CHAPTER 6

Introduction to Pharmacodynamics

Reza Karimi

OBJECTIVES

1. Understand the physiology behind the gastrointestinal tract and the route of oral drug administration and physiological influences on pharmacodynamics.
2. Understand the dynamics and functions of the major signal transduction systems and their different biomedical and biological responses in regard to receptor–ligand interactions.
3. Learn about the dynamics and mathematical expressions behind receptor–ligand interactions.
4. Understand dose–response relationships and factors that affect a pharmacological response.
5. Learn about agonistic, antagonistic, and partial agonistic binding of drugs to receptors.
6. Learn about different concepts such as addition, synergism, and potentiation that lead to an enhancement effect of drugs.
7. List a few regulatory mechanisms for receptors.
8. Implement a series of Learning Bridge assignments at your experiential sites to bridge your didactic learning with your experiential experiences.

KEY TERMS AND DEFINITIONS

1. **cAMP:** cyclic adenosine 3',5"-monophosphate; a second messenger that plays an important role in signal transduction.
2. **cGMP:** cyclic guanosine 3',5"monophosphate; a second messenger that plays an important role in signal transduction.
3. **Dose–response relationship:** when an endogenous or exogenous ligand binds to a receptor and produces a pharmacological effect. The effect can approach a maximum value (also called E_{max}) in which a further increase in the ligand concentration does not produce any higher response.
4. **Efficacy:** the ability of a drug to produce a pharmacological response when it interacts with its receptor.
5. **First-pass metabolism:** a type of metabolism in which drugs that are absorbed by the gastrointestinal tract go through the portal vein to the liver and are metabolized there before they are distributed to the general circulation.
6. **Homeostasis:** a balanced physiological process that protects and maintains the integrity of the internal environment (e.g., an organ, a cell).
7. **IP_3:** inositol 1,4,5-triphosphate; it is generated from the cell membrane's phospholipids, diffuses through the cytoplasm to the endoplasmic reticulum, and binds to its receptor to stimulate Ca^{2+} channels to open and release Ca^{2+} into the cytoplasm.
8. **Isoforms:** two or more protein forms that have the same function but have been expressed by different genes.

(*continues*)

(CONTINUED)

9. **Isozyme:** two or more enzymes that catalyze the same reaction but are expressed by different genes.
10. **MEC:** minimum effective concentration; the plasma drug concentration that produces the minimum pharmacological effect.
11. **MTC:** minimum toxic concentration; the plasma drug concentration that produces the minimum toxic effect.
12. **PDE:** a cyclic nucleotide phosphodiesterase enzyme that degrades either cAMP or cGMP.
13. **Pharmacokinetics (PK):** the study of the rate and extent of drug absorption, distribution, and elimination from the body.
14. **Pharmacodynamics (PD):** the study of the molecular interactions of drugs and receptors.
15. **Pharmacology:** the study of how drugs interact with the body to produce a biochemical or physiological effect.
16. **Physiological receptors:** receptors that can recognize and accept exogenous ligands.
17. **Potency:** a measure of the concentration (or dose) of a drug that produces 50% of the maximal effect.
18. **Protein kinases:** a class of enzymes that transfer a phosphate group from an ATP molecule to a protein.
19. **Receptors:** large intracellular or integral proteins that, by receiving chemical signals, play important roles in many physiological and cellular functions.
20. **Signal transduction**: the movement of signals from outside to inside of a cell, or vice versa.

Introduction

The field of pharmacodynamics studies how a ligand (endogenous or exogenous), such as a hormone or a neurotransmitter, binds to its receptor to produce a pharmacological response. In addition, pharmacodynamics is concerned with factors that affect the ligand–receptor binding. Signal transduction is the cornerstone of pharmacodynamics. This mechanism employs proteins in the form of enzymes or receptors that receive a specific signal and in a sensitive manner convert that signal to a series of biochemical and physiological events. As a result, the specificity and sensitivity of receptors and the concentration of ligands are of paramount importance in pharmacodynamics.

In this chapter, an introduction to physiology of homeostasis and the gastrointestinal (GI) tract is provided to lay the groundwork for appreciating how different factors affect the role of pharmacodynamics in oral absorption of drugs. The important roles that efficacy and potency play in producing a dose–response relationship are emphasized. In addition, different subtypes of ligand–receptor interactions, such as agonistic, antagonistic, and partial agonistic, and a few regulatory mechanisms for receptors are described.

Physiological Influences on Pharmacodynamics

Receptors are large proteins that play an important role in the field of pharmacodynamics. Receptors have the ability to amplify physiological signals and, as a result, they are potential targets for drugs. Receptors have two major functions: binding to their specific molecules (ligands) and sending signals (signal transduction) to a series of events. This dual function indicates that there must be at least two domains on a receptor: a ligand-binding domain and an effector domain. The unique characteristic of receptors is that they serve as proteins to recognize and accept endogenous and exogenous ligands. Exogenous ligands (xenobiotics or drug products) should, however, mimic the structures of the endogenous ligands. Receptors that can recognize and accept exogenous ligands are called physiological receptors.

Just as a drug's effect is produced after binding to a receptor, so can that effect be terminated when the drug dissociates from the receptor (although an exception exists in the form of "constitutive activity," a concept that is explained later on in this chapter). If a drug does not dissociate due to a very tight binding (such as a covalent bond), the effect continues to be produced as long as the receptor–drug complex remains intact. However, the latter process does not last too long because at some point the receptor–drug complex is degraded and eliminated, and a new free receptor is synthesized.

A drug molecule is considerably smaller in size than a receptor. The specific binding site on a receptor may be identified and studied. Intensive crystallography studies and genetic changes (mutations) in wild-type receptors have revealed important features of receptors, their binding sites, and their roles in pharmacodynamics. Because the affinity of a drug to a receptor and the chemical structure of a drug play important roles in producing a physiological effect, the effects of drugs depend on a combination of physiological and physicochemical factors. In addition, the pathophysiology may contribute to the effect of a drug. In other words, a drug may produce a different effect (or no effect at all) in an individual who does not have any abnormality in a cellular or physiological function. Conversely, a drug's effect can change due to a disease state. For instance, in patients who have cirrhosis accompanied by reduced hepatic metabolism, oral administration of drugs that extensively are metabolized by the liver will result in an increase in those drugs' systemic bioavailability (plasma level). Patients with liver cirrhosis also have a reduced glomerular filtration rate and renal plasma flow, which accounts for their decreased renal elimination of drugs such as fluconazole (Diflucan), lithium (Lithobid), and ofloxacin (Apo-Oflox, from Canada).

A variety of physiological and physicochemical factors affect pharmacodynamics, pharmacology, pharmacokinetics, biochemistry, and pharmaceutics. Often, these fields are integrated when a drug action is studied; thus it is not surprising when one finds overlapping factors that can affect all of the fields.

Physiology of Homeostasis

Higher-order eukaryotic organisms, including humans, are multicellular organisms with many unique and diverse cells. In fact, there are approximately 200 different cells in humans. Cells that are alike assemble to form a tissue. Several tissues, in turn, assemble to make an organ. An organ performs a specific function, and each organ belongs to an organ system. For instance, the heart is part of the cardiovascular system and the lungs are part of the respiratory system. Because there are many organ systems, there must be a coordination process between them to maintain the daily functions of the organism. This coordination process is carried out by the nervous system and the endocrine system.

Interestingly, nerve cells, after having grown and built a network with other nerve cells, do not divide (indeed, some nerve cells even die during embryonic deployment). In contrast, the cells of the endocrine system divide frequently, and some even adapt their growth based on a specific need. For instance, pancreatic cells are known to divide and grow as needed. To maintain homeostasis, the various organ systems work at different speeds. For instance, the autonomic nervous system rapidly adjusts to changes in the environment by releasing neurotransmitters to produce an effect. By comparison, the endocrine system acts much more slowly by releasing hormones into the systemic circulation, which can take hours and even days to produce any effect.

All organ systems (e.g., cardiovascular, respiratory, digestive, urinary) work together to maintain homeostasis. Homeostasis is a balanced physiological process that protects and maintains the integrity of the internal environment (e.g., an organ, a cell). For instance, when the digestive

system breaks down proteins into amino acids, the cardiovascular system distributes those amino acids to the cells to protect the integrity of cells from starvation. Another example of homeostasis occurs when you suddenly stand up from a sitting position and your blood pressure falls (hypotension). This hypotensive effect immediately forces the nervous system to constrict your blood vessels to increase the blood pressure and return it to a normal level. Another example is when you maintain your blood glucose concentration at a constant level (at 5 mM). While your muscles take up glucose from your blood, your liver supplies more glucose to the blood to maintain the level of blood glucose at a constant 5 mM.

Through evolution, organisms have developed a remarkable collection of regulatory mechanisms for maintaining homeostasis. If the homeostasis does not work correctly, however, we become sick. In the glucose uptake example, if your muscle cells (myocytes) cannot take up sufficient glucose from your blood (because of a lack of insulin), your blood glucose level will be high (diabetes). This excess level of glucose produces many severe physiological consequences—ketoacidosis, for example, is a life-threatening condition.

Understanding drug absorption, the physiology of the GI tract, and the various factors that affect drug absorption can assist students in understanding the important role that pharmacodynamics plays when drugs interact with receptors and other macromolecules in the body. Because the majority of drugs are administered orally, a brief overview of GI physiology and oral drug absorption is provided in the following subsections.

Physiology and Routes of Oral Drug Absorption

After oral drug administration, the drug, which could be in a solid form (e.g., tablets, capsules) or a liquid dosage form (e.g., emulsion, suspension), enters the esophagus. It has only a short transit time there, which makes the esophagus a poor site for absorption of orally administered drugs. After its passage through the esophagus, the drug is dissolved in the GI tract. The GI tract includes the stomach, the small intestine (which includes the duodenum, jejunum, and ileum), and the large intestine. Finally, the GI tract ends in the rectum. The various areas of the GI tract have their own characteristics (pH, surface area, and secretions) and, therefore, differently influence drug absorption. It is from one of these areas that the drug will be absorbed into the circulation and thereby reach its site of action.

The anatomy of the GI tract includes four layers of tissues: mucosa, submucosa, muscularis external, and serosa (**Figure 6.1**).

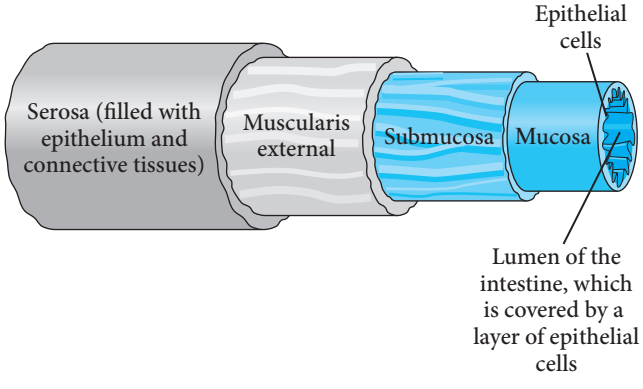

Figure 6.1 Different layers of the GI tract.

The mucosa itself is made of three layers:

- Epithelial cells, which are in contact with the intestinal content
- Lamina propria, which is the underlying supportive tissue of the epithelial cell and contains blood vessels, lymphatic vessels, and nerves
- A layer of muscle fibers (muscularis mucosa)

The absorption of drugs occurs through the epithelial cells into the blood capillaries of the lamina propria; the blood carrying these cells then travels through capillaries into the rest of the body. These epithelial cells are tightly packed (tight junction) to allow drugs (particularly un-ionized or hydrophobic drugs) to pass through a passive transcellular diffusion (see also the *Introduction to Pharmacokinetics* chapter).

Drug Absorption from the Stomach

The mucosa of the stomach wall has hundreds of gastric glands. Each gastric gland contains four major types of cells:

- *Mucous neck cells* secrete mucins. Mucins are large glycoproteins that form a mucous layer that adheres to the surface of epithelial cells of the stomach to protect those cells from gastric acid. These epithelial cells secrete HCO_3^- ions that become trapped in the mucous layer and make this layer neutral (pH = 7.0). Anything (e.g., NSAIDs, *Helicobacter pylori* bacterial infection) that destroys the mucosal integrity can cause an ulcer.
- *Parietal cells* have the H^+-K^+ ATPase enzyme on the surface of their cell membranes. The H^+-K^+ ATPase exchanges an H^+ ion (pumps it out to the stomach) for each K^+ ion (pumps it into the parietal cell). The K^+ ions that have been pumped into the cell enter the interstitial fluid by K^+ channels. It is the H^+ that makes the stomach an acidic environment. The stomach already has a high concentration of H^+, so pumping H^+ from an area of a low concentration (parietal cell) to an area of high concentration (stomach) requires an active transporter system (ATPase) and energy in the form of ATP molecules. Parietal cells also have receptors for histamine (a hormone), gastrin (a hormone), and acetylcholine (a neurotransmitter).
- *Chief cells* secrete pepsinogen. The secretion of HCl assists in converting pepsinogen (an inactive precursor or zymogen) to active pepsin. Pepsin needs to be in an acidic environment (pH of 1–3) to be in its active form. The role of pepsin is to degrade proteins into small fragments of polypeptides in the stomach. Pepsin is inactivated reversibly and irreversibly at pH 4 and 7, respectively.
- *Enteroendocrine cells* secrete four hormones: histamine (activates parietal cells), serotonin (stimulates stomach contraction and gut motility), gastrin (stimulates parietal cells to release HCl), and somatostatin (inhibits the effect of gastrin).

Due to the short transit time (less than 1 hour) of a drug in the stomach, no significant drug absorption occurs from this part of the GI tract. However, the stomach's gastric acid assists with disintegration and dissolution of many oral solid drugs, including tablets and capsules (see also the *Introduction to Pharmaceutics* chapter).

Drug Absorption from the Small Intestine

The dissolved drugs do not stay in the stomach, but rather travel to the small intestine. Due to the collectively large surface area of the villi that are lined with epithelial and goblet cells and the 4-hour transit time of drugs, many drugs are absorbed from the small intestine. Each villus of the small intestine has a supportive network of capillaries (**Figure 6.2**). Many drugs and nutrients are

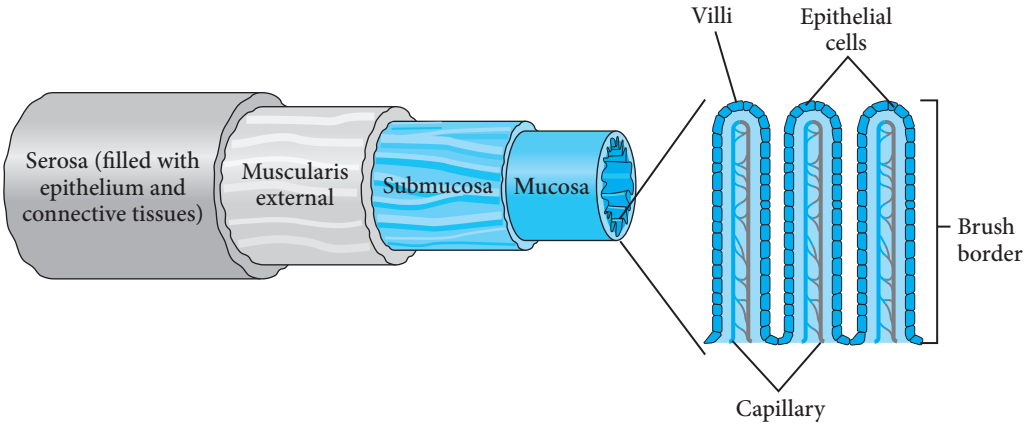

Figure 6.2 Villi occupy a significant area of the lumen of the small intestine.

absorbed into these capillaries and then move into the portal vein. The pH of the small intestine is higher due to the secretion of bicarbonate from the pancreas into the small intestine. The greatest surface areas of the small intestine are found in the duodenum and jejunum, because the villi are found in their highest concentration in these two areas. Therefore, the majority of drugs are absorbed from these two areas.

Drug Absorption from the Large Intestine

The large intestine does not have any villi, so it does not provide an adequate surface area for a drug absorption. However, the large intestine plays an important role in the absorption of water and electrolytes into the circulation. The pH of the large intestine is approximately 7. Keep in mind if a drug is not fully absorbed in the small intestine, it may be absorbed from the large intestine. However, often drugs are not absorbed in this organ, but rather are excreted in the feces.

Many factors affect the role of pharmacodynamics in oral absorption of drugs. For instance, it has been suggested that the sensitivity of β-adrenergic receptors declines as we get older. As a result, elderly patients may respond to a β-adrenergic blocker differently compared with younger patients. In addition, aging reduces the levels of testosterone, a sex hormone, in both men and women, which may in turn affect the intracellular receptor binding of steroids. Other factors that can affect the absorption of drugs (particularly orally administered drugs) include gastric acid, molecular size of drugs, first-pass metabolism, cytochrome P450 enzymes, foods, drug formulation, disintegration and dissolution rates, blood flow, solubility, pH and pK_a, intestinal microflora, efflux transporters, transcytosis, and carrier-mediated transporters. While some of these factors are discussed in this chapter, others are discussed elsewhere in this text.

Gastric Acid

Polypeptides and proteins are denatured in the acidic environment of the stomach (**Figure 6.3**). The more a drug is denatured, the more it is prone to attack by the enzyme pepsin (pepsin hydrolyzes polypeptides and proteins into small fragments). Insulin (a peptide hormone) is an example of a compound that is destroyed by the acidic milieu of the stomach. Some drugs are acid-labile (acid-sensitive). One typical example is the members of the penicillin class, which lose their effectiveness due to the acidic degradation of penicillin. Another example is erythromycin, which is destroyed by gastric acid. However, enteric-coated and esterified forms of erythromycin are stable in gastric acid.

Some Europeans and others eat a food called "sweetbread," which contains pancreas tissue. The pancreas is the source of insulin, which affects carbohydrate metabolism. However, the high insulin content in the "sweetbread" does not interfere with carbohydrate metabolism because insulin is simply destroyed by the acidic pH of the stomach. Even if insulin manages to survive this acidic milieu, it will not be able to pass through the aqueous mucus that covers the epithelial cells in the small intestine (see the discussion of the molecular size of drugs in the next subsection).

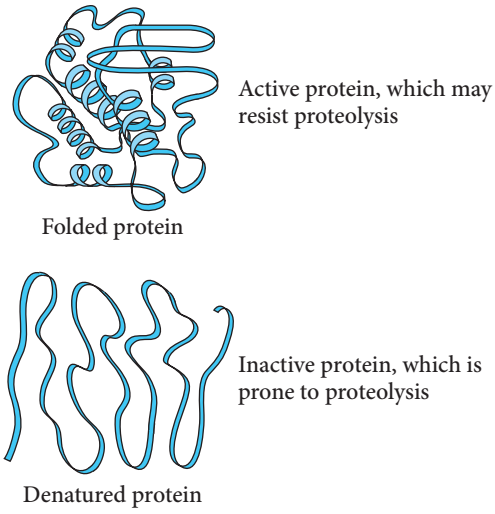

Figure 6.3 Properly folded proteins are unfolded and denatured in the acidic milieu of the stomach.

By the same token, some drugs actually need gastric acid to be effectively absorbed. One example is ferrous sulfate (Feosol), an iron supplement. Another example is oral ketoconazole (Apo-Ketoconazole, from Canada), an antifungal agent. As a result and to increase the absorption of these drugs, pharmacists should advise their patients to take these drugs with orange juice (which has citric acid) and administer them at least 2 hours before taking any antacids or acid suppressive agents.

Molecular Size of Drugs

Goblet cells are the second most abundant cells (after epithelial cells) on the villi. Goblet cells produce a mucous layer that makes a film inside the lumen of the small intestine to cover the epithelial cells. The mucous layer does not allow large drug molecules (larger than 800 daltons) to reach the epithelial cells. For instance, low-molecular-weight heparin (LMWH), also known as enoxaparin (Lovenox), is an anticoagulant that is given intravenously (IV) or subcutaneously (SC) and is used to treat thrombosis. An oral dosage form of enoxaparin will, however, have limited oral absorption because enoxaparin, due to its large molecular weight (more than 4,000 g/mol), cannot go through the mucous layer of the GI tract. Therefore, enoxaparin is administered only as an IV or SC formulation. Insulin is another example; its molecular weight is more than 5,000 g/mol, so it cannot pass through the mucous layer of the GI tract.

First-Pass Metabolism

Many drugs that are absorbed by the GI tract go through the portal vein to the liver before they are distributed to the general circulation. The reason is that all of the venous blood from the stomach, the small intestine, and the large intestine enters the portal vein and then moves to the liver. Approximately 75% of all blood that reaches the liver does so via the portal vein. In the liver, the drugs carried in this blood are metabolized (first-pass metabolism). For example, propranolol (Inderal LA), an antihypertensive agent, has a high first-pass metabolism. This effect means that a total of 100–200 mg orally administered propranolol tablets, in essence, equals 1–3 mg of an intravenous injection of propranolol.

Food

A drug may interact with a specific chemical component of a food in a manner that makes the drug–component combination an insoluble complex. For instance, absorption of digoxin

(Lanoxin), and thereby this drug's plasma concentration, is decreased by foods that contain fiber or are high in pectin. Similarly, members of the tetracycline drug class interact with calcium, magnesium, zinc, iron, and aluminum. Because these ions reduce the absorption of tetracycline, patients should take tetracycline antibiotics at least 2 hours before taking any iron supplements or antacids.

Another example of a food's effect on drug metabolism can be seen with the effect of leafy vegetables on warfarin (Coumadin). Patients who take warfarin should maintain consistency in their intake of green leafy vegetables. These vegetables contain large amounts of vitamin K; vitamin K, in turn, is important for the formation of prothrombin, a blood protein that plays a key role in the blood clotting process. Changing the amount of vitamin K (inconsistency in the intake of green leaves), therefore, changes the therapeutic effect of warfarin. For this reason, patients should be advised not to change their dietary habits once they are stabilized on warfarin.

The absorption of omeprazole is also reduced by food. Because consumption of food increases stomach acid, this drug will be released into stomach rather than at its actual site of action—that is, the parietal cells lining the stomach.

Consumption of some foods also slows gastric emptying, which in turn delays the transport of drugs from stomach to small intestine and may affect drug absorption. Conversely, consumption of fiber (fruit) and whole grains can sometimes cause diarrhea, which causes a drug to move quickly throughout the GI tract (less transition time and thereby less drug absorption). Penicillin derivatives are also known to cause diarrhea. Because the diarrhea causes a drug to move quickly throughout the GI tract, many drugs may not effectively be absorbed when they are administered concurrently with penicillin derivatives.

Effects of Age

The stomach acidity is low (achlorhydria) in infants (1 month to 1 year). This leads to an increased absorption of basic drugs (which are less ionized in a higher pH) and decreased absorption of acidic drugs (which are more ionized in a higher pH) through gastric membranes. In addition, gastric motility (gastric emptying time) is slowed in infants, which results in slow absorption of oral drugs in the small intestine. However, their gastric motility is fully developed within the first 6 to 8 months of life. Aminoglycosides (e.g., neomycin, amikacin, gentamicin) have a longer half-life ($t_{1/2}$) in children who are younger than 6 months, in part because they have a reduced renal elimination rate.

Age also affects the level of serum albumin. Serum albumin is an abundant protein in the blood that binds reversibly to many drugs. Drugs that are bound to albumin are pharmacologically inactive because only free drug can bind to receptors or act on other targets. The amount of serum albumin is low in infants, which results in higher concentrations of unbound drugs (more effect). Similarly, malnourished elderly patients have lower serum albumin, which may increase the effect (or toxicity) of certain drugs. In addition, a low serum albumin leads to a higher blood flow; in other words, the more albumin, the more viscous the blood is. Albumin has the highest affinity for weakly acidic and hydrophobic drugs.

Similar to the case for the pediatric population, members of the geriatric population tend to have decreased stomach acidity. Decreased stomach acidity leads to reduced absorption of a few drugs. As mentioned earlier, iron supplements or oral ketoconazole are best absorbed when taken with an orange juice. Atazanavir (Reyataz) and indinavir (Crixivan), two antiviral agents (see the *Introduction to Microbiology* chapter), require normal acid levels in the stomach for their optimal absorption.

The total body water (TBW) accounts for 60% of total body weight in men and 50% of total body weight in women. While two-thirds of TBW is located in the intracellular compartment, one-third is located in the extracellular compartment. TBW is reduced in elderly patients because of reduced muscle mass and increased lipid storage. Because of the higher rate of lipid storage in such individuals, the availability of hydrophobic drugs in the plasma is low (see the *Introduction to Pharmacokinetics* chapter), which results in a delay in these drugs reaching the excretory organs (e.g., liver, kidneys). The latter process results in an increased duration of action for the drugs.

Elderly individuals are also prone to be affected by physiological changes of aging or pathophysiological consequences that arise from one or more diseases. For instance, the hepatic first-pass effect is reduced in the elderly population, which results in lower hepatic metabolism and ultimately leads to increased plasma concentration of drugs. Likewise, aging reduces the density and sensitivity of receptors. For instance, it has been suggested that the sensitivity of muscarinic, β-adrenergic, α_1-adrenergic, and μ-opioid receptors declines as a consequence of aging. A change in receptors' sensitivity may cause a change in the affinity between a drug and a receptor, a change in the binding of a drug to a receptor, or a change in the structure and function of a receptor.

Obesity

Obesity affects both pharmacokinetics and pharmacodynamics by altering the volume of distribution. The more lipid storage that occurs, the higher the volume of distribution will be for hydrophobic drugs. In turn, the higher the volume of distribution, the longer the half-life of drugs (lower elimination rate; see also the *Introduction to Pharmacokinetics* chapter). For instance, hydrophobic drugs such as benzodiazepines have lower elimination rates in obese patients. Moreover, weight gain often results in insulin resistance. It has been suggested that obese individuals who are not diabetic may have the same level of insulin resistance as patients who have type 2 diabetes. One explanation for this phenomenon could be too much circulating fatty acid in the blood. Fatty acids interfere with glucose uptake into muscle (insulin resistance). This effect could also explain why pregnant women, who produce large amounts of fatty acids, are prone to develop insulin resistance (i.e., gestational diabetes).

Receptors

Many important physiological and biological functions are accomplished by chemical messengers—that is, hormones or ions that arrive via the bloodstream or neurotransmitters that are released by nerve cells. These messengers are received by receptors. Many hydrophilic hormones (e.g., peptide hormones such as insulin and glucagon) interact with receptors located in the plasma membrane and transmit signals to the cytoplasm. The entire process is called transmembrane signaling. In contrast, hydrophobic hormones (thyroid, steroid, and retinoid hormones) are able to cross the cell membrane and interact with their intracellular receptors located inside the cells. As a result, these hydrophobic ligands do not have any transmembrane receptors, but rather have intracellular receptors.

Most often the interaction between the messenger and the receptor is achieved noncovalently, similar to an enzyme–substrate interaction. Hormones are ligands that are the "first messengers," whereas Ca^{2+}, cAMP, IP_3, diacylglycerol, and cGMP are the "second messengers" that are released or synthesized when the hormone binds to its receptor. The roles of second messengers are to stimulate or inhibit a series of biochemical events in the cytoplasm or nucleus.

Many drugs mimic the endogenous ligands and, as a result, are able to bind to physiological receptors. By binding to receptors, these drugs—much like the endogenous ligands—initiate a series of events that leads to biochemical changes in a cell and ultimately results in

pharmacological responses. Because receptors play important roles in receiving extracellular signals and turn those signals into intracellular signals, any alteration in receptors' affinity for their ligands can have immediate biochemical and physiological impacts.

Not all proteins in the plasma membranes are receptors. While some proteins are receptors, others serve as transporters, ion channels, or enzymes. These proteins have essential functions as well. For instance, due to the hydrophobic nature of the lipid bilayer of the plasma membrane, anions and cations cannot readily cross plasma membranes. The influx and outflow of ions are critical regulatory events in both excitable and nonexcitable cells. To maintain the electrochemical gradients required to maintain or produce a membrane potential, all cells express ion transporters for Na^+, K^+, Ca^{2+}, and Cl^-. For instance, the human body expresses more than 200 different ion channels to regulate precisely the flow of Na^+, K^+, Ca^{2+}, and Cl^- across the cell membranes.

The names of receptors are often designated based on the names of the ligands (or a class of ligands) that they bind. For instance, while insulin (ligand) binds to an insulin receptor, norepinephrine (ligand) binds to adrenergic receptors. A single cell may have different receptors for different ligands—for example, a hepatocyte has receptors for insulin and glucagon ligands. In addition, the same receptor for a ligand could be expressed in different cells. For example, insulin receptors are largely expressed on hepatocytes, myocytes, and adipocytes.

Signal transduction refers to the movement of chemical signals (e.g., hormones, neurotransmitters, second messengers) from outside a cell to inside the cell. The movement of signals can follow a simple path, like the ion channels of the plasma membrane that open and close in response to a chemical ligand, or can be more complex, like the coupling of ligand–receptor interactions that leads to a series of protein activation and phosphorylation steps that change enzyme activities and protein conformations in the cytoplasm of a cell.

Transmembrane Signaling and Signal Transduction

The terms *transmembrane signaling* and *signal transduction* have often been used interchangeably, even though there is a slight distinction between the two. Transmembrane signaling refers to sending an extracellular signal through a cell membrane receptor. Signal transduction is a much broader concept, referring to sending a signal through a cell membrane where the receptor may not be an embedded cell membrane receptor, but rather an intracellular receptor. For simplicity, the term "signal transduction" is used throughout this chapter.

Six signal transduction systems are employed by receptors:

1. Gated ion channels
2. Receptor enzymes
3. Serpentine receptors
4. Receptors for hydrophobic ligands
5. Receptors without intrinsic enzyme activity
6. Adhesion receptors

Before these receptors are described and their signaling roles are elucidated, it is necessary to explain a few important molecules that are involved in the signaling pathways.

Adenylyl Cyclase

Adenylyl cyclase (AC) is a large membrane-bound enzyme that is folded multiple times inside the cell membrane. It has 12 transmembrane regions, with two bundles of 6 transmembrane

components. Adenylyl cyclase catalyzes the synthesis of cyclic adenosine-3',5'-monophosphate (cAMP) by acting on an ATP molecule. Ten isoforms have been identified to date, including a recently identified free isoform (soluble) in mammalian sperm. Each isoform has a unique tissue expression and distribution, which may indicate AC catalyzes the formation of cAMP in many different tissues. Each AC has two regulatory components—stimulatory G proteins (designated as Gs) and inhibitory G proteins (designated as Gi)—that stimulate and inactivate the catalytic activity of AC, respectively. Due to AC's involvement in many signaling pathways, certain hormones stimulate AC (e.g., calcitonin, glucagon, epinephrine, antidiuretic hormone) while others inhibit AC (e.g., acetylcholine, somatostatin, angiotensin II).

Guanylyl Cyclase

Guanylyl cyclase, similar to AC, catalyzes formation of a cyclic nucleotide, cyclic guanosine monophosphate (cGMP). Guanylyl cyclase exists in two forms: type 1 (transmembrane) and type 2 (a heme-containing soluble enzyme). There are seven isoforms of type 1 and four isoforms of type 2. Guanylyl cyclases are found in most tissues.

Cardiac atrial tissue (atrial myocytes) express a short peptide (28 amino acids) called atrial natriuretic peptide (ANP) that has important roles in natriuresis, diuresis, vasodilation, and inhibition of aldosterone secretion. The ANP activates the membrane-bound guanylyl cyclase, which results in an increased rate of synthesis of cGMP. In addition, nitric oxide hormone (and even compounds that contain NO) activates the soluble guanylyl cyclase to synthesize cGMP.

Cyclic Adenosine Monophosphate

The second messenger cAMP is synthesized by the adenylyl cyclase enzyme. Cyclic AMP plays important roles in many cellular functions, including cell growth and differentiation, regulation of gene expression, and apoptosis. While cAMP has many targets, perhaps the most relevant one in this chapter is the cyclic AMP-dependent protein kinase (PKA) that is involved in the adrenergic receptors' roles and functions (see the discussion of the role of epinephrine in serpentine receptors later in this chapter).

cAMP is rapidly degraded by an enzyme called cyclic nucleotide phosphodiesterase. As will be discussed in conjunction with serpentine receptors in this chapter, cAMP is involved in activation of glycogen phosphorylase *a* and glycogenolysis and, as a result, plays a significant role in increasing the blood glucose levels.

Cyclic Guanosine Monophosphate

The guanylyl cyclase enzyme catalyzes formation of cyclic GMP by acting on a GTP molecule. Similar to the inactivation of cAMP, cGMP phosphodiesterase (PDE) enzyme degrades cGMP. A few agents, such as sildenafil (Viagra) and tadalafil (Cialis), inhibit cGMP phosphodiesterase (PDE) to increase the level of cGMP. The cyclic GMP plays a major role in promoting smooth muscle relaxation by inhibiting calcium influx, activating potassium channels, and stimulating cGMP-dependent kinase (PKG)—mechanisms that will be discussed further later in this chapter.

Calcium

Calcium is another important second messenger that plays important roles in the signal transduction. The concentration of Ca^{2+} in the cytoplasm is very low (10^{-6} M), while the concentration outside the cell and inside the endoplasmic reticulum (ER) is 10^{-3} M. Calcium regulates a series of responses that include gene expression, contraction, secretion, and metabolism. It can enter a cell through ion channels (Ca^{2+} channels) that are located in the plasma membrane or it can be released

from intercellular storage by hormones or growth factors. For instance, when the calcium channels in the ER open, Ca^{2+} is released into the cytoplasm. Intracellular Ca^{2+} levels cause skeletal muscle cells to contract and stimulate some endocrine cells to secrete hormones.

Because calcium plays an important role in many cellular functions, disruption of calcium influx or efflux can have severe physiological consequences. For instance, damage to the ligand-gated Ca^{2+} channel or voltage-gated Ca^{2+} channel (discussed later) causes Ca^{2+} to move down its concentration gradient into the cytoplasm of cells. The resulting increase in intracellular Ca^{2+} inhibits the ATPase enzyme during oxidative phosphorylation (which results in less synthesis of ATP), alters the function of microfilaments, enhances the activation of hydrolytic enzymes, and increases the generation of reactive species such as reactive nitrogen species (RNS) and reactive oxygen species (ROS), which ultimately damage many other cells.

Some of the serpentine receptors (see the discussion of serpentine receptors later in this chapter), by binding to their ligands, activate their G protein, Gq. For example, when the oxytocin or vasopressin hormones bind to their receptors, the α subunit of Gq in turn binds to and activates a plasma membrane enzyme called phospholipase C. Phospholipase C catalyzes the production of two other second messengers, inositol 1,4,5-triphosphate (IP_3) and diacylglycerol, from the phosphatidylinositol 4,5-bisphosphate found in the plasma membrane. IP_3 has its own receptor. This IP_3 receptor is a large protein and is found in high concentrations in the membrane of the ER. The role of IP_3 molecules is to diffuse through the cytoplasm to the ER and bind to the specific IP_3 receptor. The binding of IP_3 to its receptor stimulates Ca^{2+} channels to open and releases Ca^{2+} into the cytoplasm (**Figure 6.4**). The released Ca^{2+}, together with the diacylglycerol, activates another protein kinase, PKC, which in turn phosphorylates a variety of proteins to change their activities and, consequently, to mediate a wide range of signaling events.

Calcium regulates a number of enzymes, often through a Ca^{2+} binding protein called calmodulin. This small protein is found in all eukaryotic cells. Due to its unique structure, calmodulin is able

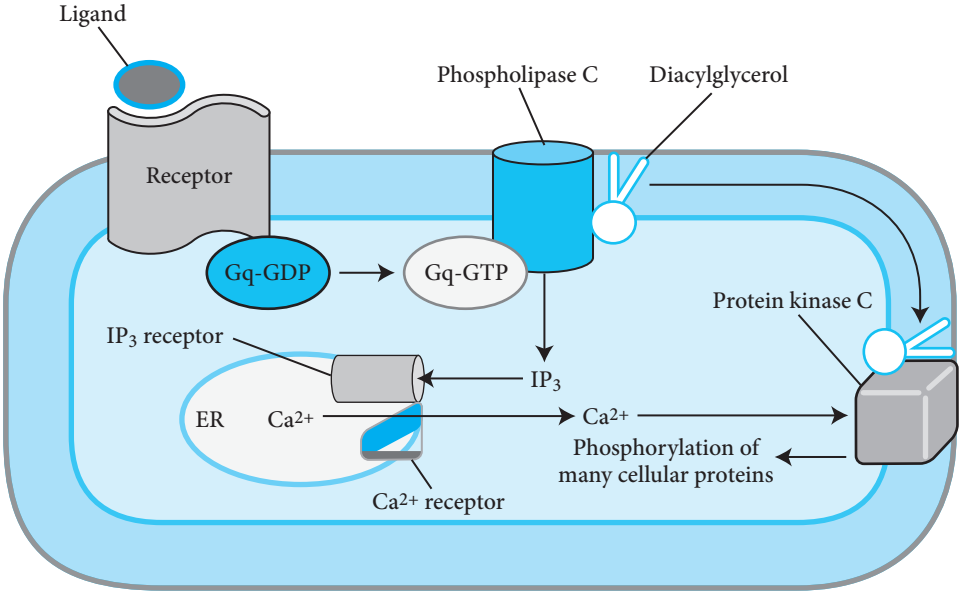

Figure 6.4 Binding of a ligand to its receptor activates phospholipase C, which in turn releases diacylglycerol and IP_3 from the phospholipids of the cell membrane. The overall results are to increase intracellular Ca^{2+} and activate protein kinase C.

Adapted from: Nelson DL, Cox MM, and Lehninger AL. *Principles of biochemistry*, 5th ed. New York: W. H. Freeman and Company; 2008: Chapter 12.

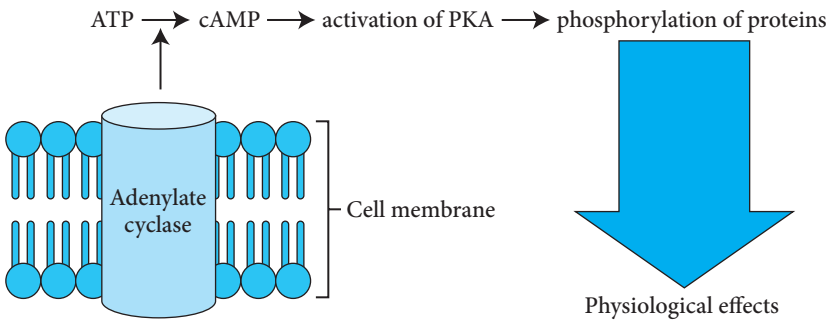

Figure 6.5 The cAMP-dependent protein kinase A (PKA) is activated by cAMP that is synthesized by the adenylate cyclase enzyme.

to bind four calcium ions and, therefore, serves as an intracellular receptor for regulatory calcium signals. An increase in cytosolic Ca^{2+} concentration allows Ca^{2+} to bind to the four binding sites on calmodulin. As a consequence of the Ca^{2+} binding, calmodulin undergoes a conformational change that allows it to interact with other cytoplasmic proteins to influence a series of cellular responses. The activation of calmodulin by Ca^{2+} is similar to the activation of PKA by cAMP, except here the Ca^{2+}–calmodulin complex has no enzymatic activity on its own, but rather activates other proteins and enzymes.

Protein Kinases

Protein kinases are a class of enzymes that transfer a phosphate group from an ATP molecule to a protein—a reversible process called protein phosphorylation. Protein phosphorylation is primarily directed at serine, threonine, and tyrosine amino acid residues in proteins (all of these amino acid residues have an OH group; see also the *Introduction to Cell Biology* chapter). Nearly one-third of our proteins inside of cells are phosphorylated by protein kinases. More than 100 protein kinases have been identified in humans; indeed, the human genome has about 500 protein kinase genes. Protein phosphorylation plays an important role in the regulation of protein kinases. For instance, protein kinase A (PKA) is not activated until a cAMP molecule participates in PKA phosphorylation (**Figure 6.5**). While the overall function of kinases is to phosphorylate other proteins, there are considerable differences among them in regard to their size, subunit composition, autophosphorylation, kinetic parameters (e.g., K_m, k_{cat}), and substrate specificity.

One of the major roles of protein kinases is the regulation of the cell cycle. The passage of a cell from one phase to another through the cell cycle is controlled by proteins called cyclins. Cyclins are regulatory subunits of a series of protein kinases called cyclin-dependent protein kinases (CDKs). These proteins have no kinase activities unless they are tightly bound to the cyclins. For instance, while CDK1 facilitates the transition from the G_2 phase to the M phase during the cell cycle, CDK2 initiates DNA synthesis in the early S phase (**Figure 6.6**). To date, 9 CDKs (referred to as CDK1 through CDK9) and 11 cyclins have been identified in humans.

Gated Ion Channels

Many drugs act on their receptors to stimulate or inhibit the influx of ions through cell membrane channels. For instance, a few drugs that cause calcium influx can affect the release of neurotransmitters and gene expression. Similarly, endogenous ligands can bind to their receptors and open ion channels. For instance, the endogenous ligand known as acetylcholine binds to acetylcholine receptor and increases the Na^+ influx from the extracellular fluid into the cells. Based on gating

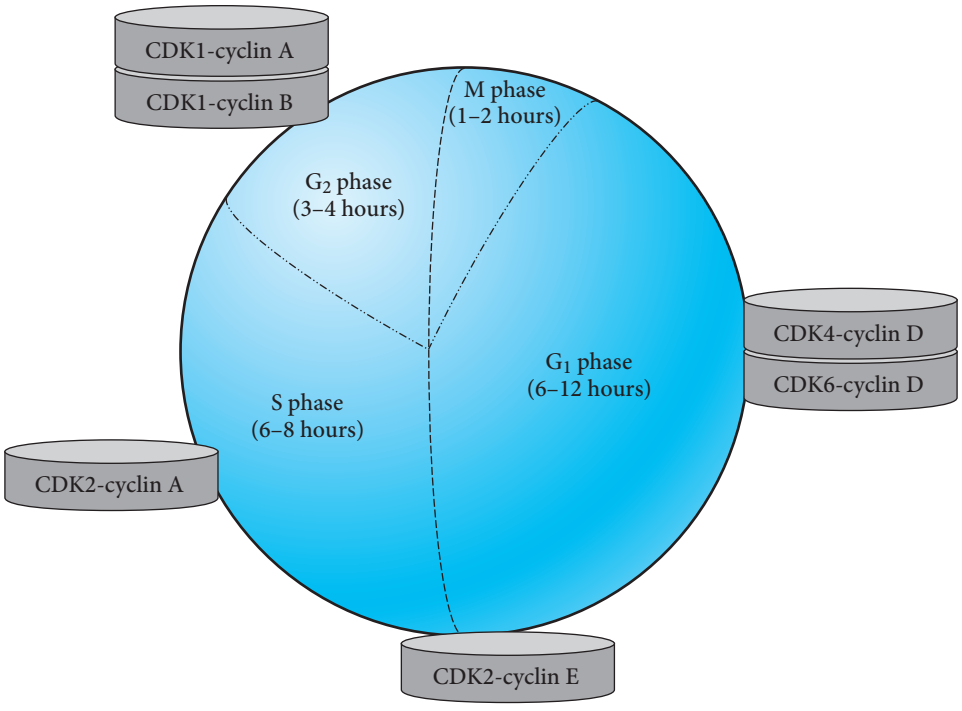

Figure 6.6 The eukaryotic cell cycle and the points where cyclin-dependent protein kinases (CDKs) are activated by different cyclins.

Adapted from: Murray RK, Jacob M, Varghese J. Cancer: an overview. In: Murray RK, Kennelly PJ, Rodwell VW, et al., eds. *Harper's illustrated biochemistry*, 29th ed. New York: McGraw-Hill; 2011. Available at: http://www.accesspharmacy.com/content.aspx?aID=55887057.

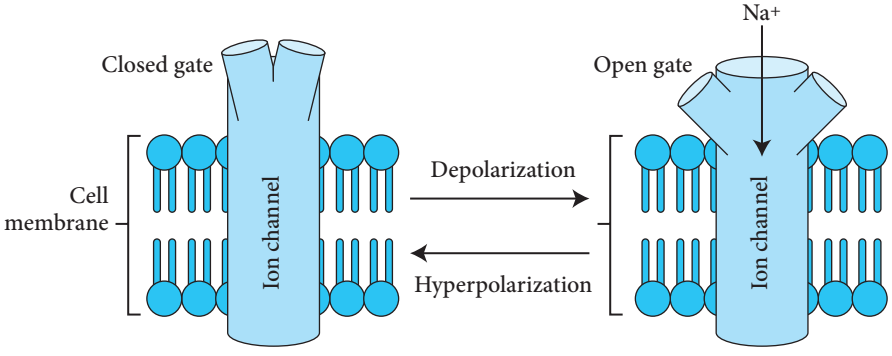

Figure 6.7 Voltage-gated ion channels open and close as a result of changes in the cell membrane potential.

mechanisms, the gated ion channels are classified as either voltage-gated ion channels or ligand-gated ion channels. The movement of ions across these two gates results in an influx of cations into the cell to cause depolarization or an influx of anions to cause hyperpolarization.

Voltage-Gated Ion Channels

Voltage-gated ion channels do not bind to their ligands directly, but rather (and as their name indicates) respond and are controlled by a change in the membrane potential. In other words, the opening probability of these channels depends on the membrane voltage (**Figure 6.7**). Voltage-gated ion channels provide a major pathway for K^+, Cl^-, Na^+, and Ca^{2+} ions in many types of cells. For example, K^+, Na^+, and Ca^{2+} gates open when the membrane potential becomes more positive inside

(i.e., when the membrane potential is depolarized). For instance, the voltage-gated Na^+ channels are closed when the voltage of the membrane is –60 mV (i.e., when the membrane is at rest), but are open when the membrane is depolarized (i.e., when the inside of the cell is positive). The depolarization effect arrives when acetylcholine binds to its receptor. These ion channels are very selective and the rate of influx is high. For instance, the Na^+ channel is 100 times more selective for Na^+ than for Ca^{2+} or K^+ ions, and the rate of ion influx is more than 10 million Na^+ ions per second.

Another example of how the voltage-gated ion channels work, and indeed communicate with other channels, is when the opening of Na^+ channels in the previous example causes depolarization of nerve cells, which in turn opens the voltage-gated Ca^{2+} channels and results in an influx of Ca^{2+} ions. An increased entry of intracellular Ca^{2+} into the presynaptic neuron results in the release of acetylcholine neurotransmitter from the secretory vesicles containing acetylcholine into the synaptic cleft. The acetylcholine binds to its receptor on the next neuron and causes opening of voltage-gated Na^+ channels. The entire cycle is then repeated to send the action potential from one neuron to another.

Use of the calcium-channel blocker agent, verapamil (Calan), demonstrates how blocking these channels causes a physiological change. Verapamil inhibits voltage-gated calcium channels in the vascular smooth muscle and myocardium so as to reduce blood pressure and produce antiarrhythmic effects, respectively. A series of anticonvulsant agents act through the voltage-gated sodium channels. For instance, phenytoin (Dilantin) and carbamazepine (TEGretol) decrease seizure activity by decreasing the influx of Na^+ ions across cell membranes.

Ligand-Gated Ion Channels

To explain this class of gated ion channels, one can return to the neurotransmitter acetylcholine as an example. The acetylcholine receptor and its ligand acetylcholine, and their roles in signal transduction, have been studied extensively. The nicotinic acetylcholine receptor is found in the postsynaptic membrane of neurons at certain synapses and in myocytes at neuromuscular junctions. Synaptic vesicles in synaptic knobs contain approximately 10,000 acetylcholine molecules per vesicle. The released acetylcholine binds to its receptor on the postsynaptic neuron, which in turn results in the influx of Na^+ into the cell.

The acetylcholine receptor serves as an ion channel and opens in response to the neurotransmitter acetylcholine (a ligand—compare it with the voltage-gated ion channel, in which the channel opens as a result of a change in the membrane potential). The receptor (ion channel) is formed from five subunits (two α subunits and one subunit each of β, γ, and δ), where the N termini of the two α subunits bind the neurotransmitter acetylcholine. The effects of acetylcholine on the postsynaptic ion channel are mainly due to protein conformational changes. By binding to its receptor, acetylcholine changes the receptor conformation from a closed to an opened form. As a result, Na^+, Ca^{2+}, or K^+ moves in (other ions cannot move in). The ligand binding is positively cooperative, such that binding to the first α subunit increases the binding affinity of other α subunits to acetylcholine (**Figure 6.8**).

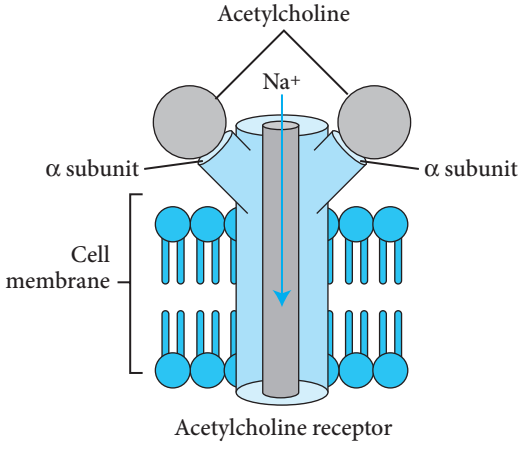

Figure 6.8 A ligand-gated ion channel reacting to its ligand, acetylcholine.

While the role of the acetylcholine receptor channel is to facilitate influx of ions and is a typical example of a ligand-gated ion channel, similar ion channels have been studied for the serotonin

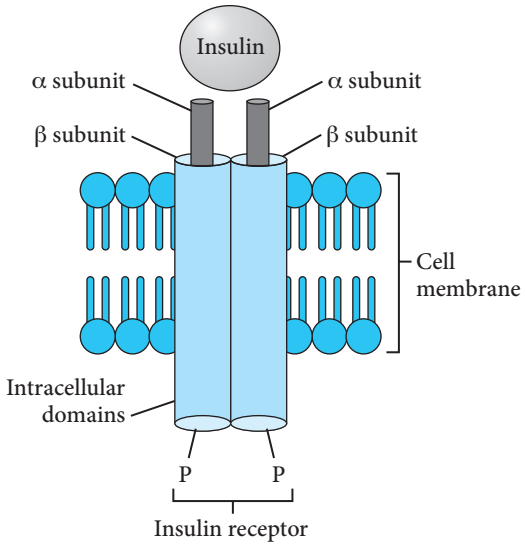

Figure 6.9 A receptor enzyme. The intercellular domains of the insulin receptor undergo auto-phosphorylation upon binding of insulin to its receptor.

neurotransmitter and amino acid ligands such as glutamate and glycine. For instance, binding of the serotonin or glutamate ligands to their receptor channels facilitates ion entry of K^+, Na^+, and Ca^{2+}; in contrast, binding of the glycine ligand to its receptor channel facilitates ion entry of Cl^-. Another example is binding of the major inhibitory neurotransmitter gamma-aminobutyric acid (GABA) to its receptor channel, which facilitates entry of Cl^- ion, causing hyperpolarization of the neuron. Because GABA has an inhibitory effect on the action potential (through entry of Cl^- and hyperpolarization of neurons), benzodiazepines have been suggested to increase the entry of Cl^- ions through the GABA receptor as well. However, benzodiazepines do not compete with GABA for the GABA's binding site, but rather bind to their own binding site on the GABA receptor.

Receptor Enzymes

Receptor enzymes have two domains: a single membrane-spanning domain that faces the extracellular environment for receiving a signal and a catalytic domain that faces the cytosol of a cell. The catalytic domain (or the intracellular domain) could be a protein tyrosine kinase, a serine kinase, or a guanylyl cyclase. The binding of the ligand activates the enzymatic domain, which in turn affects cellular metabolism by phosphorylation of different target proteins (**Figure 6.9**).

In the receptor enzyme signal transduction system, important receptors include epidermal growth factor (EGF), insulin receptor, and many other trophic hormones. Insulin receptor is a glycoprotein that has two α and two β subunits, all of which are stabilized by disulfide bonds. The intracellular domain of the β chains are the site of tyrosine kinase activity. The tyrosine kinase activity of the β subunit is triggered by auto-phosphorylation of Tyr residues in the C-terminal domain of the β subunit. This tyrosine kinase then transfers a phosphoryl group from ATP molecules to the hydroxyl group of Tyr residues of other target proteins such as insulin receptor substrate (IRS).

Because many of the tyrosine kinases are involved in signaling pathways in neoplastic diseases, some of the receptor enzymes are good targets for therapeutic agents that seek to inhibit the signaling pathways. For example, the monoclonal antibody trastuzumab (Herceptin) binds to the extracellular domain of the human epidermal growth factor receptor 2 (HER-2) and thereby produces cellular cytotoxicity by inhibiting the S phase of the cell cycle (recall from the *Introduction to Cell Biology* chapter that the S phase is where DNA, RNA, and protein synthesis occur). In doing so, it suppresses the expression of HER-2 receptors on cancerous cells. The HER-2 receptor is overexpressed in approximately 30% of patients who have developed breast cancer. In addition, this receptor is found, albeit to different degrees, in other malignancies such as ovarian, lung, and prostate cancers. Due to pharmacogenomics and genetic variations among patients, it is imperative to check whether the patient is overexpressing the gene for HER-2 to ensure that he or she will be able to benefit from a trastuzumab treatment, a process that is discussed more in detail in the *Introduction to Pharmacogenomics* chapter.

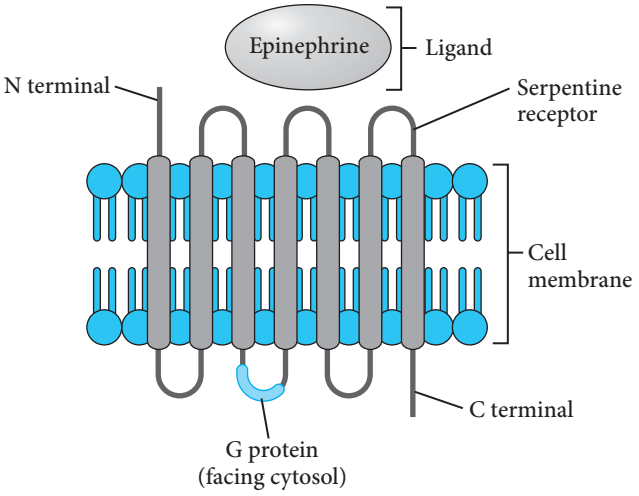

Figure 6.10 A serpentine receptor that has been folded back and forth seven times inside of the cell membrane.

Serpentine Receptors

Serpentine receptors bind to extracellular ligands and enhance intracellular concentrations of second messengers such as cAMP, Ca^{2+}, and phosphoinositides. The second messengers that are small molecules or ions bind to a variety of proteins and thereby change the shapes and functions of those proteins. Upon binding of the ligand to a serpentine receptor, a G protein that is located on the cytoplasmic face of the plasma membrane becomes activated. Consequently, these receptors are called G-protein receptors. A unique characteristic of a serpentine receptor (as indicated by its name) is that the receptor is folded and embedded back and forth seven times across the cell membrane (**Figure 6.10**).

Serpentine receptors are targets of many drugs, including antihistamines, neuroleptics, antidepressants, and antihypertensive agents. An example of a serpentine receptor is the β-adrenergic receptor. In 2012, two scientists, Brian Kobilka and Robert Lefkowitz, won the Nobel Prize in chemistry for their work elucidating the signaling mechanism for serpentine receptors.

The following discussion briefly describes the role of a serpentine receptor. Epinephrine is an adrenergic ligand that binds to its specific receptor (β-adrenergic receptor) on the cell surface. This binding promotes a conformational change in the cytosolic domain of the receptor, which in turn binds to a Gs protein. The α subunit of the Gs protein has the binding site for either GDP or GTP. The ligand-bound receptor causes GTP to replace GDP, such that the Gs protein becomes activated. The Gs protein then activates adenylate cyclase, which catalyzes the synthesis of cAMP. The cAMP-dependent protein kinase, PKA, becomes activated by cAMP. PKA activates phosphorylase *b* kinase, which in turn converts glycogen phosphorylase *b* to glycogen phosphorylase *a*, which finally leads to glycogenolysis (**Figure 6.11**). PKA is able to phosphorylate many physiological targets, including metabolic enzymes, transport proteins, regulatory proteins, other protein kinases, ion channels, and transcription factors.

Different serpentine receptors may utilize the G proteins differently. Indeed, it is known that G proteins facilitate the sending of signals for more than 500 receptors. Whereas Gs activates

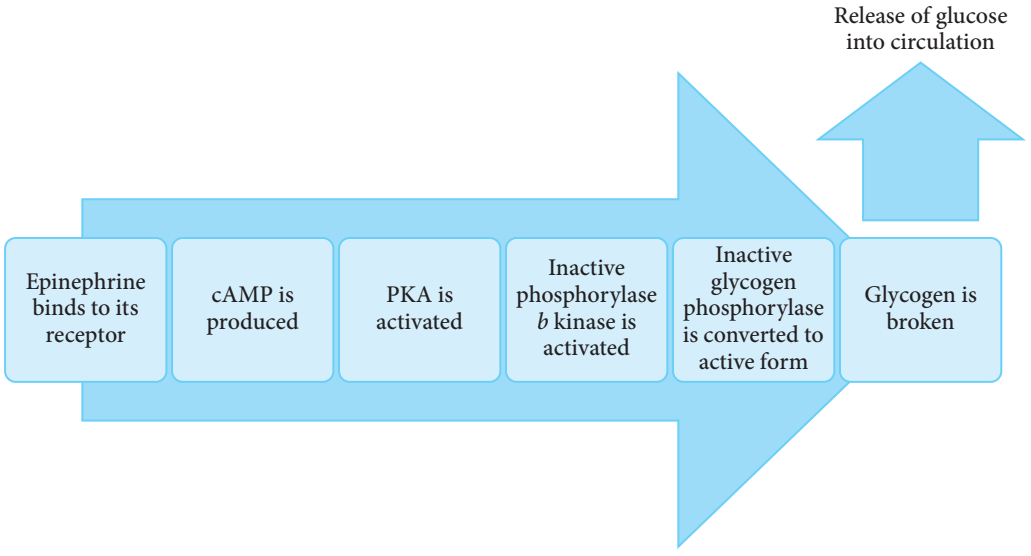

Figure 6.11 The cascade of reactions that occur when epinephrine binds to a serpentine receptor (β-adrenergic receptor).

adenylate cyclase when epinephrine binds to its β-adrenergic receptors, Gq activates phospholipase C to generate diacylglycerol and IP_3 when oxytocin or vasopressin hormones bind to their serpentine receptors. In addition, when epinephrine binds to an α_2-adrenergic receptor, the enzymatic activity of AC is inhibited because the α_2-adrenergic receptor is not coupled to Gs, but rather to Gi, which is an inhibitory G protein.

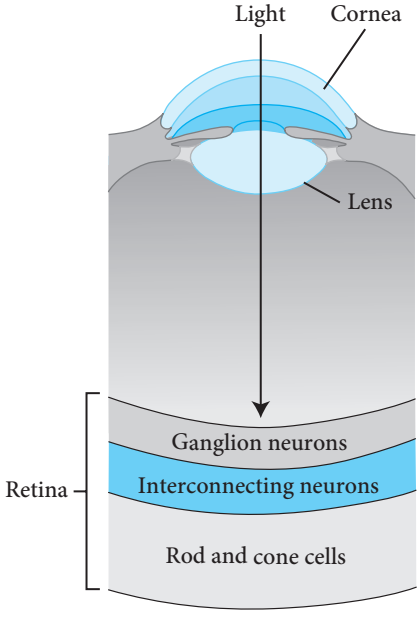

Figure 6.12 The anatomy of the eye where light reaches the retina to induce a signal transduction.

Adapted from: Nelson DL, Cox MM, and Lehninger AL. *Principles of biochemistry*, 5th ed. New York: W. H. Freeman and Company; 2008; Chapter 12.

The detection of light (vision), odors (olfaction), and tastes (gustation) in vertebrates also employs serpentine receptors, which act through the G proteins to change the sensory neurons' electrical potential. The retina is made of three layers of cells: (1) rods and cones; (2) interconnecting neurons (bipolar cells); and (3) ganglion cells (**Figure 6.12**).

Cone cells function in bright light and are responsible for color vision. A human retina contains approximately 6 million cone cells. Because cones require a reasonable amount of light to be stimulated, they function only during the day. This is why we are able to see colors only during the daytime—in other words, when the light intensity is high enough to stimulate the cone receptors.

The light must first pass through both the ganglion cells and the bipolar cells before it can reach and affect the photoreceptors

(rod and cone cells). Rod and cone cells in the retina contain rhodopsin and photopsin, respectively. Rhodopsin and photopsin, which are structurally very similar, contain a light-absorbing pigment. Rhodopsin has a typical serpentine architecture. It consists of a protein called opsin linked to a prosthetic group called 11-*cis* retinal, a vitamin A derivative. When a photon of energy is absorbed by the retinal component of rhodopsin, the conformation of the prosthetic group is altered, which in turn changes the conformation of rhodopsin—the first stage in visual transduction. The conformational changes in rhodopsin (upon receiving photons of energy) result in a decrease in the concentration of cyclic GMP. A decrease in cGMP in the outer segment of the rod cell causes Na^+- and Ca^{2+}-gated ion channels to close. This leads to hyperpolarization of the cell membrane, which triggers an electrical signal to be sent to the brain. The drugs sildenafil and tadalafil, both of which inhibit cGMP phosphodiesterase (PDE), increase the amount of cGMP and cause dose-related impairment of color discrimination.

Interruption of Signaling Pathways

Toxins produced by *Bordetella pertussis* (which causes whooping cough) and *Vibrio cholerae* (which causes cholera, a life-threatening dehydration caused by watery diarrhea) are able to increase the synthesis of cAMP and thereby interrupt many hosts' cellular functions. The cholera toxin, for example, catalyzes the addition of the ADP-ribose moiety of nicotinamide adenine dinucleotide (NAD) to the α subunit of the Gs protein and inhibits the Gs protein's GTPase activity, thereby making the Gs protein remain attached to GTP (which is the active form of Gs). The activated Gs protein stimulates adenylate cyclase to produce cAMP continuously, which in turn stimulates water, bicarbonate, and Cl^- secretion into the intestinal lumen.

Similarly, *B. pertussis* catalyzes the addition of the ADP-ribose moiety of NAD to the Gi protein and inhibits displacement of GDP by GTP. This action finally blocks the effect of Gi to inhibit adenylate cyclase.

Both Gs and Gi are active when they are in their GTP-bound forms and inactive when they are in their GDP-bound forms. Both toxins interfere with signal transduction and affect a series of the metabolic events that are dependent on the signaling pathways.

Graves's disease is another example in which the signaling pathway is interrupted. Graves's disease causes production of antibodies that serve as agonists to the thyroid-stimulating hormone (TSH) protein, a serpentine receptor. Binding of the antibodies activates the AC enzyme, leading to increased synthesis of cAMP. The cAMP stimulates the expression of the thyroglobulin and thyroid peroxidase genes. Ultimately, the overall effect is to cause the thyroid pathophysiology (excessive secretion of the thyroid hormone, T_4) evident in Graves's disease. In addition, in many patients with McCune-Albright syndrome (a rare genetic syndrome characterized by bone disorders, skin hyperpigmentation, and endocrine dysfunction), the G-protein signaling in the TSH receptor is interrupted, such that these patients produce excessive thyroid hormones similar to the case in Graves's disease.

Xanthine derivatives such as caffeine and theophylline are known to inhibit cyclic nucleotide phosphodiesterase and increase the amount of cAMP present. The elevated cAMP level stimulates activation of glycogen phosphorylase *a* and glycogenolysis; and increased blood glucose level in the body soon follows (see also **Figure 6.11**).

Learning Bridge 6.1

Joe is a 28-year-old biochemist who works for the United Nations in its International Affairs division. He just returned from a trip abroad. Joe comes to your pharmacy and complains about dehydration that has been caused by watery diarrhea. One of the attending physicians at a local clinic, which Joe visited yesterday, mentioned that he has contracted some bacterial infections overseas. To Joe's surprise, the attending physician did not prescribe any antibiotic. However, the physician asked him to contact a pharmacist and buy an oral rehydration solution (ORS). You also notice that Joe has sunken eyes and appears weak.

Joe comes to the counter and asks you a few questions:

A. Which kind of infection do I have that does not require an antibiotic?
B. Why have I been asked to buy an ORS?
C. I have a master of science in biochemistry. Can you please tell me, on the cellular level, why I have watery diarrhea?

Receptors for Hydrophobic Ligands

A few ligands are hydrophobic and, as a result, do not have any plasma membrane receptors; instead, their targeted proteins are intracellular receptors. In this class, one can find steroids (corticosteroids, mineralocorticoids, sex steroids, and vitamin D) and thyroid hormones. These receptors are involved in the expression of genes. Because these ligands are hydrophobic, they are strictly dependent on other proteins to assist them in traveling from the site of their synthesis to the site of their action through the blood circulation. For instance, transcortin, androgen-binding proteins, and sex-hormone-binding proteins assist steroid hormones in the blood to travel and reach their target cells, and transthyretin assists thyroid hormones in doing the same.

Once the steroid hormones reach their target cells, they dissociate from their transport proteins, spontaneously pass through the cell membrane and enter the cytoplasm, bind to their receptor, and then migrate to the nucleus. In the nucleus, their function is to induce (or, to a lesser extent, repress) transcription of specific genes. The receptors that bind steroid hormones have a high-affinity ligand-binding property. These receptors belong to a large family of structurally similar DNA-binding proteins. Depending on the nature of the steroid hormones, the receptor-binding complex may occur in the cytoplasm (e.g., for glucocorticoids) or in the nucleus (e.g., for estrogen and androgen).

Binding of steroids to their receptors changes the conformation of these receptors in which the hormone–receptor complex can bind to specific DNA sequences called hormone response element (HRE). The binding to HRE can either activate or repress the transcription of adjacent genes (**Figure 6.13**).

Steroid receptors share some structural similarities. All of the known steroid receptors contain a small DNA-binding domain that contains zinc, which is an essential element for the DNA binding. The zinc stabilizes the structure of the domain; without it, the domain would unfold.

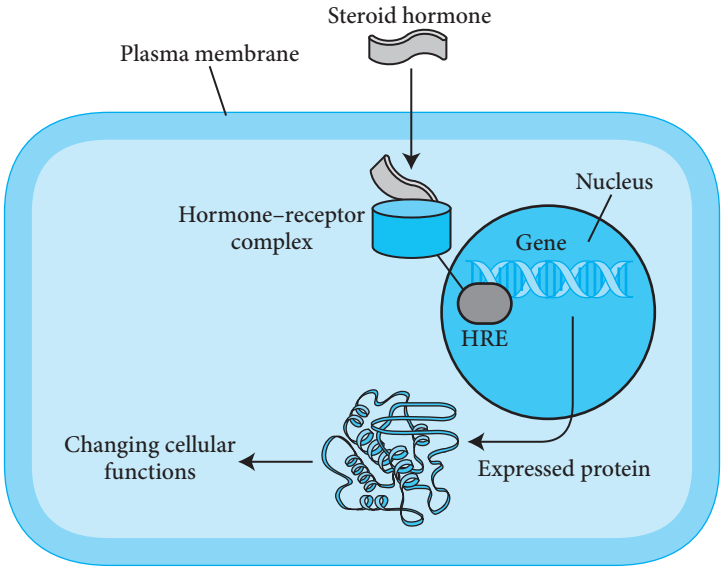

Figure 6.13 Due to steroid hormones' hydrophobic structures, they can readily span the plasma membrane so as to enter the cytoplasm or nucleus.

A hormone–receptor complex can bind to several HREs and, as a result, many gene expressions can be changed by the same hormone. Each type of HRE can influence the transcription of a large number of genes (50–100 genes). For this reason, binding of a steroid hormone–receptor complex to its HRE has a large amplified effect on cellular function.

Steroid hormones—in contrast to peptide hormones, which rapidly act on membrane receptors (recall the roles of glucagon and insulin)—are relatively slow acting. The reason for their slow action is that it takes longer (30 minutes to several hours) to activate the expression of a gene for the synthesis of an enzyme or a protein than to act directly on preexisting enzymes. The slow action partially explains why steroids (e.g., budesonide, fluticasone) are not appropriate for rapidly relieving breathing difficulty in acute bronchial asthma. However, the action of steroid hormones lasts for a longer duration (hours to days) than that of peptide hormones. The reason for the longer effect is that the turnover of most enzymes and proteins expressed by these ligands is slow.

Learning Bridge 6.2

Amy Johnson is a 48-year-old fashion designer who works for a well-known clothing company on the West Coast. Amy was diagnosed with type 2 diabetes six months ago. She comes to your pharmacy for a refill of her prescription, metformin (Glucophage), 500 mg twice daily. Upon a routine review of her medical records, you notice that despite filling her prescription for metformin since her diagnosis, Amy's blood glucose has remained high (120 mg/dL).

You decide to talk to her to learn more about her medical adherence. Amy admits that she occasionally forgets to take the second metformin tablet, although that occurs only rarely. She also mentions that she drinks three cups of tea and three cups of coffee every day.

What is your reaction to Amy's responses? Use your knowledge about signal transduction to justify your answer.

Receptors Without Intrinsic Activity

Some receptor proteins do not have any intrinsic enzyme activities. For instance, cells express cytokine receptors that respond to growth hormone, erythropoietin, and γ-interferon. The intracellular domain of these receptors binds a soluble tyrosine kinase called Janus kinase (JAK). Upon the ligand binding, JAK phosphorylates other proteins that are signal transducers and activators of transcription (STAT, for short) to regulate transcription of a gene. The entire pathway is referred to as the JAK-STAT pathway. Mammals contain four JAKs and six STATs that activate gene transcription differently in different cell types.

Perhaps the most relevant process to describe in this chapter is the binding of erythropoietin (EPO) to its receptor. Erythropoietin is a hormone built of 165 amino acid residues that is synthesized in the kidney. The binding of EPO to its receptor stimulates production of erythrocytes. The EPO receptor has no enzymatic activity. However, the intercellular domain of the EPO-bound receptor complex binds a soluble JAK, which in turn phosphorylates several of the tyrosine residues of the EPO receptor. These phosphorylated tyrosine residues bind a domain of STAT. This process brings the STAT domain close enough to JAK to be phosphorylated by JAK as well. The phosphorylated STAT domain then enters the cell nucleus and promotes expression of genes that are essential for erythrocyte maturation. An erythropoietin deficiency is the underlying cause for anemia in 90% of the patients who have end-stage renal failure and are on dialysis.

Adhesion Receptors

Adhesion molecules such as integrins, cadherins, and selectins (see also the *Introduction to Cell Biology* chapter) and immunoglobulin-like cell adhesion molecules (Ig-CAM) are involved in a variety of signaling pathways. These receptors have both extracellular and intracellular domains. While the extracellular domain is attached to macromolecule proteins such as collagen and fibronectin, the intracellular domain is attached to the cytoskeleton (largely to actin and filaments) of the cytoplasm. Upon receiving the appropriate signals, these adhesion receptors facilitate cell migration, cell–cell adhesion, and platelet aggregation at the site of tissue injury; mediate tissue repair; and perform other critical functions to restore the body to homeostasis. For instance, an integrin receptor has two subunits, α and β (the β subunit is also known as CD18). A genetic mutation that encodes a nonfunctional β subunit results in the immunological disease known as leukocyte adhesion deficiency (LAD). In this disease, the nonfunctional β subunit does not allow leukocytes to adhere to the blood vessel so that they can migrate through the blood vessel to reach the site of an infection. LAD is a genetic disease. Affected infants often suffer from recurrent infections, and many die of infections before the age of 2.

Learning Bridge 6.3

Amy works as a cashier in a grocery store in Hillsboro, Oregon. Her daughter Emily is a 7-year-old first grader who has been diagnosed with occasional bronchospasm (difficulty with breathing). Today, Amy comes to your pharmacy to receive Emily's Proventil. She mentions to you that Amy's sister, Jennifer, also has breathing difficulty. Jennifer is not using Proventil, but rather Pulmicort. Jennifer is very happy with Pulmicort and has suggested that Amy ask her physician to prescribe Pulmicort for Emily. Amy asks you to call Emily's physician and request a prescription for Pulmicort.

(*continues*)

(*continued*)

Your preceptor asks you for your reaction and also asks you to answer the following questions:

A. What would be your answer to Amy's question?
B. What is the major difference between Proventil and Pulmicort?
C. How will you explain to Amy which drug is better for Emily?

Learning Bridge 6.4

At an airport in the Midwest, while you are waiting for your flight to go home over the Christmas break, you observe that one of the pilots is very angry and is arguing with a TSA officer. The pilot claims that he does not understand why, according to the airline policy, he cannot take sildenafil (Viagra) before he flies the plane (some airlines nowadays restrict their pilots from flying for 24 hours after using sildenafil).

You notice that the pilot's argument with the TSA officer has put him in a stressful situation, and he has made what seems to be a gesture indicating chest pain. While you are approaching him, you see that the pilot takes one tablet from a tiny amber glass container and puts it under his tongue. You listen calmly to his complaint and tell him you would be happy to explain why sildenafil may not be appropriate right before his flight. What would be your explanation?

Dose–Response Relationship

Receptor–Ligand Affinity

Receptors play important roles in selecting their ligands (drugs), but it is the concentration of the ligand at a receptor site that governs a biological response. While the majority of interactions of ligands or drugs with their receptors are transient (i.e., reversible), one can find irreversible interactions as well. Here we focus first on reversible receptor–ligand binding. The binding of a receptor with its ligand produces a receptor–ligand complex (RL) that often leads to a conformational change of the receptor. This change can entail either a minor tweak or a major adjustment of the receptor's polypeptide chain.

According to the law of mass action, at equilibrium, the equilibrium constant (K_a, which here is also called the association constant) for a reaction between a receptor and a ligand (shown in the following reaction scheme) is expressed by Equation 6.1:

$$\text{Receptor (R)} + \text{Ligand (L)} \leftrightarrow \text{Receptor–Ligand (RL)}$$

$$K_a = \frac{[RL]}{[R][L]} \qquad (6.1)$$

K_a provides a measure of an affinity of the ligand for the receptor. The [RL] complex is directly related to the concentrations of free ligand (L), free receptor (R), and the association constant (K_a), as indicated in Equation 6.2:

$$K_a\,[R][L] = [RL] \qquad (6.2)$$

The fraction of receptor that is bound to ligand, θ, is shown in Equation 6.3:

$$\theta = \frac{\text{Occupied binding sites}}{\text{Total binding sites}} \tag{6.3}$$

The occupied binding site is [RL] and the total binding site is [RL] + [R]. With respect to this observation, we can rewrite Equation 6.3 as Equation 6.4:

$$\theta = \frac{[RL]}{[RL]+[R]} \tag{6.4}$$

According to Equation 6.2, we can replace [RL] with $K_a[L][R]$ and rewrite Equation 6.4 in the form of Equation 6.5:

$$\theta = \frac{K_a[R][L]}{K_a[R][L]+[R]} \tag{6.5}$$

If we divide all parameters in Equation 6.5 by [R], we obtain Equation 6.6:

$$\theta = \frac{K_a[L]}{K_a[L]+1} \tag{6.6}$$

To simplify Equation 6.6 further, we can divide all parameters in Equation 6.6 by K_a, which yields Equation 6.7:

$$\theta = \frac{[L]}{[L]+\frac{1}{K_a}} \tag{6.7}$$

K_a has units of concentration^{-1} (M^{-1}) and measures affinity; that is, the greater the value of K_a, the greater the affinity. It is, however, more practical to use the reciprocal of the association constant—the dissociation constant, K_d—which has the unit of concentration (M). Therefore Equation 6.7 can also be written as Equation 6.8:

$$\theta = \frac{[L]}{[L]+K_d} \tag{6.8}$$

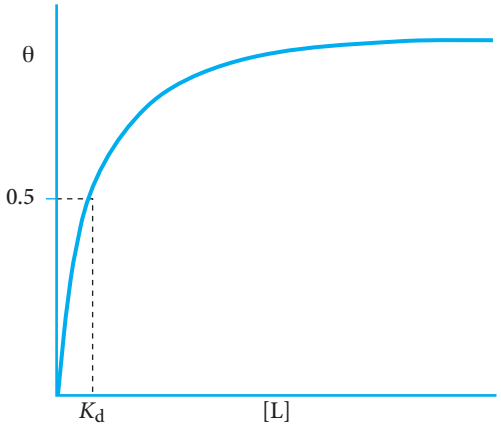

Figure 6.14 If a fixed amount of a receptor is incubated with varying amounts of a ligand, the fraction (θ) of the receptors that are bound with ligand can be determined. The parameter K_d represents the concentration of the ligand at which 50% of the available ligand-binding sites are occupied.

Adapted from: Nelson DL, Cox MM, and Lehninger AL. Principles of biochemistry, 5th ed. New York: W. H. Freeman and Company; 2008; Chapter 5.

If a fixed amount of a receptor is incubated with varying amounts of a ligand, the fraction of the receptors that are bound with ligand (θ) may then be determined. A plot of this fraction versus ligand concentration [L] should yield a hyperbolic curve. The concentration of L that matches to 50% of θ gives the value of K_d (**Figure 6.14**).

Now let's see if the parameters in Equation 6.8 make sense in regard to the ligand-binding affinity. When [L] is equal to K_d, half (50%) of the binding site is occupied. (Check this out: put in 1 for [L] and 1 for K_d in Equation 6.8.) Basically, K_d is the concentration of the ligand at which 50% of the available ligand-binding sites are occupied. For instance, the protein avidin has a binding affinity (K_d) of 1×10^{-15} M to biotin (which is the ligand for avidin). Insulin receptor

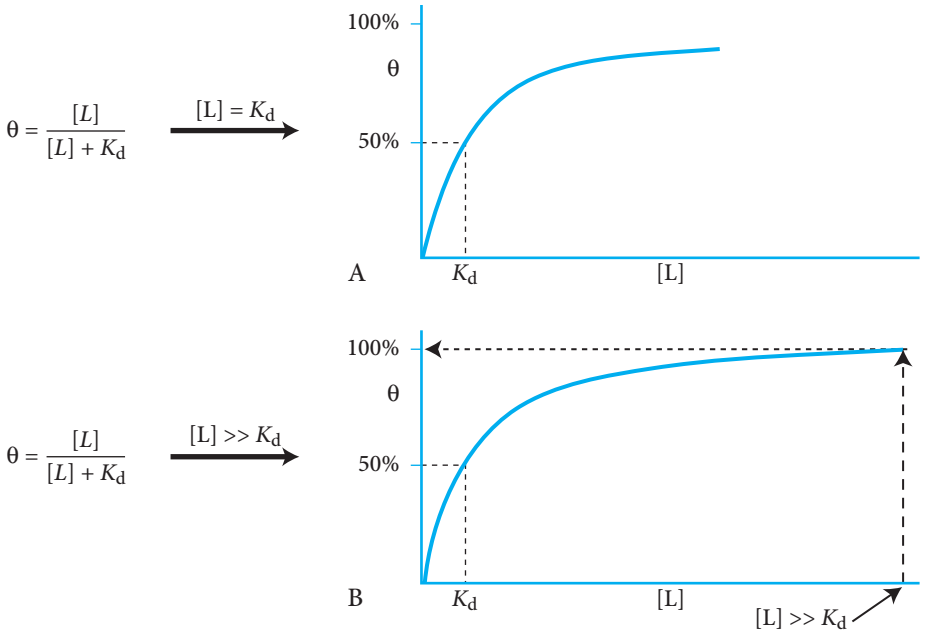

Figure 6.15 (A) When the concentration of a ligand is equal to its K_d, the ligand occupies 50% of the available ligand-binding sites. (B) When the concentration of a ligand is much more (at least 100 times more) than its K_d, the ligand occupies 100% of the available ligand-binding sites.

has a K_d value of 0.1 nM for insulin (ligand). Based on Equation 6.8, the concentration of insulin that is equal to its K_d (0.1 nM) occupies 50% of the insulin receptor binding sites. Based on the same equation, when the insulin concentration is much higher (100 times) than K_d (in our case, 10 nM), insulin hormone occupies 100% of the insulin receptor binding sites. These examples are also shown in **Figure 6.15**.

Plasma Drug Concentration Profile

Efficacy and Potency of Drugs

Efficacy is the ability of a drug to produce a pharmacological response when it interacts with its receptor. The same mathematic description that we used to explain receptor–ligand binding can be used to explain the efficacy of drugs. However, one has to make three important assumptions here:

1. The extent of the biological response is proportional to the amount of receptors that are bound to a ligand.
2. The maximum biological response (E_{max}) is achieved when all receptors are occupied (i.e., when there are no "spare receptors").
3. Binding of a drug to the receptor does not change the affinity of the receptor for another drug (i.e., it does not lead to cooperativity).

Spare receptors are those receptors that do not have to be occupied to produce a maximal response. One can express the binding of a drug to a receptor as follows:

$$\frac{E}{E_{max}} = \frac{[D]}{[D] + K_d} \qquad (6.9)$$

Notice that Equation 6.9 is very similar to Equation 6.8 $\left(\theta = \frac{[L]}{[L] + K_d}\right)$.

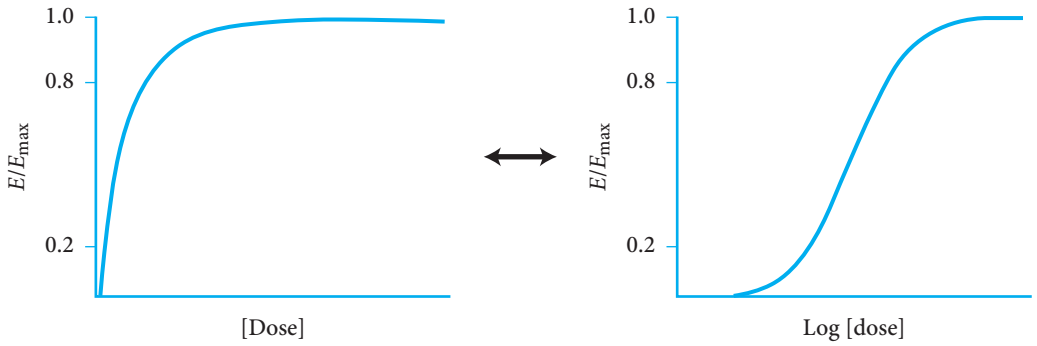

Figure 6.16 Dose–response curves. The logarithm of the dose (or drug concentration) on the *x*-axis is often used to allow the range of the drug concentrations to be more readily perceived.

Adapted from: Clark M, Finkel R, Rey J and Whalen, K. *Pharmacology*, 5th ed. Lippincott Williams and Wilkins; 2012; Chapter 2.

E is the effect of the drug at concentration [D]; E_{max} is the maximal effect of the drug (i.e., when all of the receptors are occupied by the drug); and K_d is the dissociation constant for a given drug [D]. If you plot the ratio of E/E_{max} as a function of a drug dose, you will get a hyperbolic curve. However, often the logarithm of the dose (or drug concentration) is used to allow the range of the drug concentrations to be more readily perceived (**Figure 6.16**).

Equation 6.9 and **Figure 6.17** can be used to explain two important concepts:

- If the drug concentration (or dose) is much higher than K_d, E/E_{max} becomes 1 (or 100%). This indicates the drug molecules have occupied all of the receptor sites (i.e., receptors are saturated with the drug molecules).
- If the drug concentration (or dose) is equal to its K_d, 50% of the maximum effect (EC_{50}) has been achieved by the drug. EC_{50} indicates potency. Basically, the potency is a measure of the concentration (or dose) of the drug that is necessary to produce 50% of the maximal effect (**Figure 6.18**). Keep in mind that potency and EC_{50} have a reciprocal relationship: the higher the value of EC_{50}, the lower the potency.

When EC_{50} is less than K_d, the dose that is required to produce potency is lower than the dose that is required to occupy 50% of the cell's total receptors. This indicates that the cell expresses more receptors than it needs to produce the maximal pharmacological response. In other words, the cell expresses "spare receptors." When spare receptors are present, you should not assume that

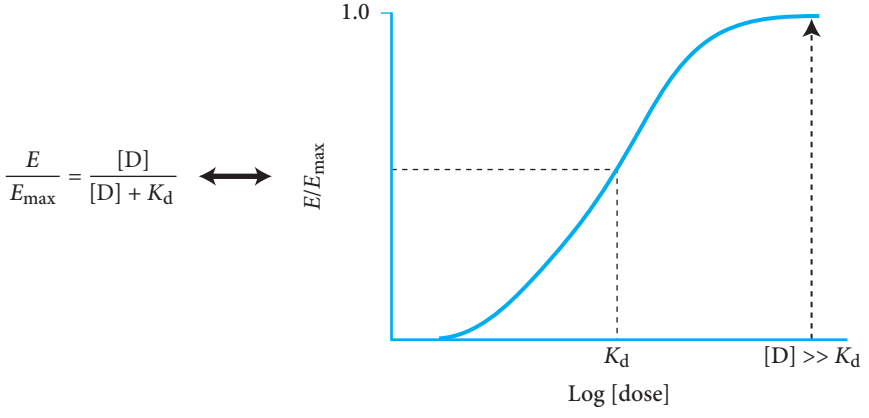

Figure 6.17 In both Equation 6.9 and this figure, when [D] is much larger (at least 100 times larger) than K_d, E/E_{max} becomes 100% and the drug molecules have occupied all of the receptor sites.

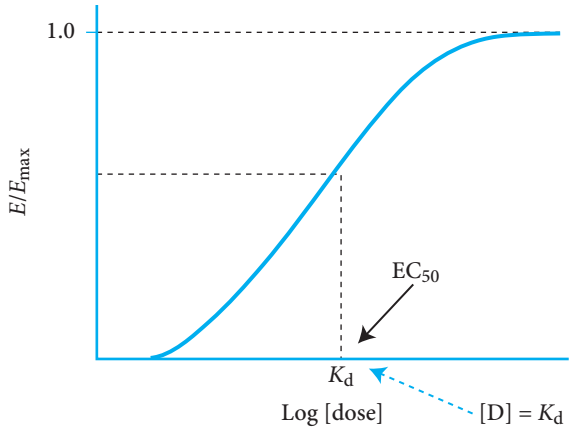

Figure 6.18 The potency of a drug is a measure of the concentration (or dose) of the drug that is necessary to produce 50% of the maximal effect.

Table 6.1 Relationship Between Affinity and Potency

Dose–Response System Without Spare Receptors	Dose–Response System with Spare Receptors
When $EC_{50} = K_d$ EC_{50} ↓; K_d ↓; Potency ↑	When $EC_{50} < K_d$ EC_{50}↓; Potency↑; here you don't know how K_d changes. In other words, you cannot utilize K_d to estimate potency.

EC_{50} is the same as K_d. **Table 6.1** summarizes the relationship between K_d and potency in a dose–response system with and without spare receptors.

Let's use a few examples to make these pharmacodynamic concepts clearer.

Example 6.1: Which of the antipsychotic drugs in **Table 6.2** has the lowest affinity to all three dopamine receptors? Which drug has the highest affinity to the D_3 dopamine receptors?

Table 6.2 Affinity of Different Antipsychotic Agents to Different Dopamine Receptors

Antipsychotic Drugs	K_d (nM)		
	Dopamine Receptor 2 (D_2)	Dopamine Receptor 3 (D_3)	Dopamine Receptor 4 (D_4)
Chlorpromazine	0.55	1.2	9.7
Clozapine	35	83	22
Haloperidol	0.53	2.7	2.3
Quetiapine	105	340	2,000

Modified from: Strange PG. Antipsychotic drugs: importance of dopamine receptors for mechanisms of therapeutic actions and side effects. *Pharmacol Rev.* **2001:53(1)119–134.**

Answer: The affinity of a receptor for a drug is measured by its dissociation constant. The higher the K_d, the lower the affinity. Quetiapine has the lowest affinity and chlorpromazine has the highest affinity for the D_3 dopamine receptors.

Example 6.2: Suppose the drugs in Table 6.2 produce the maximal effect at certain drug concentrations and there are no spare dopamine receptors. Which of these antipsychotic drugs has the lowest potency relative to all three dopamine receptors?

Answer: The potency is the concentration of a drug that produces 50% of the maximal pharmacological response. As there are no spare receptors in this example, the potency or EC_{50} is represented by K_d. The higher the K_d, the higher the EC_{50} and the lower the potency. Quetiapine has the lowest potency for all three dopamine receptors.

Example 6.3: Based on the presented information indicated in **Figure 6.19**, which of the following statements is correct?

A. Chlorpromazine has a higher potency than clozapine.

B. Chlorpromazine has a lower potency than clozapine.

C. Chlorpromazine has a higher efficacy than clozapine.

D. Clozapine has a higher efficacy than chlorpromazine.

E. Clozapine and chlorpromazine have the same efficacy.

Answer: Both A and E are correct. Estimate EC_{50} and you will see that the value for chlorpromazine is lower than the value for clozapine, which means chlorpromazine has a higher potency. As both drugs have the same maximal effect (plateau), both have the same efficacy.

A few parameters are affected by binding of a drug to a receptor. It is important briefly to emphasize their role in pharmacodynamics. Some of these parameters are indicated in **Figure 6.20**.

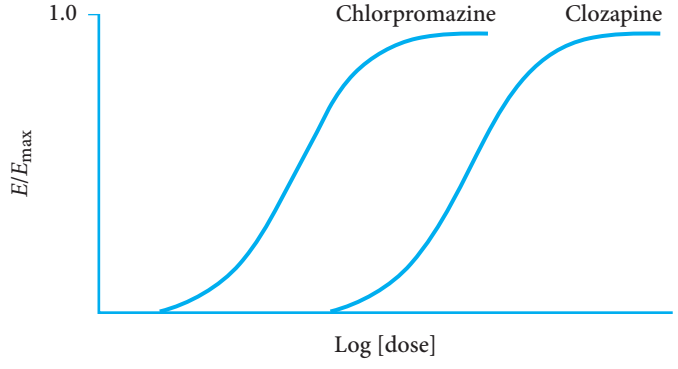

Figure 6.19 A hypothetical dose–response system for chlorpromazine and clozapine.

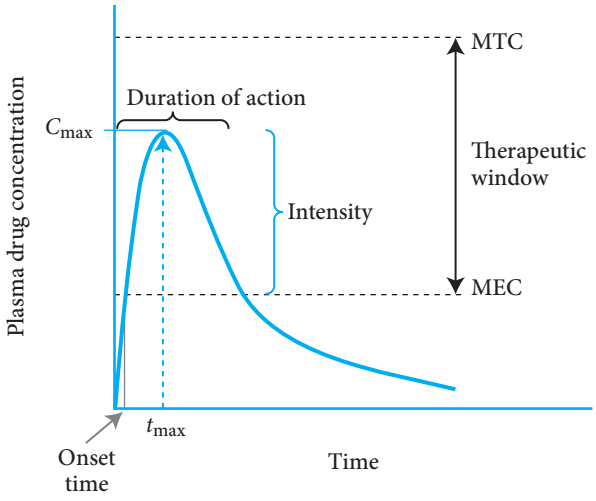

Figure 6.20 A few parameters that play important roles in a dose–response system. This plasma drug profile reflects a single oral dose administration of a drug.
Adapted from: Hedaya M. *Basic pharmacokinetics*. Boca Raton: CRC Press; 2012.

Intensity of a Response: The intensity of a pharmacological response has to do with the number of receptors occupied by a drug. The higher the plasma drug concentration, the greater the pharmacological response—at least until the maximum effect is achieved. The intensity changes in a manner paralleling the peak concentration; in other words, a high peak concentration results in a high intensity.

MEC: The minimum effective concentration is the plasma drug concentration that produces the minimum pharmacological effect.

Onset Time: The onset time is the time required for a plasma drug concentration to produce an MEC.

Duration of Effect: The duration of effect is the time for which the plasma drug concentration stays above the MEC.

Therapeutic Range: The therapeutic range identifies those points when the pharmacological effect is between the MEC and the minimum toxic concentration of the drug in plasma (MTC). Clinicians use therapeutic drug monitoring (discussed later) to maintain the plasma drug concentration within the therapeutic range.

t_{max}: The t_{max} is the time required to achieve the maximum plasma drug concentration.

Therapeutic Drug Monitoring (TDM): TDM is a process in which clinicians use plasma drug concentrations and apply pharmacokinetics and pharmacodynamics to monitor a patient's response to a drug therapy. TDM is often applied to drugs for which a direct relationship has been identified between plasma drug concentration and pharmacological effect. For instance, TDM for digoxin has resulted in a significant reduction in digoxin toxicity.

Therapeutic Index: The ratio between the minimum dose that is toxic for 50% of the population (TD_{50}) and the minimum dose that is effective for 50% of the population (ED_{50}) is referred to as the therapeutic index. The higher the therapeutic index for a drug and the wider the margin between doses, the safer the drug is. In other words, a safe drug is expected to have a large toxic dose and a small effective dose. The therapeutic index can be expressed as follows:

$$\text{Therapeutic index} = \frac{TD_{50}}{ED_{50}} \tag{6.10}$$

For instance, one can compare warfarin with ibuprofen (Advil) to identify which drug has a higher therapeutic index (i.e., which drug is safer). The data in **Figure 6.21** apply to different doses of warfarin and ibuprofen. As shown in the figure, ibuprofen has a higher therapeutic index. Pay attention to the doses that produce the desired therapeutic effect and a toxic effect for 50% of patients.

Therapeutic Window: This parameter is clinically more useful than the therapeutic index; it describes the difference between the MEC and the MTC for a particular drug (**Figure 6.20**). For instance, the MEC for total phenytoin (Dilantin) is 10 μg/mL and the observed MTC is 20 μg/mL. Accordingly, the therapeutic window for phenytoin is 10–20 μg/mL. In other words, 10–20 μg/mL is a safe and acceptable range of plasma concentrations when designing a dosing regimen.

Keep in mind that a receptor can be in its active form even if a ligand is not bound to the receptor. Receptor activity in the absence of ligand is referred to as constitutive activity. If a higher than expected dose of a drug must be administered to produce a pharmacological response, the response is called hyporeactive or tolerance. While endogenous ligands act as the natural ligands to stimulate a receptor, many drugs available on the market either stimulate a receptor or block a receptor from binding its natural ligand. It is important to expand the roles of these drugs in pharmacodynamics.

Agonists

A drug that binds to a receptor and mimics the effects of an endogenous molecule is an agonist drug. For instance, the drug fluticasone (Flovent HFA) mimics corticoid and binds to corticoid steroid receptors to produce an anti-inflammatory effect. Not all agonists for the same receptor have the same affinity, however. For instance, fluticasone has 18 times higher affinity (18 times lower K_d) for the corticoid receptor compared to dexamethasone (Baycadron).

Partial Agonists

A drug that binds to a receptor but is not as effective as the endogenous ligands is called a partial agonist. Even if all of the receptor sites are occupied, the E_{max} will not be produced by a partial agonist. **Figure 6.22** demonstrates dose–response curves for agonist and partial agonist agents.

The reason that a partial agonist does not produce a full effect may not be related to its decreased affinity to the receptor. Instead, it may not be able to fully activate the receptor. There are two scenarios that can happen with partial agonists:

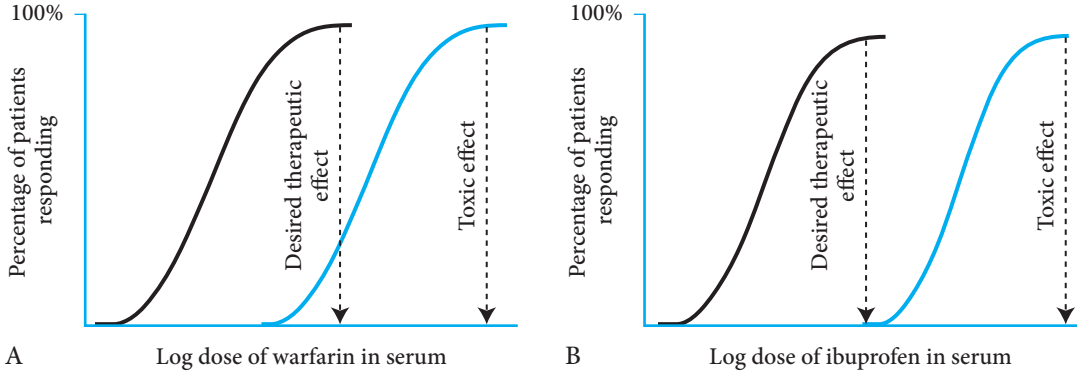

Figure 6.21 Dose–response plots for (A) warfarin and (B) ibuprofen.

Adapted from: Clark M, Finkel R, Rey J and Whalen, K. *Pharmacology*, 5th ed. Lippincott Williams and Wilkins; 2012; Chapter 2.

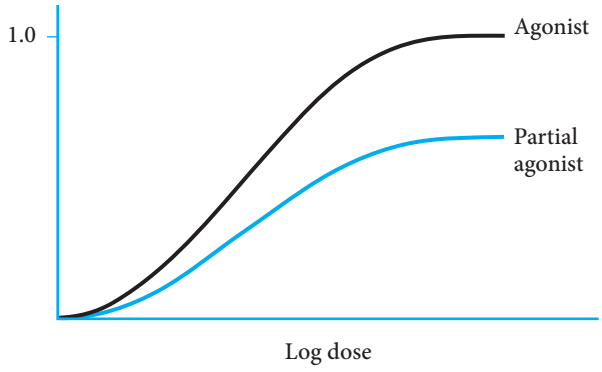

Figure 6.22 Dose–response curves for agonist and partial agonist agents.

1. A partial agonist in the absence of a full agonist serves as a partial agonist.
2. A partial agonist in the presence of a full agonist competes with the full agonist and blocks the agonist's effect.

Antagonists

A drug that binds to a receptor and blocks the binding of the endogenous ligand or another drug is called an antagonist. An antagonist has affinity for a receptor but does not have any intrinsic activity. Four classes of antagonists are distinguished: (1) competitive antagonists, (2) noncompetitive antagonists, (3) chemical antagonists, and (4) physiologic antagonists.

Competitive Antagonists

These drugs bind reversibly to a receptor and compete with an agonist seeking to bind to the same binding site. A competitive antagonist's effects, however, are diminished if the dose of the agonist is increased sufficiently. In the presence of a full agonist, a competitive antagonist shifts the agonist's dose–response curve to the right but does not change the maximum response. A typical example of this type of drug is prazosin (Minipress, an antihypertensive agent), which competes with norepinephrine for binding to α_1-adrenergic receptors. **Figure 6.23** demonstrates how the dose–response curve is shifted for an agonist with and without a competitive antagonist. At higher doses of norepinephrine, the maximum effect from the agonist is achieved.

Noncompetitive Antagonists

A noncompetitive antagonist binds to the receptor at a site different from the agonist binding site (i.e., to an allosteric binding site). It is also possible for an antagonist to bind irreversibly to the

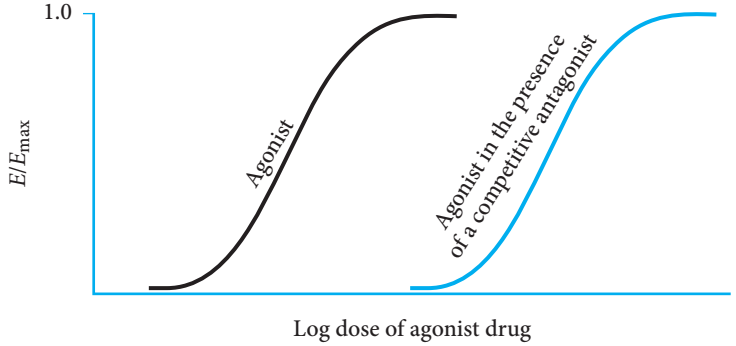

Figure 6.23 A dose–response system in the presence and the absence of a competitive antagonist.

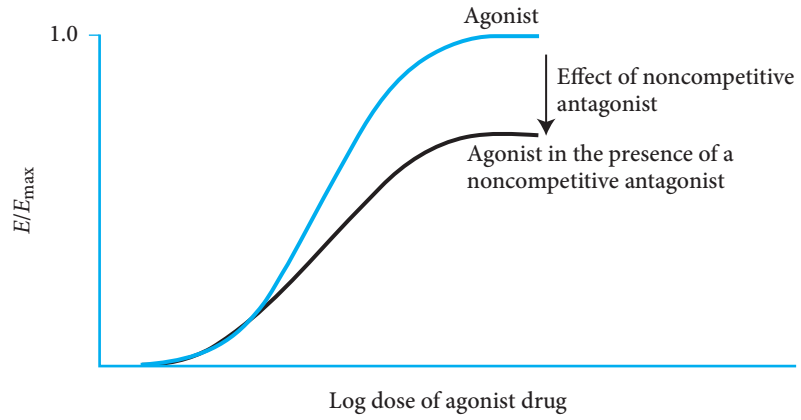

Figure 6.24 A dose–response system in the presence and the absence of a noncompetitive antagonist.

active site and thereby reduce the ability of the agonist to compete and produce a response. Thus, no matter how high the concentration of the agonist dose, the agonist cannot compete with the noncompetitive antagonist (**Figure 6.24**). Phenoxybenzamine (Dibenzyline) is a noncompetitive antagonist to adrenergic receptors that reduces the hypertension risk associated with excess release of catecholamines from an adrenal tumor (pheochromocytoma—a rare neuroendocrine tumor).

The release of a noncompetitive antagonist may be a slow process that results in a prolonged antagonistic effect. For instance, amlodipine (Norvasc) dissociates slowly from calcium channels so that it has a lengthy duration of antihypertensive action (24 hours).

Chemical Antagonists

A chemical antagonist mediates the effect of another drug by binding to and inactivating that drug. One example is protamine sulfate (an antidote for heparin), a highly positively charged molecule that binds to the negatively charged heparin molecule and makes heparin inactive. Because there is no receptor involved in a chemical antagonism process, these agents are also called nonreceptor antagonists.

Physiologic Antagonists

A physiologic antagonist acts by counteracting a regulatory pathway. For instance, propranolol (Inderal LA) is used to treat tachycardia in patients with hyperthyroidism. Although the exact mechanism is not known, an increased level of thyroid hormones (recall the hyperthyroidism observed in Graves's disease) results in an increased number of the beta-adrenergic receptors. To counter this effect, propranolol is often given to patients with hyperthyroidism.

Learning Bridge 6.5

Joe is a 71-year-old retired teacher who used to teach physics in a high school. Today, he comes to your pharmacy to ask you a few questions about his medication, tamsulosin (Flomax), which he started to take 2 days ago. This drug has been prescribed for his prostatic hyperplasia, to treat his bladder outlet obstruction symptoms. Tamsulosin is known to block α_1-receptors in the smooth muscle of the bladder and causes relaxation that reduces resistance to urinary outflow.

(*continues*)

(*continued*)

Joe comes to the counter and asks you three questions:

A. I feel dizzy whenever I stand up from a sitting position. Does my dizziness have anything to do with tamsulosin?
B. When I was looking up information about tamsulosin, I noticed that it was called a competitive antagonist. What does that mean?
C. The information I found stated that this drug inhibits a signaling pathway. Can you please explain what that means?

Assist Joe by answering his questions.

Enhancement of Drug Effects

Three concepts are used to describe an enhancement effect for drugs: addition, synergism, and potentiation.

Addition

In addition, when two different drugs with the same pharmacological effect that either have the same or different strengths are combined, the combined effect is equal to the sum of the individual effect. Such an effect is seen with trimethoprim-sulfamethoxazole (Septra DS), which is used to inhibit bacterial infection. Both trimethoprim and sulfamethoxazole have antibiotic effects, but when they are combined the total effect is equal to the sum of both drugs' effects. Folic acid is reduced to dihydrofolate and then to tetrahydrofolate (THF; the active form of folic acid); sulfamethoxazole inhibits the synthesis of bacterial folic acid. In contrast, trimethoprim inhibits bacterial formation of THF. **Figure 6.25** depicts this addition effect.

Synergism

In synergism, when two different drugs with the same pharmacological effect (that either have the same or different strengths) are combined, the combined effect is greater than the sum of the individual effects (**Figure 6.26**). For example, when penicillin is combined with gentamicin, the penicillin enhances the cellular uptake of gentamicin by bacteria.

A side effect of a medication may also be affected by a synergistic mechanism. For instance, diclofenac sodium (Cambia, an NSAID used to treat osteoarthritis) produces synergistic GI bleeding effects when it is combined with warfarin. In addition, co-administration of two drugs, each with a toxic effect that is tolerable by an organ, can enhance the toxicity and result in organ damage. For instance, combining administration of two nephrotoxic drugs can result in kidney failure, even though the dose of each individual drug alone does not produce toxicity to any significant extent.

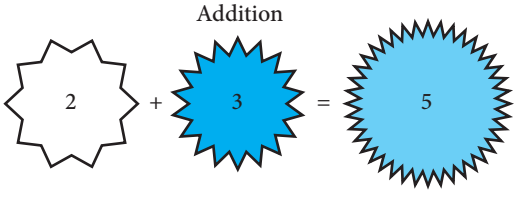

Figure 6.25 The combination of trimethoprim with sulfamethoxazole results in an effect that is equal to the total effect received from the individual trimethoprim and sulfamethoxazole antibiotics. The numbers are arbitrary here.

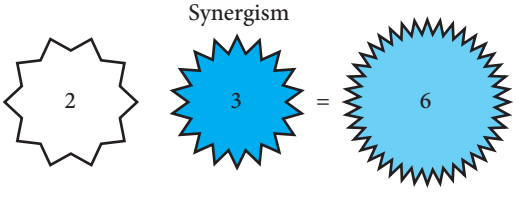

Figure 6.26 The combination of penicillin and gentamicin results in an effect that is greater than the total effect received from these two individual antibiotics. The numbers are arbitrary here.

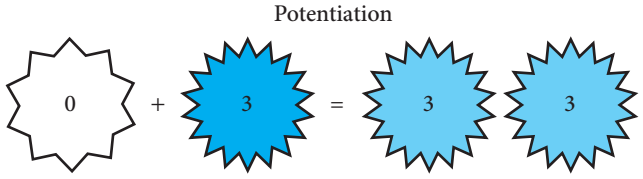

Figure 6.27 The combination of amoxicillin and clavulanic acid results in a better effect compared with using amoxicillin alone. In this example, clavulanic acid has no antibiotic effect. The numbers are arbitrary here.

Potentiation

In potentiation, an ineffective drug increases the effect of another effective drug. For instance, carbidopa alone does not have any significant effect in treating Parkinson's disease. When it is combined with dopa, however, the half-time of dopa is prolonged. Similarly, the agent known as Augmentin includes two drugs: amoxicillin and clavulanic acid. While Augmentin's bactericidal effect is derived from amoxicillin, clavulanic acid has no antimicrobial effect on its own. However, clavulanic acid is important for protecting amoxicillin from destruction. In this way, the effect of Augmentin is enhanced by potentiation (**Figure 6.27**). A third example involves Stribild, which includes four active ingredients: elvitegravir (a protease inhibitor), emtricitabine (a nucleotide reverse transcriptase inhibitor [NRTI]), tenofovir disoproxil fumarate (a NRTI), and cobicistat. Cobicistat has no pharmacological activity of its own, but rather is used to inhibit CYP3A4 and thereby reduce metabolic inactivation of the elvitegravir component in Stribild.

Regulation of Receptors

Because receptors play a crucial role in the signaling pathways, their number, intensity, location, and sensitivity are regulated within the body. While the regulation occurs within seconds for some receptors, it may take days for others.

If a receptor is continuously receiving a ligand (agonist), there is a chance that receptor responses might be reduced, a phenomenon referred to as tachyphylaxis. For instance, a molecule called β-arrestin binds to an intracellular component of the β-adrenergic receptors when these receptors are constantly binding to their agonistic ligands. The role of β-arrestin is to block access of the Gs protein, which might otherwise desensitize the receptor within minutes. When the agonist is removed, β-arrestin also dissociates from the intracellular component of the β-adrenergic receptors, allowing restoration of these receptors back to their full sensitivity.

Another example of regulation arises when an agonist-bound receptor is internalized by an endocytosis process. The endocytosis prevents the ligand from accessing the receptor. The removal of the receptor from the membrane may be only temporary because the internalized receptor may be relocalized into the membrane (which is the case for morphine receptors), or the receptor may be permanently degraded (which is the case for epidermal growth factor receptors). In the latter case, a new receptor will emerge upon expressing its gene, which may take anywhere from hours to a day.

In addition to these mechanisms, both down-regulation and up-regulation of receptors can occur. Down-regulation of receptor expression may take place when a receptor is continuously exposed to an agonistic ligand. Conversely, up-regulation of receptor expression may occur if the receptor is blocked by an antagonist for several days. However, these changes in receptor expressions can be caused by pathophysiological factors as well. As mentioned earlier in this chapter, approximately 30% of patients with breast cancer overexpress the HER-2 receptor. Similarly,

some patients with hyperthyroidism express a higher level of β-adrenergic receptors, which explains why untreated patients with hyperthyroidism show symptoms such as palpitation and tremor. However, the underlying cause for the elevation in β-adrenergic receptors is not fully understood.

Golden Keys for Pharmacy Students

1. Receptors have two major functions: binding to their specific molecules (ligands) and sending signals (signal transduction). Thus there must be at least two domains on a receptor: a ligand-binding domain and an effector domain.
2. A change in drug sensitivity may be the result of a change in the affinity between a drug and a receptor, a change in the binding to a receptor, or a change in the structure and function of a receptor.
3. The large intestine does not have any villi, so it does not provide an adequate surface area for drug absorption.
4. Due to the large surface areas of villi that are lined with epithelial and goblet cells and the 4-hour transit time of drugs, many drugs are absorbed from the small intestine.
5. Polypeptides and proteins are denatured in the acidic environment of the stomach. The more a drug is denatured, the more it is prone to proteolytic hydrolysis.
6. Goblets cells are the second most abundant cells (after epithelial cells) on the villi. Goblet cells produce a mucous layer that makes a film inside the lumen of the small intestine to cover the epithelial cells. The mucous layer does not allow large drug molecules (larger than 800 daltons) to reach the epithelial cells.
7. Many drugs that are absorbed by the GI tract travel through the portal vein to the liver before they are distributed to the general circulation. The venous blood from the stomach, the small intestine, and the large intestine passes into the portal vein and then moves to the liver.
8. The presence of food in the stomach slows gastric emptying, which in turn delays the transport of a drug from the stomach to the small intestine.
9. The stomach acidity is low (achlorhydria) in infants (1 month to 1 year). This leads to increased absorption of basic drugs (which are less ionized in a higher pH) and decreased absorption of acidic drugs (which are more ionized in a higher pH) through the gastric membranes.
10. Drugs that are bound to albumin are pharmacologically inactive because only free drug can act on receptors or other targets. The amount of serum albumin is low in infants, which results in higher levels of unbound drugs in these patients.
11. Albumin has the highest affinity for weak acidic and hydrophobic drugs.
12. The hepatic first-pass effect is reduced in the elderly population, which results in lower hepatic metabolism and, ultimately, increased plasma concentrations of many drug products.
13. The sensitivity of muscarinic, β-adrenergic, α_1-adrenergic, and μ-opioid receptors declines as a consequence of aging.
14. The more lipid storage of hydrophobic drugs that occurs, the higher the volume of distribution, which results in a longer half-life for these drugs (lower elimination rate).
15. Hormones are ligands that are the "first messengers"; Ca^{2+}, cAMP, IP_3, diacylglycerol, and cGMP are the "second messengers" that are released or synthesized when hormones bind to their receptor.

16. Cyclic AMP plays important roles in many cellular functions, including cell growth and differentiation, regulation of gene expression, and apoptosis.

17. The concentration of Ca^{2+} in the cytoplasm is very low (10^{-6} M); the concentration of this ion outside the cell and inside the ER is 10^{-3} M.

18. The detection of light (vision), odors (olfaction), and tastes (gustation) in vertebrates relies on serpentine receptors that act through the G proteins to change the sensory neurons' electrical potential.

19. The toxins produced by *Bordetella pertussis* (which causes whooping cough) and by *Vibrio cholerae* (which causes cholera) are able to increase the synthesis of cAMP and thereby interrupt many hosts' cellular functions.

20. When steroid hormones reach their target cells, they dissociate from their transport proteins, spontaneously pass through the cell membrane and enter the cytoplasm, bind to their receptor, and then migrate to the nucleus. In the nucleus, their function is to induce (or, to a lesser extent, repress) transcription of specific genes.

21. The physiological effect from steroid hormones occurs slowly, whereas the physiological effects of peptide hormones occurs rapidly.

22. Receptors play important roles in selecting their ligands (drugs), but it is the concentration of the ligand at a receptor binding site that governs the biological response to ligand–receptor binding.

23. Spare receptors are those receptors that do not have to be occupied for a ligand or drug to produce the maximal response.

24. Potency and EC_{50} have a reciprocal relationship: the higher the EC_{50}, the lower the potency.

25. The affinity of a receptor for a drug is measured by its dissociation constant. The higher the value of K_d, the lower the affinity.

26. An agonist is a drug that binds to a receptor and mimics the effects of an endogenous molecule.

27. A partial agonist is a drug that binds to a receptor but is not as effective as the endogenous ligands.

28. An antagonist is a drug that binds to a receptor and blocks the binding of the endogenous ligand or another drug.

29. Down-regulation of receptor expression may occur when a receptor is exposed in a continuous manner to an agonist. Up-regulation of receptor expression may occur as well if the receptor is blocked by an antagonist for several days.

30. A noncompetitive antagonist binds to the receptor at a site different from the agonist binding site (i.e., to an allosteric binding site).

Learning Bridge Answers

6.1 A. Joe has most likely been infected with *Vibrio cholerae*. If not treated, this disease can cause life-threatening dehydration and electrolyte imbalance. The causative bacterium is often found in contaminated water. Patients with *V. cholerae* infection recover fairly quickly if they are appropriately treated with an ORS. Tetracyclines such as doxycycline, trimethoprim-sulfamethoxazole, and macrolide agents have been used in severe cases, but the bacterium has developed resistance against these antibiotics.

B. Joe is losing many electrolytes (of particular concern is potassium) in his watery diarrhea, so it is important to treat his electrolyte imbalance. Rapid restoration of the fluid loss should be initiated as soon as possible. It has been suggested that rice-based ORS is more efficacious than glucose-based ORS because the rice-based ORS can reduce fluid requirements.

C. Explain to Joe that the cholera toxin interrupts signal transduction by catalyzing the addition of the ADP-ribose moiety of nicotinamide adenine dinucleotide to the α subunit of the Gs protein and inhibits the Gs protein's GTPase activity; the Gs protein then becomes attached to GTP (the active form of Gs). The activated Gs protein stimulates adenylate cyclase to produce cAMP continuously, which in turn stimulates water, bicarbonate, and Cl^- secretion into the intestinal lumen.

6.2 It seems Amy is in good compliance with her metformin therapy. However, she needs to cut down on the amount of coffee and tea that she drinks. Coffee and tea contain xanthine derivatives—namely, caffeine and theophylline, respectively. These xanthine derivatives inhibit the cyclic nucleotide phosphodiesterase enzyme and increase the amount of cAMP present. The elevated cAMP activates the cAMP-dependent protein kinase (PKA). PKA activates phosphorylase *b* kinase, which in turn converts glycogen phosphorylase *b* to glycogen phosphorylase *a*, which finally initiates glycogenolysis. Consequently, Amy's blood glucose level is increased.

6.3 A. These two drugs are two different medications that are used for different reasons. While Proventil is used for acute breathing difficulty and has a rapid action (1–2 minutes), Pulmicort is not appropriate for use in acute bronchospasm but rather is typically used for chronic breathing difficulty.

B. The active ingredient in Proventil is albuterol, a β_2-adrenergic receptor agonist that has a rapid bronchodilation effect. It stimulates the serpentine β_2-receptor, which in turn activates the adenylate cyclase enzyme to synthesize more cAMP. The cyclic AMP, in turn, activates PKA, which lowers intracellular calcium concentrations. A decrease in the intracellular Ca^{2+} concentration results in smooth muscle relaxation, thereby alleviating bronchospasm. Budesonide (Pulmicort) is also used to prevent shortness of breath and difficulty with breathing associated with lung diseases. Because it is a steroid agent, it will affect nuclear receptors so as to alter expression of a few genes. As a result, it will take hours before any effect is seen with Pulmicort.

C. Although the agent in Pulmicort can help Emily to breathe better, because Pulmicort is a steroid agent it will act slowly; thus it will not assist Emily in rapidly achieving a bronchodilation effect. In addition, it may take 2–6 weeks to see the full effect from Pulmicort—which is not helpful at all for Emily's acute breathing difficulty.

6.4 Sildenafil inhibits phosphodiesterase 5 (PDE5), the enzyme that degrades cGMP. By administering this drug, the pilot will have a higher concentration of cGMP and thereby better vasodilation. However, sildenafil may also inhibit phosphodiesterase 6, the isoenzyme that breaks down cGMP in the rod cells of the retina. A higher concentration of cGMP leads to opened Na^+ and Ca^{2+} channels, and thereby an impaired response of the rod cells to light. Basically, the pilot's vision will be impaired—which is why he should avoid flying an aircraft.

Your observation also indicates that the pilot is suffering from angina pectoris and most likely is using nitroglycerin tablets to relieve his chest pain. Angina pectoris is a very painful symptom of coronary artery disease. This disease occurs when the coronary arteries in the heart become narrowed, making it difficult for oxygen to flow.

Nitroglycerin relieves the pain when it is hydrolyzed into nitric oxide (NO) in the blood. NO activates guanylate cyclase to synthesize cGMP; cGMP, in turn, sequesters Ca^{2+} and thereby enables muscle cells lining the walls of blood vessels to relax (vasodilation). It is wise to advise the pilot to avoid taking sildenafil concurrently with his nitroglycerin tablets, as sildenafil will potentiate the effect of nitroglycerin (i.e., enhance the vasodilatory effect of nitroglycerin).

6.5 A. Tamsulosin is a competitive antagonist of α_1-receptors in the smooth muscle of the bladder and peripheral vasculature that blocks the uptake of catecholamines (norepinephrine and epinephrine). As a result, it not only reduces resistance to urinary outflow, but also results in vasodilation and reduced blood pressure. The reduced blood pressure causes orthostatic hypotension, which explains his dizziness. Advise Joe to have a support handy whenever he stands up from a lying or sitting position to avoid a fall.

B. Tamsulosin binds reversibly to α_1-receptors and competes for the same site against the agonists such as the natural ligands (catecholamines).

C. The α_1-receptors, upon binding to their ligands (catecholamines), activate Gq protein, which in turn activates phospholipase C to synthesize IP_3 and diacylglycerol. Both of these molecules are second messengers that increase intracellular concentration of calcium, thereby producing smooth muscle contraction. Obviously, blocking α_1-receptors will produce the opposite effects, such as bladder relaxation and hypotension.

Problems and Solutions

Problem 6.1 Which of the following physiological conditions is a homeostatic process?

A. Albumin is an abundant plasma protein that uptakes or releases hydrogen ions to maintain the blood's pH.
B. Cells uptake glucose (fuel) and release CO_2 (waste).
C. Termogenin proteins maintain body temperature.
D. Insulin glargine is a long-acting insulin preparation that maintains a constant insulin level for 24 hours.
E. Only A and B
F. All of the above except C
G. All of the above except D
H. All of the above

Solution 6.1 G is correct.

Problem 6.2 The mucosa of the stomach wall has hundreds of gastric glands. Each gastric gland has four major cells. Name these four cells and their functions.

Solution 6.2 Mucous neck cells (secrete mucins); parietal cells (secrete HCl); chief cells (secrete pepsinogen); and enteroendocrine cells (secrete hormones).

Problem 6.3 Which of the following characteristics plays an important role in the absorption of an oral drug? (Choose all that apply.)

A. The long transit time in the small intestine
B. The large surface area at the ileum
C. The large surface area at the duodenum
D. The large surface area at the jejunum
E. The large surface area of villi in the small intestine

Solution 6.3 All except B. While villi are found in the ileum, they are not concentrated there (less surface area). The transit time, which is 4 hours, provides enough time for drugs to travel through the villi and be absorbed there.

Problem 6.4 Why is gastric acid important in the synthesis of hemoglobin?

Solution 6.4 The acidic environment of the stomach converts ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). The latter form is absorbed better and will be used to form heme for the synthesis of hemoglobin. Approximately two-thirds of the body's iron is found inside hemoglobin and myoglobin proteins, and one-third is stored in the iron-storage protein, ferritin.

Problem 6.5 True or false? Erythromycin and azithromycin belong to the class of macrolide antibiotics, all of which have a similar mechanism of action. The drug absorption rate for erythromycin and azithromycin is the same in all areas of the GI tract.

Solution 6.5 False. Different areas of the GI tract have different surface areas and different pH. Having the same mechanism of action and coming from the same class does not mean that drugs are similarly absorbed from the GI tract. For instance, while the acidic pH of stomach destroys erythromycin, the low pH does not affect azithromycin.

Problem 6.6 Haloperidol has a K_d of 0.53 nM for dopamine receptor 2. Suppose haloperidol can produce its maximum antipsychotic effect at a certain concentration. Based on the information given here, suggest an EC_{50} and a haloperidol concentration that would produce the E_{max} for haloperidol. Suppose there are no spare dopamine receptors.

Solution 6.6 Based on the given information, the concentration of haloperidol that is equal to its K_d (0.53 nM) is the EC_{50} or potency. When the drug concentration is much higher (100 times) than K_d (in our case, 53 nM), haloperidol occupies 100% of the D_2 receptor binding sites and produces an E_{max} (maximal effect). Remember, if the spare receptors exist, you cannot assume that EC_{50} and K_d are the same.

Problem 6.7 Penicillin doses are commonly given in amounts 10 times higher than penicillin's MEC. Penicillin is a typical example of a drug with: (Choose all that apply.)

A. a large therapeutic index.
B. a narrow therapeutic index.
C. a large therapeutic window.
D. a small therapeutic window.
E. a partial agonistic effect.

Solution 6.7 A and C are correct.

Problem 6.8 Probenecid is an NSAID that is an acidic drug. Diazepam (which is used to treat anxiety disorders) is a hydrophobic drug. Which of the following statements is (are) correct?

A. Probenecid is more free (unbound to albumin) than diazepam in the plasma.
B. Diazepam is more free (unbound to albumin) than probenecid in the plasma.
C. Diazepam has a lower elimination rate in obese patients.
D. Diazepam has an increased duration of action in elderly patients.

Solution 6.8 All except B are correct. C and D are correct because while the volume of distribution is decreased for water-soluble drugs, it is increased for hydrophobic drugs. This results in an increased $t_{1/2}$ (or lower elimination rate) for diazepam.

Problem 6.9 Ranitidine is an inhibitor of histamine H_2-receptors of the gastric parietal cells that inhibits HCl secretion into the stomach. However, the presence of a large amount of histamine will overcome ranitidine's inhibitory effect. Which of the following statements is (are) correct?

A. Histamine is a full agonist.
B. Histamine is a partial agonist.
C. Ranitidine is a competitive antagonist.
D. Ranitidine is a noncompetitive antagonist.
E. Ranitidine is a chemical antagonist.

Solution 6.9 A and C are correct. Histamine is the natural (endogenous) ligand for the H_2-receptors. Because the presence of a large amount of histamine reverses the inhibitory effect of ranitidine (i.e., histamine forces ranitidine to leave the receptor binding site), ranitidine must be a competitive antagonist.

Problem 6.10 True or false? Aripiprazole (an antipsychotic agent) is a partial agonist agent for dopamine receptors that competes with the full agonist (dopamine) for binding to D_2 receptors. An increasing concentration of aripiprazole reduces the response from dopamine to zero.

Solution 6.10 False. Remember, the characteristic in this problem is for a competitive antagonist—not for a partial agonist. A partial agonist in the presence of a full agonist acts as a partial antagonist. Therefore, increasing the concentration of aripiprazole will inhibit the response to only a certain level (a "partial" level and not to a zero level). A competitive antagonist, however, will reduce the response to zero.

Problem 6.11 Which of the following statements is correct regarding a dose–response system with no spare receptor?

A. If the drug concentration is higher than the K_d, then E/E_{max} becomes 1 (or 100%).
B. If the drug concentration is equal to the K_d, 50% of the maximum effect (EC_{50}) has been achieved by the drug.
C. The higher the EC_{50}, the lower the potency.
D. All of the above.

Solution 6.11 C is correct.

Problem 6.12 Give an example of a ligand-gated channel.

Solution 6.12 Acetylcholine receptor is an example of a ligand-gated channel.

Problem 6.13 Describe how binding of the epinephrine hormone to a β-adrenergic receptor can increase the blood glucose level.

Solution 6.13 Upon binding of the epinephrine hormone to its receptor, GTP is exchanged for GDP on a GTP-binding protein (Gs), which in turn activates adenylate cyclase to synthesize cAMP. cAMP activates the cAMP-dependent protein kinase (PKA). PKA activates phosphorylase *b* kinase, which in turn converts glycogen phosphorylase *b* to glycogen phosphorylase *a*, which finally initiates glycogenolysis.

Problem 6.14 One day when you are completing one of your rotations in a hospital, you decide to take a break and go to the cafeteria. One of the researchers at the same hospital comes to the cafeteria, too. She knows that you have finished all those basic and clinical sciences and thinks you might be able to help her with her question. She has cultured some cells from a tumor biopsy but is not sure how she should look for any oncogene in her cell culture. Using your signal transduction knowledge, how would you respond?

Solution 6.14 Because the researcher is looking for an oncogene, the fastest way to figure out if such a gene exists is to look for a G protein. When a mutation in a G protein destroys its GTPase activity, it can no longer inactivate itself by converting bound GTP to GDP. Once activated,

the mutant G protein continues to send its unregulated signal. This leads to frequent activation of many protein kinases that are involved in the cell cycle. The result will be cancer.

References

1. Barrett KE, Barman SM, Boitano S, Brooks HL. Overview of cellular physiology in medical physiology. In: Barrett KE, Barman SM, Boitano S, Brooks HL, eds. *Ganong's review of medical physiology.* 24th ed. New York, NY: McGraw-Hill; 2012. Available at: http://www.accesspharmacy.com/content.aspx?aID=56260315.
2. Barrett KE, Barman SM, Boitano S, Brooks HL. Regulation of extracellular fluid composition and volume. In: Barrett KE, Barman SM, Boitano S, Brooks HL, eds. *Ganong's review of medical physiology.* 24th ed. New York, NY: McGraw-Hill; 2012. Available at: http://www.accessmedicine.com/content.aspx?aID=56265999.
3. Bauer LA. Clinical pharmacokinetics and pharmacodynamics. In: DiPiro JT, Talbert RL, Yee GC, et al., eds. *Pharmacotherapy: a pathophysiologic approach,* 7th ed. New York, NY: McGraw-Hill; 2008. Available at: http://www.accesspharmacy.com/content.aspx?aID=3197852.
4. Blumenthal DK, Garrison JC. Pharmacodynamics: molecular mechanisms of drug action. In: Brunton LL, Chabner BA, Knollmann BC, eds. *Goodman & Gilman's the pharmacological basis of therapeutics,* 12th ed. New York, NY: McGraw-Hill; 2011. Available at: http://www.accesspharmacy.com/content.aspx?aID=16658452.
5. Chapron DJ. Drug disposition and response. In: Delafuente JC, Stewart RB, eds. *Therapeutics in the elderly*, 3rd ed. Cincinnati, OH: Harvey Whitney; 2000: 257–288.
6. Clark M, Finkel R, Rey J, Whalen, K. *Pharmacology*, 5th ed. Baltimore, MD: Lippincott Williams & Wilkins; 2012.
7. Cusack BJ. Pharmacokinetics in older persons. *Am J Geriatr Pharm*. 2004;2:274–302.
8. Diez J, Simon MA, Anton F, et al. Tubular sodium handling in cirrhotic patients with ascites as analyzed by the renal lithium clearance method. *Eur J Clin Invest.* 1990;20:266–271.
9. Gough DJ, Levy DE, Johnstone RW, Clarke CJ. IFN-signaling: does it mean JAK-STAT? *Cytokine Growth Factor Rev*. 2008;19:383–394.
10. Gregus Z. Mechanisms of toxicity. In: Gregus Z, ed. *Casarett & Doull's essentials of toxicology*, 2nd ed. New York, NY: McGraw-Hill; 2010. Available at: http://www.accesspharmacy.com/content.aspx?aID=6479256.
11. Guay D, Artz MB, Hanlon JT, Schmader KE. The pharmacology of aging. In: Tallis R, Fillit H, eds. *Brocklehurst's textbook of geriatric medicine*, 6th ed. London, UK: Churchill-Livingstone; 2003:155–161.
12. Hedaya M. *Basic pharmacokinetics*. Boca Raton, FL: CRC Press, 2012.
13. Iber FL, Murphy PA, Connor ES. Age-related changes in the gastrointestinal system: effects on drug therapy. *Drugs Aging*. 1994;5:34–48.
14. Ishizu H, Shiomi S, Kawamura E, et al. Gastric emptying in patients with chronic liver diseases. *Ann Nucl Med.* 2002;16:177–182.
15. Kane RL, Ouslander JG, Abrass IB. Clinical implications of the aging process. In: *Essentials of clinical geriatrics*, 5th ed. New York, NY: McGraw-Hill; 2004:3–15.
16. Kohlmeier M, Vitamin K. In: *AccessScience.* New York, NY: McGraw-Hill; 2008. Available at: http://www.accessscience.com.
17. Martin S, Jung R. Gastrointestinal infections and enterotoxigenic poisonings. In: Talbert RL, DiPiro JT, Matzke GR, et al., eds. *Pharmacotherapy: a pathophysiologic approach*, 8th ed. New York, NY: McGraw-Hill; 2011. Available at: http://www.accesspharmacy.com/content.aspx?aID=8003383.

18. Masoro EJ. Physiology of aging. In: Tallis R, Fillit H, eds. *Brocklehurst's textbook of geriatric medicine*, 6th ed. London, UK: Churchill-Livingstone; 2003:291–299.
19. Medina PJ, Shord SS. Cancer treatment and chemotherapy. In: Talbert RL, DiPiro JT, Matzke GR, et al., eds. *Pharmacotherapy: a pathophysiologic approach*, 8th ed. New York, NY: McGraw-Hill; 2011. Available at: http://www.accesspharmacy.com/content.aspx?aID=8007280.
20. Morshed SA, Ando T, Latif R, Davies TF. Neutral antibodies to the TSH receptor are present in Graves' disease and regulate selective signaling cascades. *Endocrinology*. 2010;151(11):5537–5549.
21. Murray RK, Jacob M, Varghese J. Cancer: an overview. In: Murray RK, Kennelly PJ, Rodwell VW, et al., eds. *Harper's illustrated biochemistry*, 29th ed. New York, NY: McGraw-Hill; 2011. Available at: http://www.accesspharmacy.com/content.aspx?aID=55887057.
22. Nelson DL, Cox MM, Lehninger AL. *Principles of biochemistry*, 5th ed. New York, NY: W. H. Freeman and Company; 2008.
23. Osgood M, Ocorr K. *The absolute, ultimate guide to Lehninger principles of biochemistry*, 4th ed. New York, NY: W. H. Freeman and Company; 2005.
24. Pandit NK, Soltis RP. *Introduction to pharmaceutical sciences: an integrated approach*, 2nd ed. Baltimore, MD: Wolters Kluwer, Lippincott Williams & Wilkins; 2012.
25. Patterson RL, Boehning D, Snyder SH. Inositol 1,4,5-trisphosphate receptors as signal integrators. *Annu Rev Biochem.* 2004;73:437–465.
26. Ruhnke M, Yeates RA, Pfaff G, et al. Single-dose pharmacokinetics of fluconazole in patients with liver cirrhosis. *J Antimicrob Chemother.* 1995;35:641–647.
27. Sauer H. Cell differentiation. In: *AccessScience.* New York, NY: McGraw-Hill; 2012. Available at: http://www.accessscience.com.
28. Sotaniemi EA, Arranto AJ, Pelkonen O, Pasanen M. Age and cytochrome P450–linked drug metabolism in humans. *Clin Pharmacol Ther.* 1997;61:331–339.
29. Starner CI, Gray SL, Guay DR, et al. Geriatrics. In: DiPiro JT, Talbert RL, Yee GC, et al., eds. *Pharmacotherapy: a pathophysiologic approach*, 7th ed. New York, NY: McGraw-Hill; 2008. Available at: http://www.accesspharmacy.com/content.aspx?aID=3190958.
30. Strange PG. Antipsychotic drugs: importance of dopamine receptors for mechanisms of therapeutic actions and side effects. *Pharmacol Rev.* 2001:119–134.
31. Treish I, Schwartz R, Lindley C. Pharmacology and therapeutic use of trastuzumab in breast cancer. *Am J Health Syst Pharm.* 2000;57:2063–2076; quiz 2077–2079.
32. Trevor AJ, Katzung BG, Masters SB. Pharmacodynamics. In: Trevor AJ, Katzung BG, Masters SB, eds. *Pharmacology: examination & board review*, 9th ed. New York, NY: McGraw-Hill; 2010. Available at: http://www.accesspharmacy.com/content.aspx?aID=6543122.
33. UpToDate. Waltham, MA: 2012. Available at: http://www.uptodate.com/online with subscription.
34. von Zastrow M. Drug receptors and pharmacodynamics. In: Katzung BG, Masters SB, Trevor AJ, eds. *Basic & clinical pharmacology*, 12th ed. New York, NY: McGraw-Hill; 2012. Available at: http://www.accesspharmacy.com/content.aspx?aID=55820126.
35. Weil P. Hormone action and signal transduction. In: Murray RK, Kennelly PJ, Rodwell VW, et al., eds. *Harper's illustrated biochemistry*, 29th ed. New York, NY: McGraw-Hill; 2011. Available at: http://www.accesspharmacy.com/content.aspx?aID=55885339.
36. Westfall TC. Neurotransmission: the autonomic and somatic motor nervous systems. In: Brunton LL, Chabner BA, Knollmann BC, eds. *Goodman & Gilman's the pharmacological basis of therapeutics*, 12th ed. New York, NY: McGraw-Hill; 2011. Available at: http://www.accesspharmacy.com/content.aspx?aID=16659803.