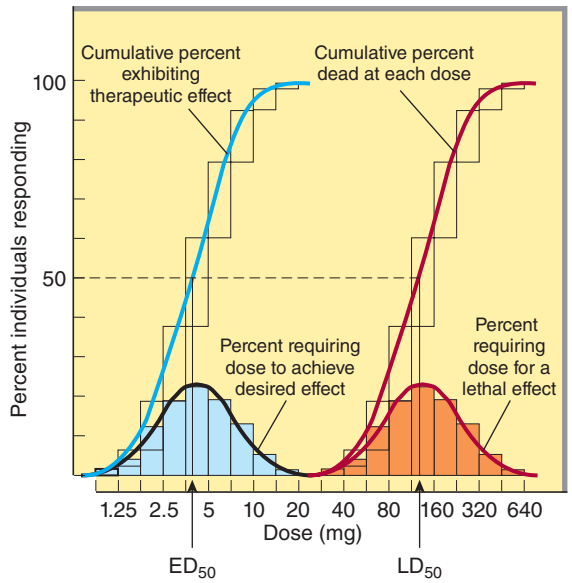


**FIGURE 2–16** Quantal dose-effect plots. Shaded boxes (and the accompanying bell-shaped curves) indicate the frequency distribution of doses of drug required to produce a specified effect; that is, the percentage of animals that required a particular dose to exhibit the effect. The open boxes (and the corresponding colored curves) indicate the cumulative frequency distribution of responses, which are lognormally distributed.

The quantal dose-effect curve is often characterized by stating the **median effective dose ($ED_{50}$),** which is the dose at which 50% of individuals exhibit the specified quantal effect. (Note that the abbreviation $ED_{50}$ has a different meaning in this context from its meaning in relation to graded dose-effect curves, described in previous text). Similarly, the dose required to produce a particular toxic effect in 50% of animals is called the **median toxic dose ($TD_{50}$).** If the toxic effect is death of the animal, a **median lethal dose ($LD_{50}$)** may be experimentally defined. Such values provide a convenient way of comparing the potencies of drugs in experimental and clinical settings: Thus, if the $ED_{50}$s of two drugs for producing a specified quantal effect are 5 and 500 mg, respectively, then the first drug can be said to be 100 times more potent than the second for that particular effect. Similarly, one can obtain a valuable index of the selectivity of a drug's action by comparing its $ED_{50}$s for two different quantal effects in a population (eg, cough suppression versus sedation for opioid drugs).

Quantal dose-effect curves may also be used to generate information regarding the margin of safety to be expected from a particular drug used to produce a specified effect. One measure, which relates the dose of a drug required to produce a desired effect to that which produces an undesired effect, is the **therapeutic index.** In animal studies, the therapeutic index is usually defined as the ratio of the $TD_{50}$ to the $ED_{50}$ for some therapeutically relevant effect. The precision possible in animal experiments may make it useful to use such a therapeutic index to estimate the potential benefit of a drug in humans. Of course, the therapeutic index of a drug in humans is almost never known with real precision; instead, drug trials and accumulated clinical experience often reveal a range of usually effective doses and a different (but sometimes overlapping) range of possibly toxic doses. The range between the minimum toxic dose and the minimum therapeutic dose is called the **therapeutic window** and is of greater practical value in choosing the dose for a patient. The clinically acceptable risk of toxicity depends critically on the severity of the disease being treated. For example, the dose range that provides relief from an ordinary headache in the majority of patients should be very much lower than the dose range that produces serious toxicity, even if the toxicity occurs in a small minority of patients. However, for treatment of a lethal disease such as Hodgkin's lymphoma, the acceptable difference between therapeutic and toxic doses may be smaller.

Finally, note that the quantal dose-effect curve and the graded dose-response curve summarize somewhat different sets of information, although both appear sigmoid in shape on a semilogarithmic plot (compare Figures 2–15 and 2–16). Critical information required for making rational therapeutic decisions can be obtained from each type of curve. Both curves provide information regarding the **potency** and **selectivity** of drugs; the graded dose-response curve indicates the **maximal efficacy** of a drug, and the quantal dose-effect curve indicates the potential **variability** of responsiveness among individuals.

## Variation in Drug Responsiveness

Individuals may vary considerably in their response to a drug; indeed, a single individual may respond differently to the same

drug at different times during the course of treatment. Occasionally, individuals exhibit an unusual or **idiosyncratic** drug response, one that is infrequently observed in most patients. The idiosyncratic responses are usually caused by genetic differences in metabolism of the drug or by immunologic mechanisms, including allergic reactions.

Quantitative variations in drug response are, in general, more common and more clinically important. An individual patient is **hyporeactive** or **hyperreactive** to a drug in that the intensity of effect of a given dose of drug is diminished or increased compared with the effect seen in most individuals. (*Note:* The term **hypersensitivity** usually refers to allergic or other immunologic responses to drugs.) With some drugs, the intensity of response to a given dose may change during the course of therapy; in these cases, responsiveness usually decreases as a consequence of continued drug administration, producing a state of relative **tolerance** to the drug's effects. When responsiveness diminishes rapidly after administration of a drug, the response is said to be subject to **tachyphylaxis.**

Even before administering the first dose of a drug, the prescriber should consider factors that may help in predicting the direction and extent of possible variations in responsiveness. These include the propensity of a particular drug to produce tolerance or tachyphylaxis as well as the effects of age, sex, body size, disease state, genetic factors, and simultaneous administration of other drugs.

Four general mechanisms may contribute to variation in drug responsiveness among patients or within an individual patient at different times.

### A. Alteration in Concentration of Drug That Reaches the Receptor

As described in Chapter 3, patients may differ in the rate of absorption of a drug, in distributing it through body compartments, or in clearing the drug from the blood. By altering the concentration of drug that reaches relevant receptors, such pharmacokinetic differences may alter the clinical response. Some differences can be predicted on the basis of age, weight, sex, disease state, and liver and kidney function, and by testing specifically for genetic differences that may result from inheritance of a functionally distinctive complement of drug-metabolizing enzymes (see Chapters 4 and 5). Another important mechanism influencing drug availability is active transport of drug from the cytoplasm, mediated by a family of membrane transporters encoded by the so-called multidrug resistance (*MDR*) genes. For example, up-regulation of *MDR* gene-encoded transporter expression is a major mechanism by which tumor cells develop resistance to anti-cancer drugs.

### B. Variation in Concentration of an Endogenous Receptor Ligand

This mechanism contributes greatly to variability in responses to pharmacologic antagonists. Thus, propranolol, a β-adrenoceptor antagonist, markedly slows the heart rate of a patient whose endogenous catecholamines are elevated (as in pheochromocytoma) but does not affect the resting heart rate of a well-trained marathon runner. A partial agonist may exhibit even more dramatically different responses: Saralasin, a weak partial agonist at angiotensin II receptors, lowers blood pressure in patients with hypertension caused by increased angiotensin II production and raises blood pressure in patients who produce normal amounts of angiotensin.

### C. Alterations in Number or Function of Receptors

Experimental studies have documented changes in drug response caused by increases or decreases in the number of receptor sites or by alterations in the efficiency of coupling of receptors to distal effector mechanisms. In some cases, the change in receptor number is caused by other hormones; for example, thyroid hormones increase both the number of β adrenoceptors in rat heart muscle and cardiac sensitivity to catecholamines. Similar changes probably contribute to the tachycardia of thyrotoxicosis in patients and may account for the usefulness of propranolol, a β-adrenoceptor antagonist, in ameliorating symptoms of this disease.

In other cases, the agonist ligand itself induces a decrease in the number (eg, down-regulation) or coupling efficiency (eg, desensitization) of its receptors. These mechanisms (discussed previously under Signaling Mechanisms & Drug Action) may contribute to two clinically important phenomena: first, tachyphylaxis or tolerance to the effects of some drugs (eg, biogenic amines and their congeners), and second, the "overshoot" phenomena that follow withdrawal of certain drugs. These phenomena can occur with either agonists or antagonists. An antagonist may increase the number of receptors in a critical cell or tissue by preventing down-regulation caused by an endogenous agonist. When the antagonist is withdrawn, the elevated number of receptors can produce an exaggerated response to physiologic concentrations of agonist. Potentially disastrous withdrawal symptoms can result for the opposite reason when administration of an agonist drug is discontinued. In this situation, the number of receptors, which has been decreased by drug-induced down-regulation, is too low for endogenous agonist to produce effective stimulation. For example, the withdrawal of clonidine (a drug whose $\alpha_2$-adrenoceptor agonist activity reduces blood pressure) can produce hypertensive crisis, probably because the drug down-regulates $\alpha_2$ adrenoceptors (see Chapter 11).

The study of genetic factors determining drug response is called **pharmacogenetics**, and the use of gene sequencing or expression profile data to tailor therapies specific to an individual patient is called **personalized** or **precision medicine**. For example, somatic mutations affecting the tyrosine kinase domain of the epidermal growth factor receptor in lung cancers can confer enhanced sensitivity to kinase inhibitors such as gefitinib. This effect enhances the antineoplastic effect of the drug, and because the somatic mutation is specific to the tumor and not present in the host, the therapeutic index of these drugs can be significantly enhanced in patients whose tumors harbor such mutations. Genetic analysis can also predict drug resistance during treatment or identify new targets for therapy based on rapid mutation of the tumor in the patient.

### D. Changes in Components of Response Distal to the Receptor

Although a drug initiates its actions by binding to receptors, the response observed in a patient depends on the functional integrity

of biochemical processes in the responding cell and physiologic regulation by interacting organ systems. Clinically, changes in these postreceptor processes represent the largest and most important class of mechanisms that cause variation in responsiveness to drug therapy.

Before initiating therapy with a drug, the prescriber should be aware of patient characteristics that may limit the clinical response. These characteristics include the age and general health of the patient and—most importantly—the severity and pathophysiologic mechanism of the disease. The most important potential cause of failure to achieve a satisfactory response is that the diagnosis is wrong or physiologically incomplete. Drug therapy is most successful when it is accurately directed at the pathophysiologic mechanism responsible for the disease.

When the diagnosis is correct and the drug is appropriate, an unsatisfactory therapeutic response can often be traced to compensatory mechanisms in the patient that respond to and oppose the beneficial effects of the drug. Compensatory increases in sympathetic nervous tone and fluid retention by the kidney, for example, can contribute to tolerance to antihypertensive effects of a vasodilator drug. In such cases, additional drugs may be required to achieve a useful therapeutic result.

## Clinical Selectivity: Beneficial versus Toxic Effects of Drugs

Although we classify drugs according to their principal actions, it is clear that *no drug causes only a single, specific effect*. Why is this so? It is exceedingly unlikely that any kind of drug molecule will bind to only a single type of receptor molecule, if only because the number of potential receptors in every patient is astronomically large. Even if the chemical structure of a drug allowed it to bind to only one kind of receptor, the biochemical processes controlled by such receptors would take place in many cell types and would be coupled to many other biochemical functions; as a result, the patient and the prescriber would probably perceive more than one drug effect. Accordingly, drugs are only *selective*—rather than specific—in their actions, because they bind to one or a few types of receptor more tightly than to others and because these receptors control discrete processes that result in distinct effects.

It is only because of their selectivity that drugs are useful in clinical medicine. Selectivity can be measured by comparing binding affinities of a drug to different receptors or by comparing $ED_{50}$s for different effects of a drug in vivo. In drug development and in clinical medicine, selectivity is usually considered by separating effects into two categories: **beneficial** or **therapeutic effects** versus **toxic** or **adverse effects.** Pharmaceutical advertisements and prescribers occasionally use the term **side effect,** implying that the effect in question is insignificant or occurs via a pathway that is to one side of the principal action of the drug; such implications are frequently erroneous.

### A. Beneficial and Toxic Effects Mediated by the Same Receptor-Effector Mechanism

Much of the serious drug toxicity in clinical practice represents a direct pharmacologic extension of the therapeutic actions of the drug. In some of these cases (eg, bleeding caused by anticoagulant therapy; hypoglycemic coma due to insulin), toxicity may be avoided by judicious management of the dose of drug administered, guided by careful monitoring of effect (measurements of blood coagulation or serum glucose) and aided by ancillary measures (avoiding tissue trauma that may lead to hemorrhage; regulation of carbohydrate intake). In still other cases, the toxicity may be avoided by not administering the drug at all, if the therapeutic indication is weak or if other therapy is available.

In certain situations, a drug is clearly necessary and beneficial but produces unacceptable toxicity when given in doses that produce optimal benefit. In such situations, it may be necessary to add another drug to the treatment regimen. In treating hypertension, for example, administration of a second drug often allows the prescriber to reduce the dose and toxicity of the first drug (see Chapter 11).

### B. Beneficial and Toxic Effects Mediated by Identical Receptors but in Different Tissues or by Different Effector Pathways

Many drugs produce both their desired effects and adverse effects by acting on a single receptor type in different tissues. Examples discussed in this book include digitalis glycosides, which act by inhibiting $Na^+/K^+$-ATPase in cell membranes; methotrexate, which inhibits the enzyme dihydrofolate reductase; and glucocorticoid hormones.

Three therapeutic strategies are used to avoid or mitigate this sort of toxicity. First, the drug should always be administered at the lowest dose that produces acceptable benefit. Second, adjunctive drugs that act through different receptor mechanisms and produce different toxicities may allow lowering the dose of the first drug, thus limiting its toxicity (eg, use of other immunosuppressive agents added to glucocorticoids in treating inflammatory disorders). Third, selectivity of the drug's actions may be increased by manipulating the concentrations of drug available to receptors in different parts of the body, for example, by aerosol administration of a glucocorticoid to the bronchi in asthma.

### C. Beneficial and Toxic Effects Mediated by Different Types of Receptors

Therapeutic advantages resulting from new chemical entities with improved receptor selectivity were mentioned earlier in this chapter and are described in detail in later chapters. Many receptors, such as catecholamines, histamine, acetylcholine, and corticosteroids, and their associated therapeutic uses were discovered by analyzing effects of the physiologic chemical signals. This approach continues to be fruitful. For example, mis-expression of microRNAs (miRNAs), small RNAs that regulate protein expression by binding to protein-coding (messenger) RNAs, was linked recently to Duchenne muscular dystrophy. Current preclinical investigations include the utility of RNA-based therapy for this and other diseases.

Other drugs were discovered by exploiting therapeutic or toxic effects of chemically similar agents observed in a clinical context. Examples include quinidine, the sulfonylureas, thiazide diuretics, tricyclic antidepressants, opioid drugs, and phenothiazine antipsychotics. Often such agents turn out to interact with receptors for endogenous substances (eg, opioids and phenothiazines for

endogenous opioid and dopamine receptors, respectively). This approach is evolving toward understanding the structural details of how chemically similar agents differ in binding to receptors. For example, X-ray crystallography of $\beta_1$ and $\beta_2$ adrenoceptors shows that their orthosteric binding sites are identical; drugs discriminate between subtypes based on differences in traversing a divergent "vestibule" to access the orthosteric site. Many GPCRs have such passages, revealing a new basis for improving the selectivity of GPCR-targeted drugs.

Thus, the propensity of drugs to bind to different classes of receptor sites is not only a potentially vexing problem in treating patients, but it also presents a continuing challenge to pharmacology and an opportunity for developing new and more useful drugs.

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## CASE STUDY ANSWER

Propranolol, a β-adrenoceptor antagonist, is a useful antihypertensive agent because it reduces cardiac output and probably vascular resistance as well. However, it also prevents β-adrenoceptor–induced bronchodilation and therefore may precipitate bronchoconstriction in susceptible individuals. Calcium channel blockers such as verapamil also reduce blood pressure but, because they act on a different target, rarely cause bronchoconstriction or prevent bronchodilation. An alternative approach in this patient would be to use a more highly selective adrenoceptor antagonist drug (such as metoprolol) that binds preferentially to the $\beta_1$ subtype, which is a major β adrenoceptor in the heart, and has a lower affinity (ie, higher $K_d$) for binding the $\beta_2$ subtype that mediates bronchodilation. Selection of the most appropriate drug or drug group for one condition requires awareness of the other conditions a patient may have and the receptor selectivity of the drug groups available.