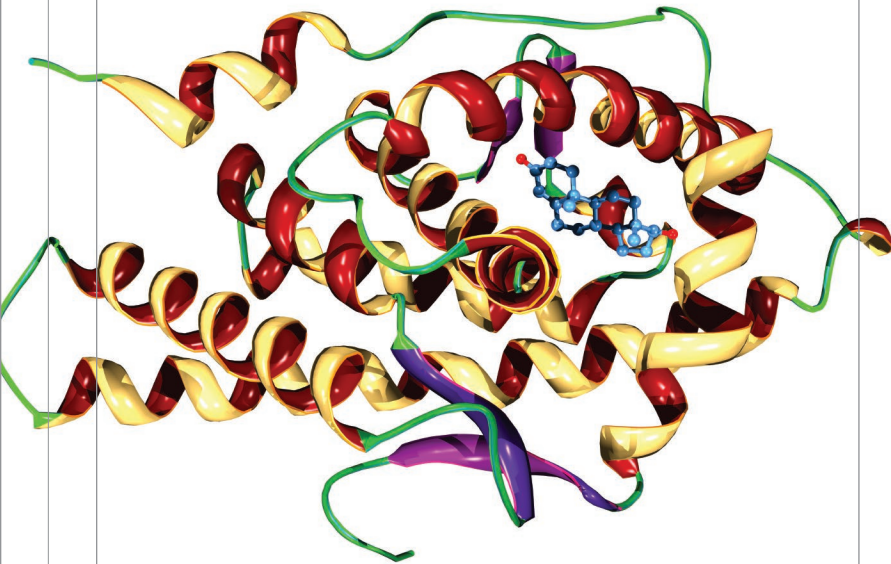


# Cell Signaling in Physiology



Computerized image of a ligand (ball and stick model in blue) binding to its receptor (ribbon diagram). ©Dr. Mark J. Winter/Science Source

**Y**ou learned in Chapter 1 how homeostatic control systems help maintain a normal balance of the body's internal environment. The operation of control systems requires that cells be able to communicate with each other, often over long distances. Much of this intercellular communication is mediated by chemical messengers. This chapter describes how these messengers interact with their target cells and how these interactions trigger intracellular signals that lead to the cell's response. Throughout this chapter, you should carefully distinguish *intercellular* (between cells) and *intracellular* (within a cell) chemical messengers and communication. The material in this chapter will provide a foundation for understanding how the nervous, endocrine, and other organ systems function. Before starting, you should review the material covered in Section C of Chapter 3 for background on ligand–protein interactions.

The material in this chapter illustrates the general principle of physiology that information flow between cells, tissues, and organs is an essential feature of homeostasis and allows for integration of physiological processes. These many and varied processes will be covered in detail beginning in Chapter 6 and will continue throughout the book, but the mechanisms of information flow that link different structures and processes share many common features, as described here. ■

## 5.1 Receptors

*Types of Receptors*

*Interactions Between Receptors and Ligands*

*Regulation of Receptors*

## 5.2 Signal Transduction Pathways

*Pathways Initiated by Lipid-Soluble Messengers*

*Pathways Initiated by Water-Soluble Messengers*

*Major Second Messengers*

*Other Messengers*

*Cessation of Activity in Signal Transduction Pathways*

## Chapter 5 Clinical Case Study

## 5.1 Receptors

In Chapter 1, you learned that several classes of chemical messengers can communicate a signal from one cell to another. These messengers include molecules such as neurotransmitters and paracrine substances, whose signals are mediated rapidly and over a short distance. Other messengers, such as hormones, communicate over greater distances and in some cases, more slowly. Whatever the chemical messenger, however, the cell receiving the signal must have a way to detect the signal's presence. Once a cell detects a signal, a mechanism is required to transduce that signal into a physiologically meaningful response, such as the cell-division response to the delivery of growth-promoting signals.

The first step in the action of any intercellular chemical messenger is the binding of the messenger to specific target-cell proteins known as **receptors** (or receptor proteins). In the general language of Chapter 3, a chemical messenger is a ligand, and the receptor has a binding site for that ligand. The binding of a messenger to a receptor changes the conformation (tertiary structure; see Figure 2.17) of the receptor, which activates it. This initiates a sequence of events in the cell leading to the cell's response to that messenger, a process called **signal transduction**. The “signal” is the receptor activation, and “transduction” denotes the process by which a stimulus is transformed into a response. In this section, we consider general features common to many receptors, describe interactions between receptors and their ligands, and give some examples of how receptors are regulated.

### Types of Receptors

What is the nature of the receptors that bind intercellular chemical messengers? They are proteins or glycoproteins located either in the cell's plasma membrane or inside the cell, either in the cytosol or the nucleus. The plasma membrane is the much more common location, because a very large number of messengers are water-soluble and therefore cannot diffuse across the lipid-rich (hydrophobic) plasma membrane. In contrast, a much smaller number of lipid-soluble messengers diffuse through membranes to bind to their receptors located inside the cell.

**Plasma Membrane Receptors** A typical plasma membrane receptor is illustrated in **Figure 5.1a**. Plasma membrane receptors are transmembrane proteins; that is, they span the entire membrane thickness. Like other transmembrane proteins, a plasma membrane receptor has hydrophobic segments within the membrane, one or more hydrophilic segments extending out from the membrane into the extracellular fluid, and other hydrophilic segments extending into the intracellular fluid. Arriving chemical messengers bind to the extracellular parts of the receptor; the intracellular regions of the receptor are involved in signal transduction events.

**Intracellular Receptors** By contrast, intracellular receptors are not located in membranes but exist in either the cytosol or the cell nucleus and have a very different structure (**Figure 5.1b**). Like plasma membrane receptors, however, they have a segment that binds the messenger and other segments that act as regulatory sites. In addition, they have a segment that binds to DNA, unlike plasma membrane receptors. This is one key distinction between the two general types of receptors; plasma membrane receptors can transduce signals without interacting with DNA, whereas all intracellular receptors transduce signals through interactions with genes.

## Interactions Between Receptors and Ligands

There are four major features that define the interactions between receptors and their ligands: specificity, affinity, saturation, and competition.

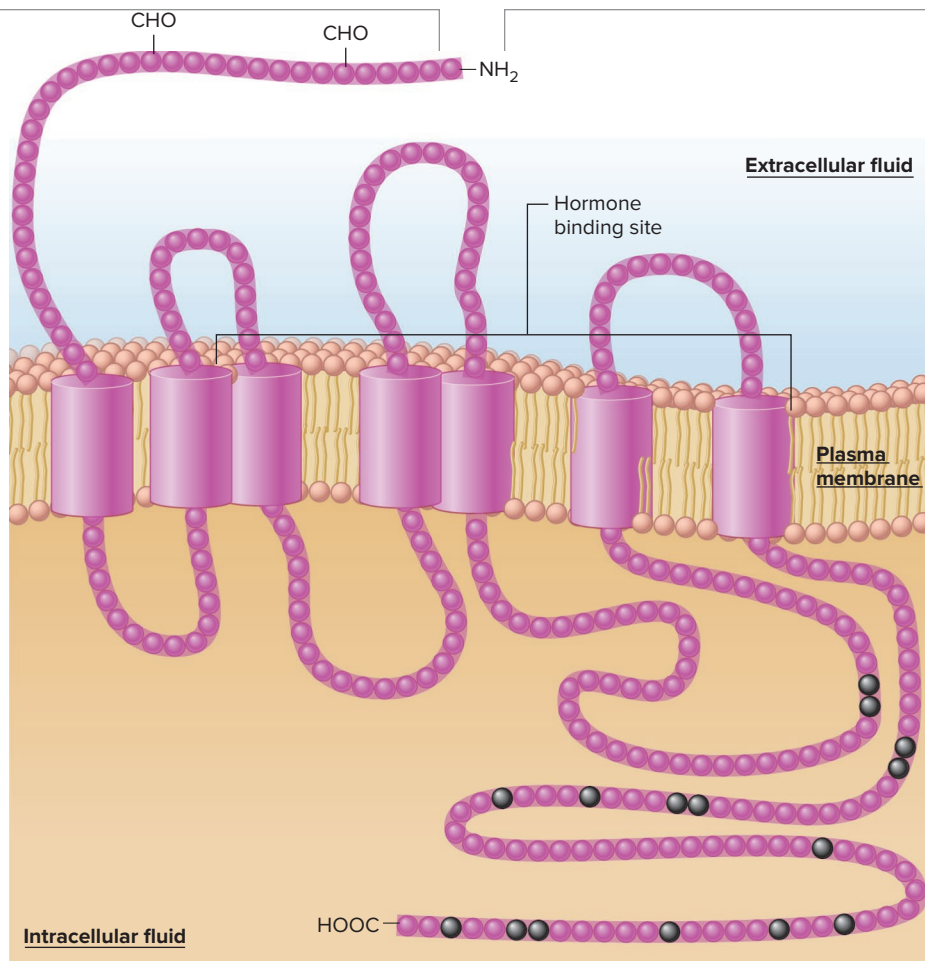
**Specificity** The binding of a chemical messenger to its receptor initiates the events leading to the cell's response. The existence of receptors explains a very important characteristic of intercellular communication—**specificity** (see **Table 5.1** for a glossary of terms concerning receptors). As described in Chapter 3 (refer back to Figures 3.28 and 3.29), a given protein binds a particular ligand and not others if its binding site for that ligand is specific. This is generally the case for chemical messengers and their receptors. Thus, although a given chemical messenger may come into contact with many different cells, it influences certain cell types and not others. This is because cells differ in the types of receptors they possess. Only certain cell types—sometimes just one—express the specific receptor required to bind a given chemical messenger (**Figure 5.2**).

In the case where many different cell types possess receptors for the same messenger, however, the responses of the various cell types to that messenger may differ from each other. For example, the neurotransmitter norepinephrine causes muscle cells of the heart to contract faster but, via the same type of receptor, regulates certain aspects of behavior by acting on neurons in the brain. In essence, then, the receptor functions as a molecular switch that elicits the cell's response when “switched on” by the messenger binding to it. Just as identical types of switches can be used to turn on a light or a radio, a single type of receptor can be used to produce different responses to the same chemical messenger in different cell types.

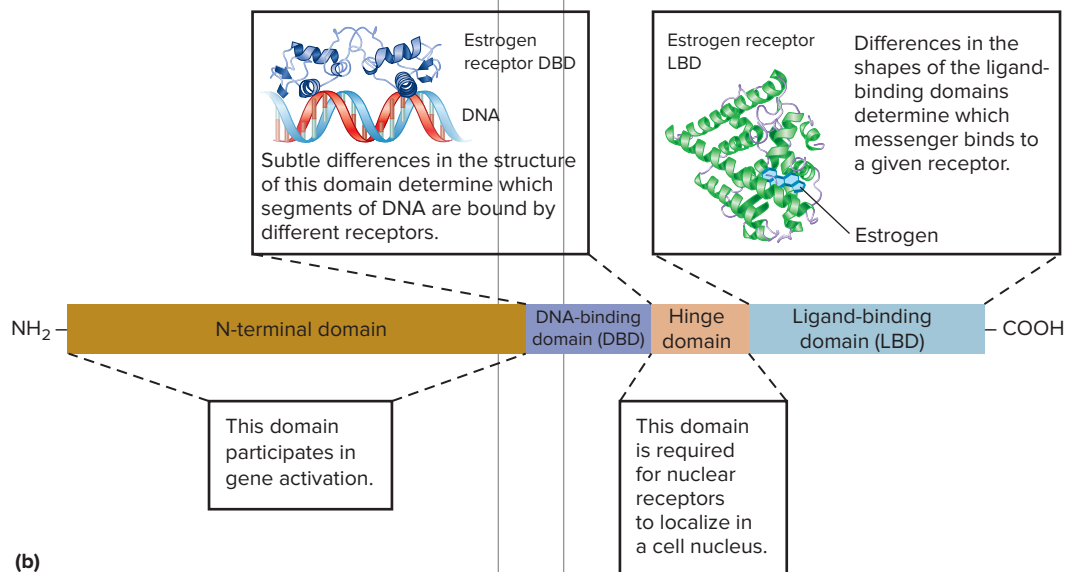
**Affinity** The remaining three general features of ligand-receptor interactions are summarized in **Figure 5.3**. The degree to which a particular messenger binds to its receptor is determined by the **affinity** of the receptor for the messenger. A receptor with high affinity will bind at lower concentrations of a messenger than will a receptor of low affinity (refer back to Figure 3.36). Differences in affinity of receptors for their ligands have important implications for the use of therapeutic drugs in treating illness; receptors with high affinity for a ligand require much less of the ligand (that is, a lower dose) to become activated.

**Saturation** The phenomenon of receptor **saturation** was described in Chapter 3 for ligands binding to binding sites on proteins, and are fully applicable here (see Figure 5.3). A cell's response to a messenger increases as the extracellular concentration of the messenger increases, because the number of receptors occupied by messenger molecules increases. There is an upper limit to this responsiveness, however, because only a finite number of receptors are available, and they become fully saturated at some point.

**Competition** **Competition** refers to the ability of a molecule to compete with a natural ligand for binding to its receptor. Competition typically occurs with messengers that have a similarity in part of their structures, and it also underlies the action of many drugs (see Figure 5.3). If researchers or physicians wish to interfere with the action of a particular messenger, they can administer competing molecules that are structurally similar enough to the endogenous messenger that they bind to the receptors for that messenger.



(a)



(b)

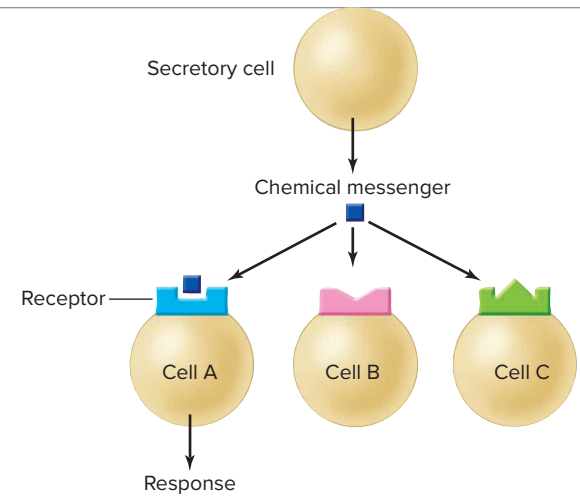
**Figure 5.1** The two major classes of receptors for chemical messengers. (a) Structure of a typical transmembrane receptor. The seven clusters of amino acids embedded in the phospholipid bilayer represent hydrophobic portions of the protein's alpha helix (shown here as cylinders). Note that the binding site for the hormone includes several of the segments that extend into the extracellular fluid. Portions of the extracellular segments can be linked to carbohydrates (CHO). The amino acids denoted by black circles represent some of the sites at which intracellular enzymes can phosphorylate, and thereby regulate, the receptor. (b) Schematic representation of the structural features of a typical nuclear receptor. The actual structures for segments of these receptors are known and are shown here for the human estrogen (a steroid hormone) receptor. (Note: The segments of proteins—including those of nuclear receptors—that perform different functions are known as “domains.”)

TABLE 5.1

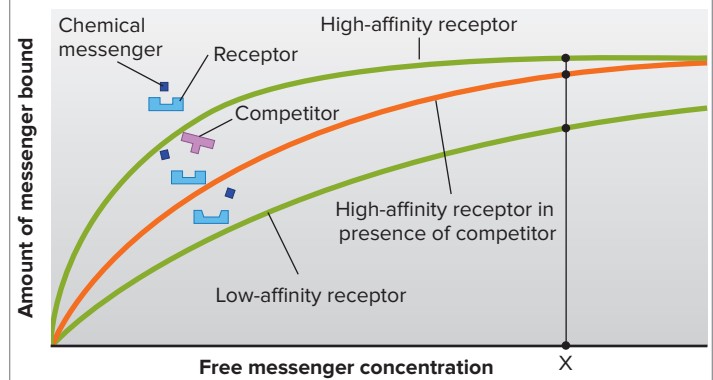
## A Glossary of Terms Concerning Receptors

<i>Receptor (receptor protein)</i>	A specific protein in either the plasma membrane or the interior of a target cell that a chemical messenger binds with, thereby invoking a biologically relevant response in that cell.
<i>Specificity</i>	The ability of a receptor to bind only one type or a limited number of structurally related types of chemical messengers. Only cells that express the correct receptor can bind a particular messenger.
<i>Saturation</i>	The degree to which receptors are occupied by messengers. If all are occupied, the receptors are fully saturated; if half are occupied, the saturation is 50%, and so on.
<i>Affinity</i>	The strength with which a chemical messenger binds to its receptor.
<i>Competition</i>	The ability of different molecules to compete with a ligand for binding to its receptor. Competitors generally are similar in structure to the natural ligand.
<i>Antagonist</i>	A molecule that competes with a ligand for binding to its receptor but does not activate signaling normally associated with the natural ligand. Therefore, an antagonist prevents the actions of the natural ligand. Certain types of antihistamines are examples of antagonists.
<i>Agonist</i>	A chemical messenger that binds to a receptor and triggers the cell's response; often refers to a drug that mimics a normal messenger's action. Some decongestants are examples of agonists.
<i>Down-regulation</i>	A decrease in the total number of target-cell receptors for a given messenger; may occur in response to chronic high extracellular concentration of the messenger.
<i>Up-regulation</i>	An increase in the total number of target-cell receptors for a given messenger; may occur in response to a chronic low extracellular concentration of the messenger.
<i>Increased sensitivity</i>	The increased responsiveness of a target cell to a given messenger; may result from up-regulation of receptors.

However, the competing molecules are different enough in structure from the native ligand that, although they bind to the receptor, they cannot activate it. This blocks the endogenous messenger from binding and yet does not induce signal transduction or trigger the cell's response. The general term for a compound that blocks the action of a chemical messenger is **antagonist**; when an antagonist works by competing with a chemical messenger for its binding site, it is known as a competitive antagonist. One example is a type of drug called a **beta-adrenergic receptor blocker** (also called beta-blocker),



**Figure 5.2** Specificity of receptors for chemical messengers. Only cell A has the appropriate receptor for this chemical messenger; therefore, it is the only one among the group that is a target cell for the messenger.



**Figure 5.3** Characteristics of receptors binding to messengers. The receptors with high affinity will have more bound messenger at a given messenger concentration (e.g., concentration X). The presence of a competitor will decrease the amount of messenger bound, until at very high concentrations the receptors become saturated with messenger and cannot bind any additional messenger. Note in the illustration that the low-affinity receptor in this case has a slightly different shape in its ligand-binding region compared to the high-affinity receptor, which makes it less able to bind the messenger. Also note the similarity in parts of the shapes of the natural messenger and its competitor.

## PHYSIOLOGICAL INQUIRY

- The general principle of physiology that structure is a determinant of—and has coevolved with—function can be considered at the molecular, cellular, and organ levels. How is this principle illustrated by the binding of messengers to their receptors?

*Answer can be found at end of chapter.*

which is sometimes used in the treatment of high blood pressure and other diseases. Beta-blockers compete with epinephrine and norepinephrine to bind to one of their receptors—the beta-adrenergic receptor. Because epinephrine and norepinephrine normally act to increase blood pressure (Chapter 12), beta-blockers tend to decrease blood pressure by acting as competitive antagonists.



**Antihistamines** are another example and are useful in treating allergic symptoms brought on due to excess histamine secretion from cells known as mast cells (Chapter 18). Certain antihistamines are competitive antagonists that block histamine from binding to its receptors on mast cells and triggering an allergic response.

On the other hand, some drugs that compete with natural ligands for a particular receptor type do activate the receptor and trigger the cell's response exactly as if the true (endogenous) chemical messenger had combined with the receptor. Such drugs, known as **agonists**, are used therapeutically to mimic the messenger's action. For example, the common decongestant drugs **phenylephrine** and **oxymetazoline**, found in many types of nasal sprays, mimic the action of epinephrine on a related but different subtype of receptors, called alpha-adrenergic receptors, in blood vessels. When alpha-adrenergic receptors are activated, the smooth muscles of inflamed, dilated blood vessels in the nose contract, resulting in narrowing of those vessels; this helps open the nasal passages and decrease fluid leakage from blood vessels.

### Regulation of Receptors

Receptors are themselves subject to physiological regulation. The number of receptors a cell has, or the affinity of the receptors for their specific messenger, can be increased or decreased in certain systems. An important example is the phenomenon of **down-regulation**. When a high extracellular concentration of a messenger is maintained for some time, the total number of the target cell's receptors for that messenger may decrease—that is, down-regulate. Down-regulation has the effect of reducing the target cells' responsiveness to frequent or intense stimulation by a messenger—that is, desensitizing them—and thus represents a local negative feedback mechanism.

Down-regulation is possible because there is a continuous synthesis and degradation of receptors. The main mechanism of down-regulation of plasma membrane receptors is **internalization**. The binding of a messenger to its receptor can stimulate the internalization of the complex; that is, the messenger-receptor complex is taken into the cell by receptor-mediated endocytosis (see Chapter 4). This increases the rate of receptor degradation inside the cell. Consequently, at increased messenger concentrations, the number of plasma membrane receptors of that type gradually decreases during down-regulation.

Change in the opposite direction, called **up-regulation**, also occurs. Cells exposed for a prolonged period to very low concentrations of a messenger may come to have many more receptors for that messenger, thereby developing increased sensitivity to it. The greater the number of receptors available to bind a ligand, the greater the likelihood that such binding will occur. For example, when the nerves to a muscle are damaged, the delivery of neurotransmitters from those nerves to the muscle is decreased or eliminated. With time, under these conditions, the muscle will contract in response to a much smaller amount of neurotransmitter than normal. This happens because the receptors for the neurotransmitter have been up-regulated, resulting in increased sensitivity.

One way in which this may occur is by recruitment to the plasma membrane of intracellular vesicles that contain within their membranes numerous receptor proteins. The vesicles fuse with the plasma membrane, thereby inserting their receptors into the plasma membrane. Receptor regulation in both directions (up- and down-regulation) is an excellent example of the general

physiological principle of homeostasis, because it acts to return signal strength toward normal when the concentration of messenger molecules varies above or below normal.

## 5.2 Signal Transduction Pathways

What are the sequences of events by which the binding of a chemical messenger to a receptor causes the cell to respond in a specific way?

The binding of a messenger to its receptor causes a change in the conformation (tertiary structure) of the receptor. This event, known as **receptor activation**, is the initial step leading to the cell's responses to the messenger. These cellular responses can take the form of changes in (1) the permeability, transport properties, or electrical state of the plasma membrane; (2) metabolism; (3) secretory activity; (4) rate of proliferation and differentiation; or (5) contractile or other activities.

Despite the variety of responses, there is a common denominator: They are all directly due to alterations of particular cell proteins. Let us examine a few examples of messenger-induced responses, all of which are described more fully in subsequent chapters. For example, the neurotransmitter-induced generation of electrical signals in neurons reflects the altered conformation of membrane proteins (ion channels) through which ions can diffuse between extracellular and intracellular fluid. Similarly, changes in the rate of glucose secretion by the liver induced by the hormone epinephrine reflect the altered activity and concentration of enzymes in the metabolic pathways for glucose synthesis. Finally, muscle contraction induced by the neurotransmitter acetylcholine results from the altered conformation of contractile proteins.

Thus, receptor activation by a messenger is only the first step leading to the cell's ultimate response (contraction, secretion, and so on). The diverse sequences of events that link receptor activation to cellular responses are termed **signal transduction pathways**. "Pathways" denotes the cell-specific mechanisms linked with different messengers.

Signal transduction pathways differ between lipid-soluble and water-soluble messengers. As described earlier, the receptors for these two broad chemical classes of messenger are in different locations—the former inside the cell and the latter in the plasma membrane of the cell. The rest of this chapter describes the major features of the signal transduction pathways that these two broad categories of messengers initiate.

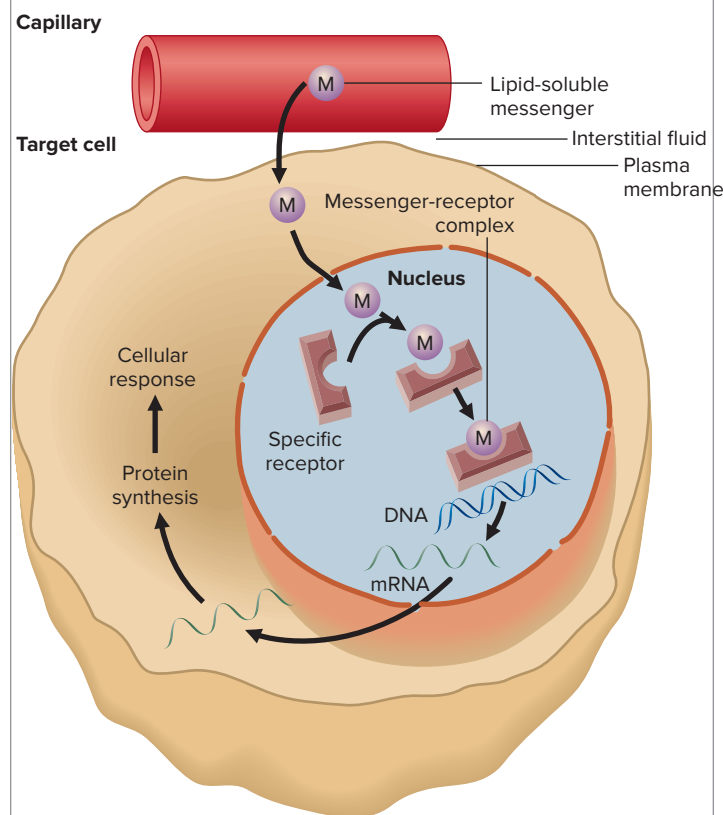
### Pathways Initiated by Lipid-Soluble Messengers

Lipid-soluble messengers include hydrophobic substances such as steroid hormones and thyroid hormone. Their receptors belong to a large family of intracellular receptors called **nuclear receptors** that share similar structures (see Figure 5.1b) and mechanisms of action. Although plasma membrane receptors for a few of these messengers have been identified, most of the receptors in this family are intracellular. In a few cases, the inactive receptors are located in the cytosol and move into the nucleus after binding their ligand. Most of the inactive receptors, however, already reside in the cell nucleus, where they bind to and are activated by their respective ligands. In both cases, receptor activation leads to altered rates of transcription of one or more genes in a particular cell.

In the most common scenario, the messenger diffuses out of capillaries from plasma to the interstitial fluid (refer back to

Figure 1.3). From there, the messenger diffuses across the lipid bilayers of the plasma membrane and nuclear envelope to enter the nucleus and bind to the receptor there (Figure 5.4). The activated receptor complex then functions in the nucleus as a transcription factor, defined as a regulatory protein that directly influences gene transcription. The hormone–receptor complex binds to DNA at a regulatory region of a gene, an event that typically increases the rate of that gene’s transcription into mRNA. The mRNA molecules move out of the nucleus to direct the synthesis, on ribosomes, of the protein the gene encodes. The result is an increase in the cellular concentration of the protein and/or its rate of secretion, accounting for the cell’s ultimate response to the messenger. For example, if the protein encoded by the gene is an enzyme, the cell’s response is an increase in the rate of the reaction catalyzed by that enzyme.

Two other points are important. First, more than one gene may be subject to control by a single receptor type. For example,



**AP|R** **Figure 5.4** Mechanism of action of lipid-soluble messengers. This figure shows the receptor (simplified in this view) for these messengers in the nucleus. In some cases, the unbound receptor is in the cytosol rather than the nucleus, in which case the binding occurs there, and the activated messenger-receptor complex then moves into the nucleus. For simplicity, a single messenger is shown binding to a single receptor. In many cases, however, two messenger-receptor complexes must bind together in order to activate a gene.

#### PHYSIOLOGICAL INQUIRY

- How does the chemical nature of lipid-soluble messengers relate to the general principle of physiology that physiological processes are dictated by the laws of chemistry and physics?

*Answer can be found at end of chapter.*

the adrenal gland hormone cortisol acts via its intracellular receptor to activate numerous genes involved in the coordinated control of cellular metabolism and energy balance. Second, in some cases, the transcription of a gene or genes may be *decreased* rather than increased by the activated receptor. Cortisol, for example, inhibits transcription of several genes whose protein products mediate inflammatory responses that occur following injury or infection; for this reason, cortisol has important anti-inflammatory effects.

#### Pathways Initiated by Water-Soluble Messengers

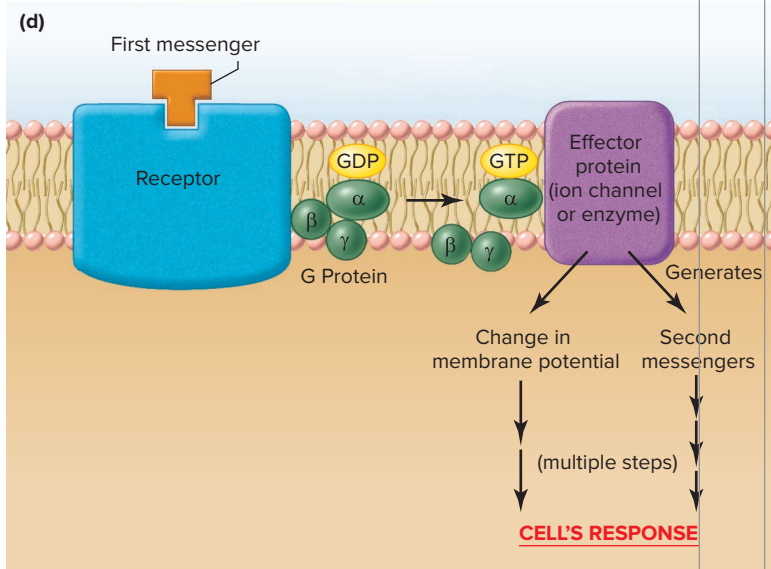
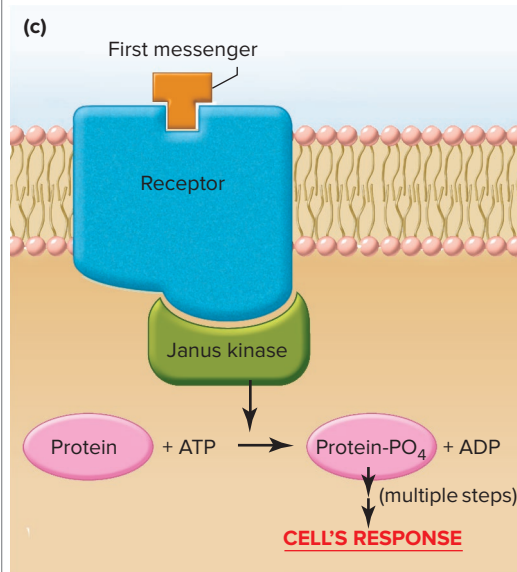
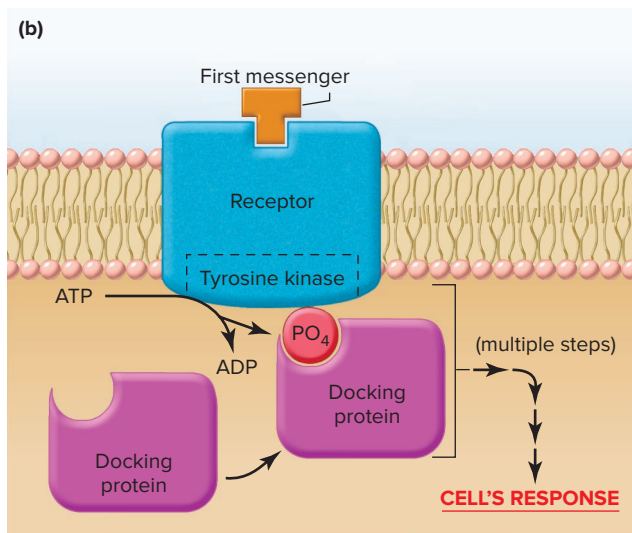
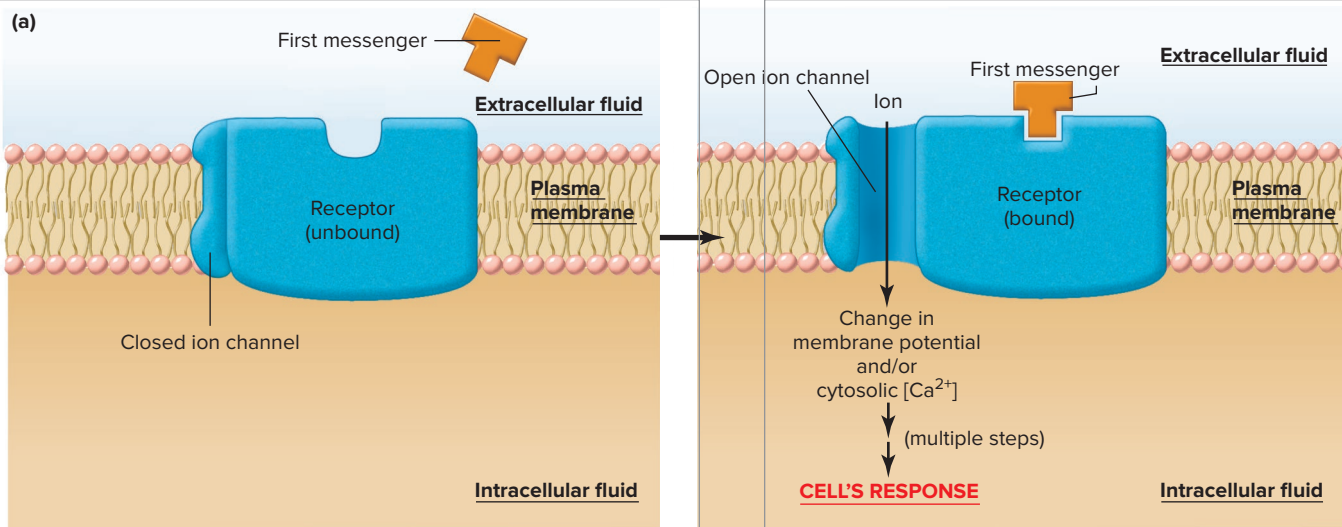
Water-soluble messengers cannot readily enter cells by diffusion through the lipid bilayer of the plasma membrane. Instead, they exert their actions on cells by binding to the extracellular portion of receptor proteins embedded in the plasma membrane. Water-soluble messengers include most polypeptide hormones, neurotransmitters, and paracrine and autocrine compounds. The signal transduction mechanisms initiated by water-soluble messengers can be classified into the types illustrated in Figure 5.5.

Some notes on general terminology are essential for this discussion. First, the extracellular chemical messengers (such as hormones or neurotransmitters) that reach the cell and bind to their specific plasma membrane receptors are often referred to as **first messengers**. **Second messengers**, then, are substances that enter or are generated in the cytoplasm as a result of receptor activation by the first messenger. The second messengers diffuse throughout the cell to serve as chemical relays from the plasma membrane to the biochemical machinery inside the cell. The third essential general term is **protein kinase**, which is the name for an enzyme that phosphorylates other proteins by transferring a phosphate group to them from ATP. Phosphorylation of a protein allosterically changes its tertiary structure and, consequently, alters the protein’s activity. Different proteins respond differently to phosphorylation; some are activated and some are inactivated (inhibited). There are many different protein kinases, and each type is able to phosphorylate only specific proteins. The important point is that a variety of protein kinases are involved in signal transduction pathways. These pathways may involve a series of reactions in which a particular inactive protein kinase is activated by phosphorylation and then catalyzes the phosphorylation of another inactive protein kinase, and so on. At the ends of these sequences, the ultimate phosphorylation of key proteins, such as transporters, metabolic enzymes, ion channels, and contractile proteins, underlies the cell’s response to the first messenger. Although all cells contain many of the same protein kinases, different cells often express specific proteins that are not necessarily found in other cells. Thus, a given protein kinase may have different substrates in different cell types.

As described in Chapter 3, other enzymes do the reverse of protein kinases; that is, they dephosphorylate proteins. These enzymes, termed protein phosphatases, also participate in signal transduction pathways; they can also serve to stop a signal once a cell response has occurred.

#### Signaling by Receptors That Are Ligand-Gated Ion Channels

In one type of plasma membrane receptor for water-soluble messengers, the protein that acts as the receptor is also an ion channel (refer back to Figure 4.7). Recall from Chapter 3 (see Figure 3.32) that normally when a ligand binds to a protein, a shape change is induced in the protein. Activation of the receptor by a first messenger (the ligand) results in a conformational change of



**AP|R** **Figure 5.5** Mechanisms of action of water-soluble messengers (noted as “first messengers” in this and subsequent figures). (a) Signal transduction mechanism in which the receptor complex includes an ion channel. Note that the receptor exists in two conformations in the unbound and bound states. It is the binding of the first messenger to its receptor that triggers the conformational change that leads to opening of the channel. *Note: Conformational changes also occur in panels b–d but only the bound state is shown for simplicity.* (b) Signal transduction mechanism in which the receptor itself functions as an enzyme, usually a tyrosine kinase. (c) Signal transduction mechanism in which the receptor activates a janus kinase in the cytoplasm. (d) Signal transduction mechanism involving G proteins. When GDP is bound to the alpha subunit of the G protein, the protein exists as an inactive trimeric molecule. Binding of GTP to the alpha subunit causes dissociation of the alpha subunit, which then activates the effector protein.

#### PHYSIOLOGICAL INQUIRY

- Many cells express more than one of the four types of receptors depicted in this figure. Can you think of any benefits that this might confer in terms of the regulation of cell function?

*Answer can be found at end of chapter.*



the receptor such that it forms an open channel through the plasma membrane (**Figure 5.5a**). Because the opening of ion channels has been compared to the opening of a gate in a fence, these types of channels are known as ligand-gated ion channels, as described in Chapter 4. They are particularly prevalent in the plasma membranes of neurons and skeletal muscle, as you will learn in Chapters 6 and 9.

The opening of ligand-gated ion channels in response to binding of a first messenger results in an increase in the net diffusion across the plasma membrane of one or more types of ions specific to that channel. As introduced in Chapter 4 (see **Figure 4.6**), such a change in ion diffusion results in a change in the electrical charge, or membrane potential, of a cell. This change in membrane potential, then, is the cell's response to the messenger. In addition, when the channel is a  $\text{Ca}^{2+}$  channel, its opening results in an increase by diffusion in cytosolic  $\text{Ca}^{2+}$  concentration. Increasing cytosolic  $\text{Ca}^{2+}$  is another essential event in the transduction pathway for many signaling systems.

**Signaling by Receptors That Function as Enzymes** Other plasma membrane receptors for water-soluble messengers have intrinsic enzyme activity. With one major exception (discussed later), the many receptors that possess intrinsic enzyme activity are all protein kinases (**Figure 5.5b**). Of these, the great majority specifically phosphorylate tyrosine residues. Consequently, these receptors are known as **receptor tyrosine kinases**.

The typical sequence of events for receptors with intrinsic tyrosine kinase activity is as follows. The binding of a specific messenger to the receptor changes the conformation of the receptor so that its enzymatic portion, located on the cytoplasmic side of the plasma membrane, is activated. This results in autophosphorylation of the receptor; that is, the receptor phosphorylates some of its own tyrosine residues. The newly created phosphotyrosines on the cytoplasmic portion of the receptor then serve as docking sites for cytoplasmic proteins. The bound docking proteins then bind and activate other proteins, which in turn activate one or more signaling pathways within the cell. The common denominator of these pathways is that they all involve activation of cytoplasmic proteins by phosphorylation.

There is one physiologically important exception to the generalization that plasma membrane receptors with inherent enzyme activity function as protein kinases. In this exception, the receptor functions both as a receptor and as a **guanylyl cyclase** to catalyze the formation, in the cytoplasm, of a molecule known as **cyclic GMP (cGMP)**. In turn, cGMP functions as a second messenger to activate a protein kinase called **cGMP-dependent protein kinase**. This kinase phosphorylates specific proteins that then mediate the cell's response to the original messenger. As described in Chapter 7, receptors that function both as ligand-binding molecules and as guanylyl cyclases are abundantly expressed in the retina of the eye, where they are important for processing visual inputs. This signal transduction pathway is used by only a small number of messengers. Also, in certain cells, guanylyl cyclase enzymes are present in the cytoplasm. In these cases, a first messenger—the gas nitric oxide (NO)—diffuses into the cytosol of the cell and combines with the guanylyl cyclase to trigger the formation of cGMP. Nitric oxide is a lipid-soluble gas produced from the amino acid arginine by the action of an enzyme called nitric oxide synthase, which is present in numerous cell types including the cells that line the interior of blood vessels. When

released from such cells, NO acts locally in a paracrine fashion to relax the smooth muscle component of certain blood vessels, which allows the blood vessel to dilate, or open, more. As you will learn in Chapter 12, the ability of certain blood vessels to dilate is an important part of the homeostatic control of blood pressure.

**Signaling by Receptors That Interact with Cytoplasmic Janus Kinases** Recall that in the previous category, the receptor itself has intrinsic enzyme activity. In the next category of signal transduction mechanisms for water-soluble messengers (**Figure 5.5c**), the enzymatic activity—again, tyrosine kinase activity—resides not in the receptor but in a family of separate cytoplasmic kinases, called **janus kinases (JAKs)**, which are associated with the receptor. In these cases, the receptor and its associated janus kinase function as a unit. The binding of a first messenger to the receptor causes a conformational change in the receptor that leads to activation of the janus kinase. Different receptors associate with different members of the janus kinase family, and the different janus kinases phosphorylate different target proteins, many of which act as transcription factors. The result of these pathways is the synthesis of new proteins, which mediate the cell's response to the first messenger. One significant example of signals mediated primarily via receptors linked to janus kinases are those of the cytokines—proteins secreted by cells of the immune system that have a critical function in immune defenses (Chapter 18).

**Signaling by G-Protein-Coupled Receptors** The fourth category of signaling pathways for water-soluble messengers is by far the largest, including hundreds of distinct receptors (**Figure 5.5d**). Bound to the inactive receptor is a protein complex located on the cytosolic surface of the plasma membrane and belonging to the family of proteins known as **G proteins**. G proteins contain three subunits, called the alpha, beta, and gamma subunits. The alpha subunit can bind GDP and GTP. The beta and gamma subunits help anchor the alpha subunit in the membrane. The binding of a first messenger to the receptor changes the conformation of the receptor. This activated receptor increases the affinity of the alpha subunit of the G protein for GTP. When bound to GTP, the alpha subunit dissociates from the beta and gamma subunits of the trimeric G protein. This dissociation allows the activated alpha subunit to link up with still another plasma membrane protein, either an ion channel or an enzyme. These ion channels and enzymes are effector proteins that mediate the next steps in the sequence of events leading to the cell's response.

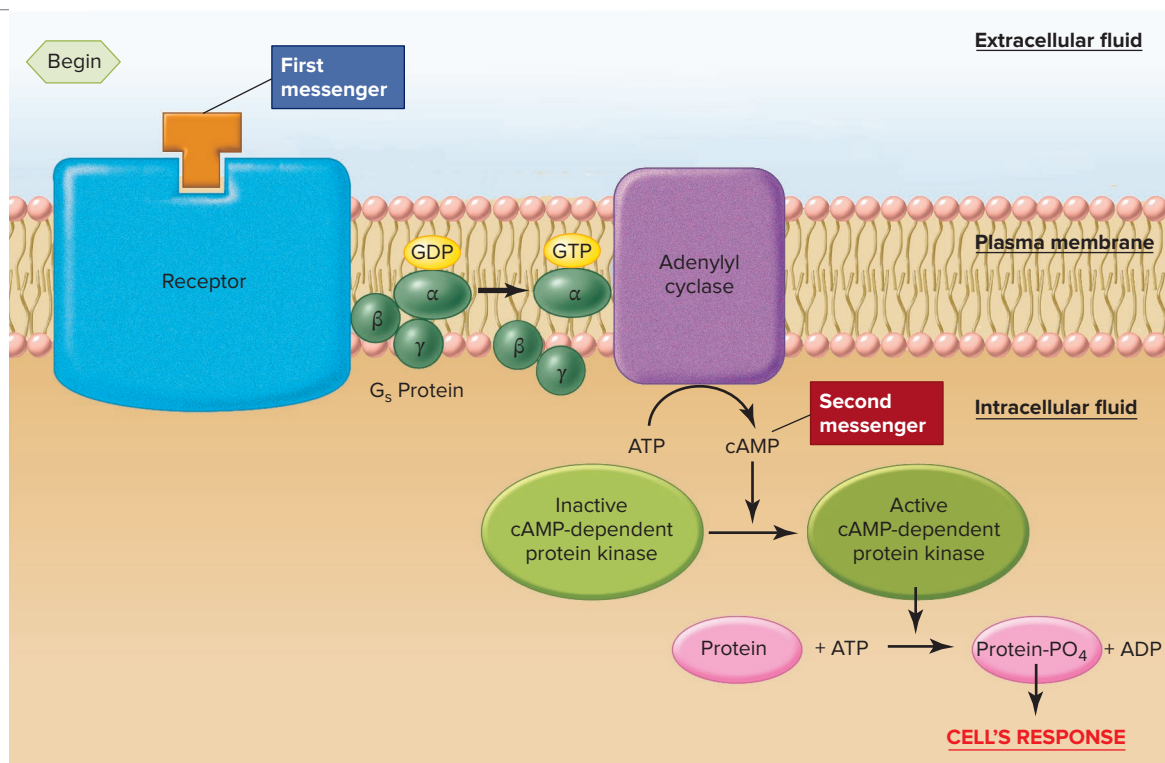
In essence, then, a G protein serves as a switch to couple a receptor to an ion channel or to an enzyme in the plasma membrane. Consequently, these receptors are known as **G-protein-coupled receptors**. The G protein may cause the ion channel to open, with a resulting change in electrical signals or, in the case of  $\text{Ca}^{2+}$  channels, changes in the cytosolic  $\text{Ca}^{2+}$  concentration. Alternatively, the G protein may activate or inhibit the membrane enzyme with which it interacts. Such enzymes, when activated, cause the generation of second messengers inside the cell.

Once the alpha subunit of the G protein activates its effector protein, a GTPase activity inherent in the alpha subunit cleaves the GTP into GDP and  $\text{P}_i$ . This cleavage renders the alpha subunit inactive, allowing it to recombine with its beta and gamma subunits.



## AP|R Figure 5.6

Cyclic AMP second-messenger system. Not shown in the figure is the existence of another regulatory protein,  $G_i$ , which certain receptors can react with to cause inhibition of adenylyl cyclase.

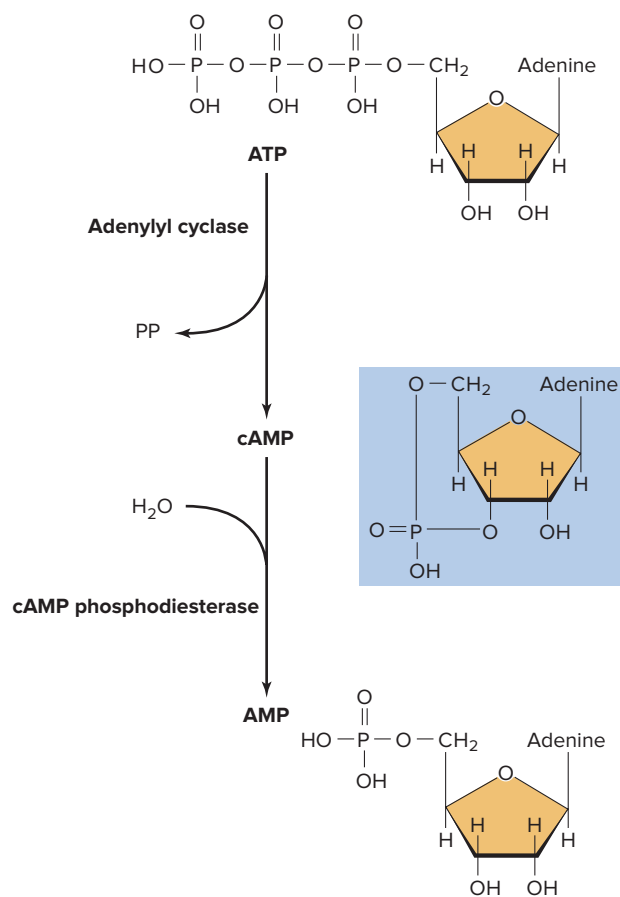


There are several subfamilies of plasma membrane G proteins, each with multiple distinct members, and a single receptor may be associated with more than one type of G protein. Moreover, some G proteins may couple to more than one type of plasma membrane effector protein. In this way, a first-messenger-activated receptor, via its G-protein couplings, can call into action a variety of plasma membrane proteins such as ion channels and enzymes. These molecules can, in turn, induce a variety of cellular events.

To illustrate some of the major points concerning G proteins, plasma membrane effector proteins, second messengers, and protein kinases, the next two sections describe the two most common effector protein enzymes regulated by G proteins—adenylyl cyclase and phospholipase C. In addition, the subsequent portions of the signal transduction pathways in which they participate are described.

## Major Second Messengers

**Cyclic AMP** In this pathway (Figure 5.6), activation of the receptor by the binding of the first messenger (for example, the hormone epinephrine) allows the receptor to activate its associated G protein, in this example known as  $G_s$  (the subscript  $s$  denotes “stimulatory”). This causes  $G_s$  to activate its effector protein, the plasma membrane enzyme called **adenylyl cyclase** (also known as adenylyl cyclase). The activated adenylyl cyclase, with its catalytic site located on the cytosolic surface of the plasma membrane, catalyzes the conversion of cytosolic ATP to 3',5'-cyclic adenosine monophosphate, or **cyclic AMP** (cAMP) (Figure 5.7). Cyclic AMP then acts as a second messenger (see Figure 5.6). It diffuses throughout the cell to trigger the sequence of events leading to the cell's ultimate response to the first messenger. The action of cAMP eventually terminates when it is broken down to AMP, a reaction catalyzed by the enzyme **cAMP phosphodiesterase** (see Figure 5.7).



**Figure 5.7** Formation and breakdown of cAMP. ATP is converted to cAMP by the action of the plasma membrane enzyme adenylyl cyclase. cAMP is inactivated by the cytosolic enzyme cAMP phosphodiesterase, which converts cAMP into the noncyclic form AMP.

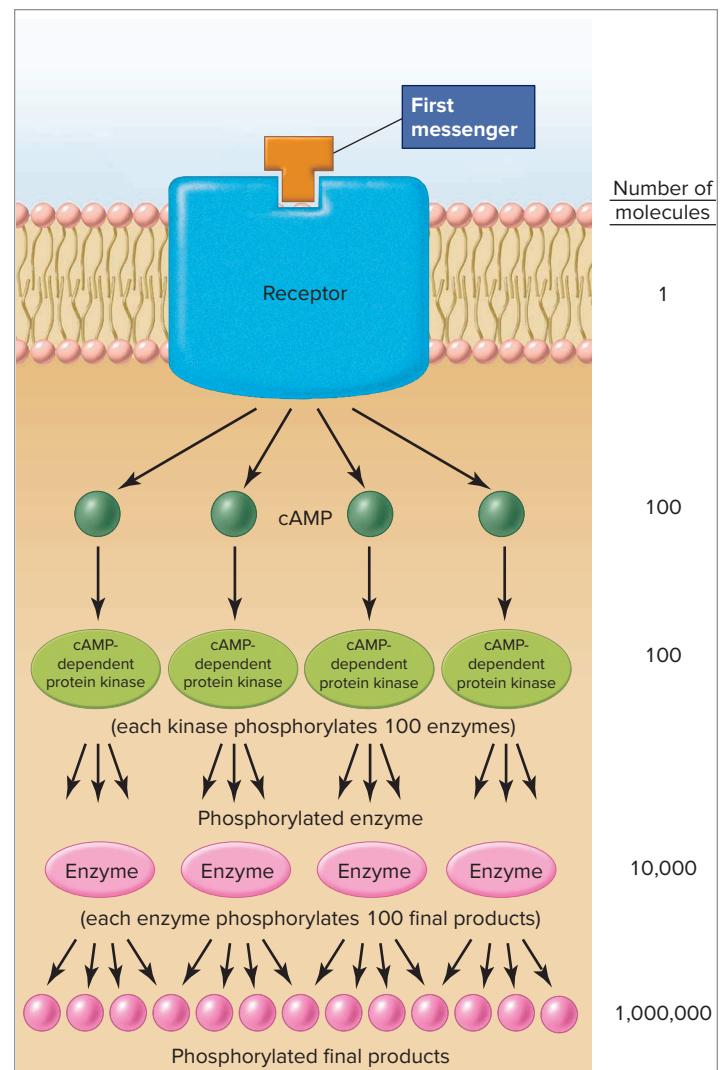
This enzyme is also subject to physiological control. Thus, the cellular concentration of cAMP can be changed either by altering the rate of its messenger-mediated synthesis or the rate of its phosphodiesterase-mediated breakdown. Caffeine and theophylline, the active ingredients of coffee and tea, are widely consumed stimulants that work partly by inhibiting cAMP phosphodiesterase activity, thereby prolonging the actions of cAMP within cells. In many cells, such as those of the heart, an increased concentration of cAMP triggers an increase in function (for example, an increase in heart rate).

What does cAMP actually do inside the cell? It binds to and activates an enzyme known as **cAMP-dependent protein kinase**, also called protein kinase A (see Figure 5.6). Recall that protein kinases phosphorylate other proteins—often enzymes—by transferring a phosphate group to them. The changes in the activity of proteins phosphorylated by cAMP-dependent protein kinase bring about a cell's response (secretion, contraction, and so on). Again, recall that each of the various protein kinases that participate in the multiple signal transduction pathways described in this chapter has its own specific substrates.

In essence, then, the activation of adenylyl cyclase by the  $G_s$  protein initiates an “amplification cascade” of events that converts proteins in sequence from inactive to active forms. **Figure 5.8** illustrates the benefit of such a cascade. While it is active, a single enzyme molecule is capable of transforming into product not one but many substrate molecules, let us say 100. Therefore, one active molecule of adenylyl cyclase may catalyze the generation of 100 cAMP molecules (and thus 100 activated cAMP-dependent protein kinase A molecules). At each of the two subsequent enzyme-activation steps in our example, another 100-fold amplification occurs. Therefore, the end result is that a single molecule of the first messenger could, in this example, cause the generation of 1 million product molecules. This helps to explain how hormones and other messengers can be effective at extremely low extracellular concentrations. To take an actual example, one molecule of the hormone epinephrine can cause the liver to generate and release  $10^8$  molecules of glucose.

In addition, activated cAMP-dependent protein kinase can diffuse into the cell nucleus, where it can phosphorylate a protein that then binds to specific regulatory regions of certain genes. Such genes are said to be cAMP-responsive. Therefore, the effects of cAMP can be rapid and independent of changes in gene activity, as in the example of epinephrine and glucose production, or slower and dependent upon the formation of new gene products.

How can cAMP's activation of a single molecule, cAMP-dependent protein kinase, be common to the great variety of biochemical sequences and cell responses initiated by cAMP-generating first messengers? The answer is that cAMP-dependent protein kinase can phosphorylate a large number of different proteins (**Figure 5.9**). In this way, activated cAMP-dependent protein kinase can exert multiple actions within a single cell and different actions in different cells. For example, epinephrine acts via the cAMP pathway on adipose cells to stimulate the breakdown of triglyceride, a process that is mediated by one particular phosphorylated enzyme that is chiefly expressed in adipose cells. In the liver, epinephrine acts via cAMP to stimulate both glycogenolysis and gluconeogenesis, processes that are mediated by phosphorylated enzymes that differ from those expressed in adipose cells.



**AP|R Figure 5.8** Example of signal amplification. In this example, a single molecule of a first messenger results in 1 million final products. Other second-messenger pathways have similar amplification processes. The steps between receptor activation and cAMP generation are omitted for simplicity.

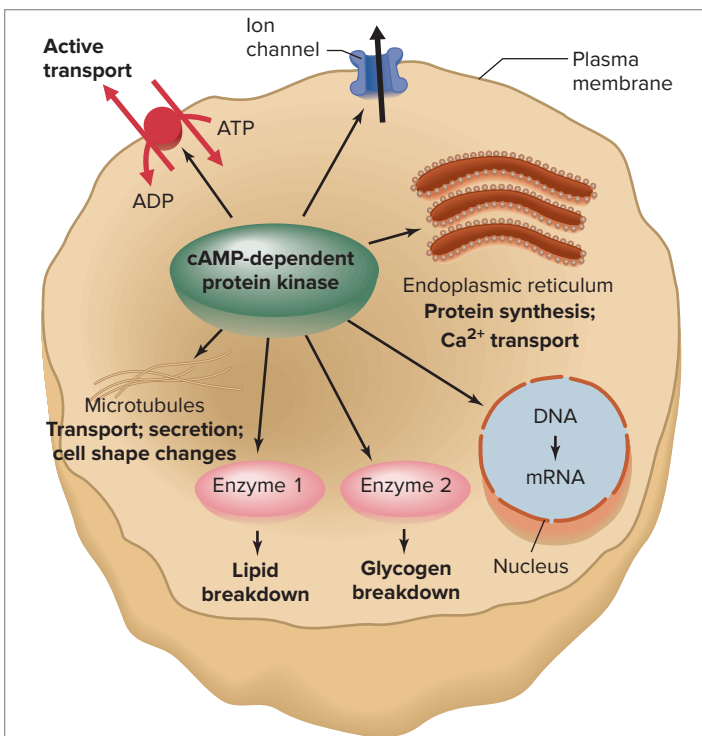
### PHYSIOLOGICAL INQUIRY

- What are the advantages of having an enzyme (like adenylyl cyclase) involved in the initial response to receptor activation by a first messenger? (*Hint*: Recall one of the key characteristics of enzymes described in Sections 3.11–3.13 of Chapter 3. Does a given activated enzyme catalyze a reaction only once, or can it act many times?)

*Answer can be found at end of chapter.*

Whereas phosphorylation mediated by cAMP-dependent protein kinase activates certain enzymes, it inhibits others. For example, the enzyme catalyzing the rate-limiting step in glycogen synthesis is inhibited by phosphorylation. This explains how epinephrine inhibits glycogen synthesis at the same time it stimulates glycogen breakdown by activating the enzyme that catalyzes the latter response.

Not mentioned thus far is the fact that receptors for some first messengers, upon activation by their messengers, *inhibit*



**AP|R** **Figure 5.9** The variety of cellular responses induced by cAMP is due mainly to the fact that activated cAMP-dependent protein kinase can phosphorylate many different proteins, activating or inhibiting them. In this figure, the protein kinase is shown phosphorylating seven different proteins—a microtubular protein, an ATPase, an ion channel, a protein in the endoplasmic reticulum, a protein involved in stimulating the transcription of a gene into mRNA, and two enzymes.

### PHYSIOLOGICAL INQUIRY

- Does a given protein kinase, such as cAMP-dependent protein kinase, only phosphorylate the same proteins in all cells in which the kinase is present?

*Answer can be found at end of chapter.*

adenylyl cyclase. This inhibition results in less, rather than more, generation of cAMP. This occurs because these receptors are associated with a different G protein known as  $G_i$  (the subscript  $i$  denotes “inhibitory”). Activation of  $G_i$  causes the inhibition of adenylyl cyclase. The result is to decrease the concentration of cAMP in the cell and thereby the phosphorylation of key proteins inside the cell. Many cells express both stimulatory and inhibitory G proteins in their membranes, providing a means of tightly regulating intracellular cAMP concentrations. This common cellular feature highlights the general principle of physiology that most physiological functions are controlled by multiple regulatory systems, often working in opposition. It provides for fine-tuning of cellular responses and, in some cases, the ability to override a response.

Finally, as indicated in Figure 5.9, cAMP-dependent protein kinase can phosphorylate certain plasma membrane ion channels, thereby causing them to open or in some cases to close. As we have seen, the sequence of events leading to the activation of

**TABLE 5.2**

**Summary of Common Mechanisms by Which Receptor Activation Influences Ion Channels**

The ion channel is part of the receptor.

A G protein directly gates the ion channel.

A G protein gates the ion channel indirectly via production of a second messenger such as cAMP.

cAMP-dependent protein kinase proceeds through a G protein, so it should be clear that the opening of such channels is indirectly dependent on that G protein. This is distinct from the direct action of a G protein on an ion channel, mentioned earlier. To generalize, the indirect G-protein gating of ion channels utilizes a second-messenger pathway for the opening or closing of the channel. **Table 5.2** summarizes the three ways by which receptor activation by a first messenger leads to opening or closing of ion channels, causing a change in membrane potential.

**Phospholipase C, Diacylglycerol, and Inositol Trisphosphate** In this system, a G protein called  $G_q$  is activated by a receptor bound to a first messenger. Activated  $G_q$  then activates a plasma membrane effector enzyme called **phospholipase C**. This enzyme catalyzes the breakdown of a plasma membrane phospholipid known as phosphatidylinositol biphosphate, abbreviated  $PIP_2$ , to **diacylglycerol (DAG)** and **inositol trisphosphate ( $IP_3$ )** (**Figure 5.10**). Both DAG and  $IP_3$  then function as second messengers but in very different ways.

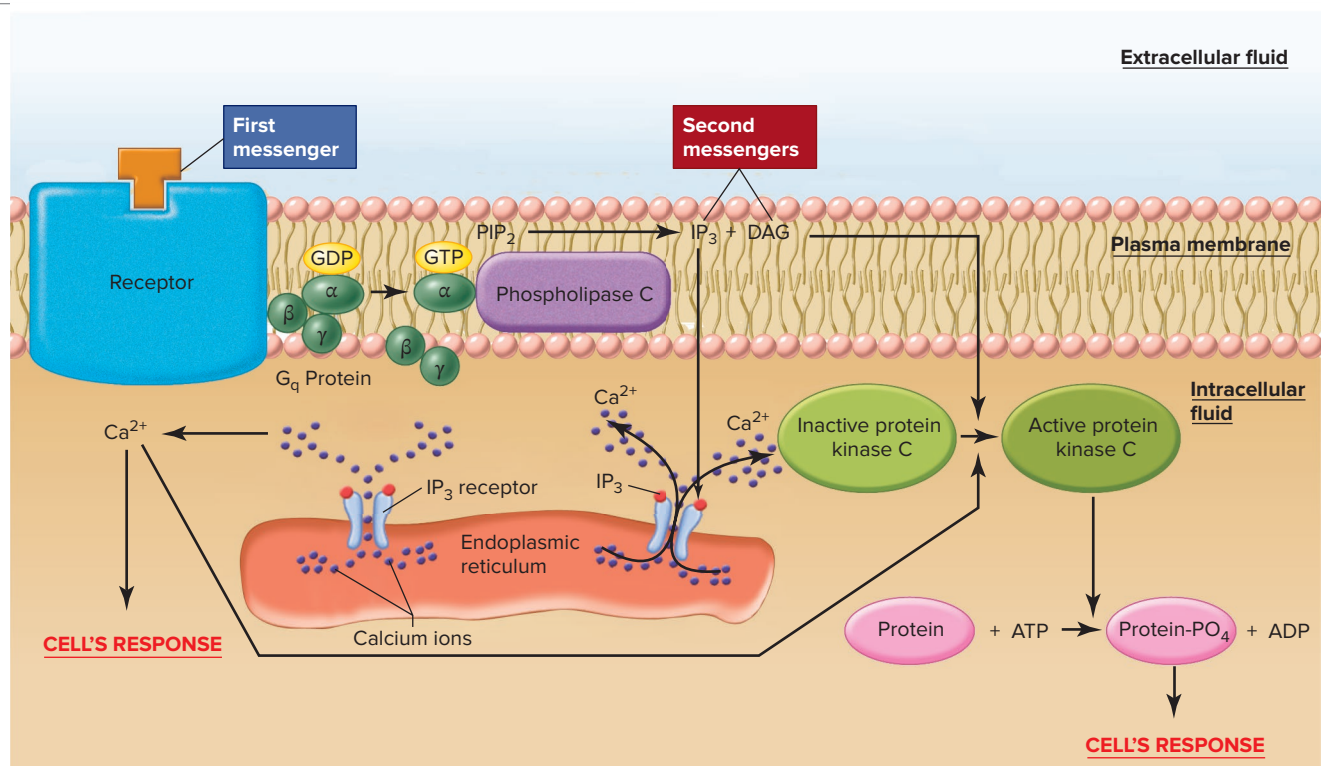
DAG activates members of a family of related protein kinases known collectively as **protein kinase C**, which, in a fashion similar to cAMP-dependent protein kinase, then phosphorylates a large number of other proteins, leading to the cell’s response.

$IP_3$ , in contrast to DAG, does not exert its second-messenger function by directly activating a protein kinase. Rather, cytosolic  $IP_3$  binds to receptors located on the endoplasmic reticulum. These receptors are ligand-gated  $Ca^{2+}$  channels that open when bound to  $IP_3$ . Because the concentration of  $Ca^{2+}$  is much greater in the endoplasmic reticulum than in the cytosol,  $Ca^{2+}$  diffuses out of this organelle into the cytosol, significantly increasing the cytosolic  $Ca^{2+}$  concentration. This increased  $Ca^{2+}$  concentration then continues the sequence of events leading to the cell’s response to the first messenger. We will pick up this thread in more detail shortly. However, it is worth noting that one of the actions of  $Ca^{2+}$  is to help activate some forms of protein kinase C (which is how this kinase got its name—C for “calcium”).

**$Ca^{2+}$**  The calcium ion functions as a second messenger in a great variety of cellular responses to stimuli, both chemical and electrical. The physiology of  $Ca^{2+}$  as a second messenger requires an analysis of two broad questions: (1) How do stimuli cause the cytosolic  $Ca^{2+}$  concentration to increase? (2) How does the increased  $Ca^{2+}$  concentration elicit the cells’ responses?

By means of active-transport systems in the plasma membrane and membranes of certain cell organelles,  $Ca^{2+}$  is





**AP|R Figure 5.10** Mechanism by which an activated receptor stimulates the enzymatically mediated breakdown of  $\text{PIP}_2$  to yield  $\text{IP}_3$  and DAG.  $\text{IP}_3$  then binds to a receptor on the endoplasmic receptor. This receptor is a ligand-gated ion channel that, when opened, allows the release of  $\text{Ca}^{2+}$  from the endoplasmic reticulum into the cytosol. Together with DAG,  $\text{Ca}^{2+}$  activates protein kinase C.

maintained at an extremely low concentration in the cytosol. Consequently, there is always a large electrochemical gradient favoring diffusion of  $\text{Ca}^{2+}$  into the cytosol via  $\text{Ca}^{2+}$  channels found in both the plasma membrane and, as mentioned earlier, the endoplasmic reticulum. A stimulus to the cell can alter this steady state by influencing the active-transport systems and/or the ion channels, resulting in a change in cytosolic  $\text{Ca}^{2+}$  concentration. The most common ways that receptor activation by a first messenger increases the cytosolic  $\text{Ca}^{2+}$  concentration have, in part, been presented in this chapter and are summarized in the top part of **Table 5.3**.

Now we turn to the question of how the increased cytosolic  $\text{Ca}^{2+}$  concentration elicits the cells' responses (see bottom of Table 5.3). The common denominator of  $\text{Ca}^{2+}$  actions is its ability to bind to various cytosolic proteins, altering their conformation and thereby activating their function. One of the most important of these is a protein found in all cells known as **calmodulin** (**Figure 5.11**). On binding with  $\text{Ca}^{2+}$ , calmodulin changes shape, and this allows  $\text{Ca}^{2+}$ -calmodulin to activate or inhibit a large variety of enzymes and other proteins, many of them protein kinases. Activation or inhibition of these **calmodulin-dependent protein kinases** leads, via phosphorylation, to activation or inhibition of proteins involved in the cell's ultimate responses to the first messenger.

Calmodulin is not, however, the only intracellular protein influenced by  $\text{Ca}^{2+}$  binding. For example, you will learn in Chapter 9 how  $\text{Ca}^{2+}$  binds to a protein called troponin in certain types of muscle to initiate contraction.

Finally, for reference purposes, **Table 5.4** summarizes the production and functions of the major second messengers described in this chapter.

## Other Messengers

In a few places in this text, you will learn about messengers that are not as readily classified as those just described. Among these are the eicosanoids. The **eicosanoids** are a family of molecules

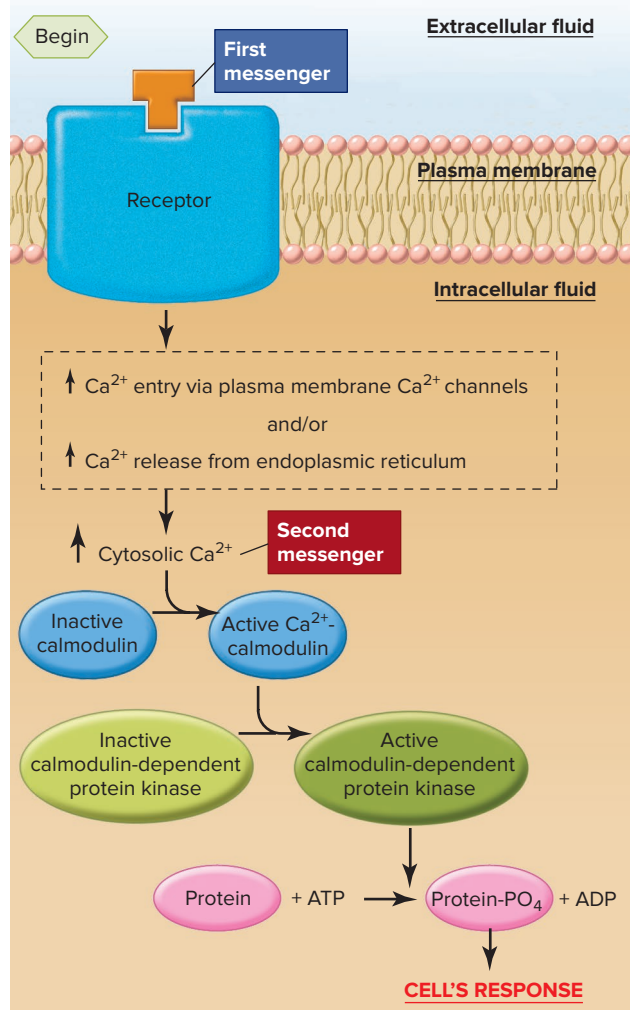
**TABLE 5.3**  $\text{Ca}^{2+}$  as a Second Messenger

### Common Mechanisms by Which Stimulation of a Cell Leads to an Increase in Cytosolic $\text{Ca}^{2+}$ Concentration

- I. Receptor activation
  - A. Plasma-membrane  $\text{Ca}^{2+}$  channels open in response to a first messenger; the receptor itself may contain the channel, or the receptor may activate a G protein that opens the channel via a second messenger.
  - B.  $\text{Ca}^{2+}$  is released from the endoplasmic reticulum; this is typically mediated by  $\text{IP}_3$ .
  - C. Active  $\text{Ca}^{2+}$  transport out of the cell is inhibited by a second messenger.
- II. Opening of voltage-gated  $\text{Ca}^{2+}$  channels

### Major Mechanisms by Which an Increase in Cytosolic $\text{Ca}^{2+}$ Concentration Induces the Cell's Responses

- I.  $\text{Ca}^{2+}$  binds to calmodulin. On binding  $\text{Ca}^{2+}$ , the calmodulin changes shape and becomes activated, which allows it to activate or inhibit a large variety of enzymes and other proteins. Many of these enzymes are protein kinases.
- II.  $\text{Ca}^{2+}$  combines with  $\text{Ca}^{2+}$ -binding proteins other than calmodulin, altering their functions.



**Figure 5.11**  $\text{Ca}^{2+}$ , calmodulin, and the calmodulin-dependent protein kinase system. (There are multiple calmodulin-dependent protein kinases.) Table 5.3 summarizes the mechanisms for increasing cytosolic  $\text{Ca}^{2+}$  concentration.

produced from the polyunsaturated fatty acid arachidonic acid, which is present in plasma membrane phospholipids. The eicosanoids include the **cyclic endoperoxides**, the **prostaglandins**, the **thromboxanes**, and the **leukotrienes** (Figure 5.12). They are generated in many kinds of cells in response to different types of extracellular signals; these include a variety of growth factors, immune defense molecules, and even other eicosanoids. Thus, eicosanoids may act as both extracellular and intracellular messengers, depending on the cell type.

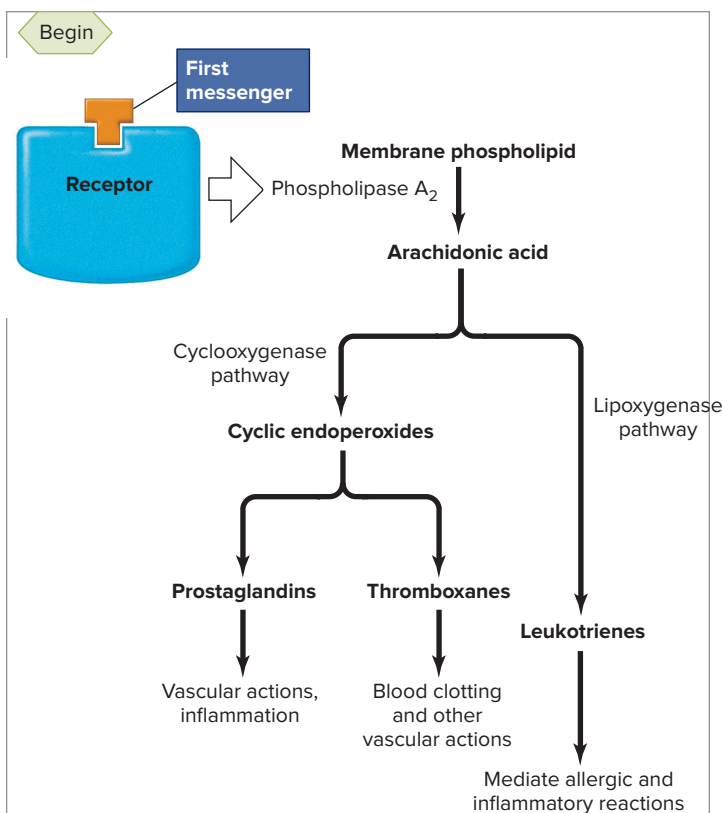
The synthesis of eicosanoids begins when an appropriate stimulus—hormone, neurotransmitter, paracrine substance, drug, or toxic agent—binds its receptor and activates **phospholipase  $\text{A}_2$** , an enzyme localized to the plasma membrane of the stimulated cell. As shown in Figure 5.12, this enzyme splits off arachidonic acid from the membrane phospholipids, and the arachidonic acid can then be metabolized by two pathways. One pathway is initiated by an enzyme called **cyclooxygenase (COX)** and leads ultimately to formation of the cyclic endoperoxides, prostaglandins, and thromboxanes. The other pathway is initiated by the enzyme **lipoxygenase** and leads to formation of the leukotrienes. Within both of these pathways, synthesis of the various specific eicosanoids is enzyme-mediated. Thus, beyond phospholipase  $\text{A}_2$ , the eicosanoid-pathway enzymes expressed in a particular cell determine which eicosanoids the cell synthesizes in response to a stimulus.

Each of the major eicosanoid subdivisions contains more than one member, as indicated by the use of the plural in referring to them (*prostaglandins*, for example). On the basis of structural differences, the different molecules within each subdivision are designated by a letter—for example, PGA and PGE for prostaglandins of the A and E types, which then may be further subdivided—for example,  $\text{PGE}_2$ .

Once they have been synthesized in response to a stimulus, the eicosanoids may in some cases act as intracellular messengers, but more often they are released immediately and act locally. For this reason, the eicosanoids are usually categorized as paracrine and autocrine substances. After they act, they are quickly

**TABLE 5.4** Reference Table of Important Second Messengers

Substance	Source	Effects
$\text{Ca}^{2+}$	Enters cell through plasma membrane ion channels or is released into the cytosol from endoplasmic reticulum.	Activates protein kinase C, calmodulin, and other $\text{Ca}^{2+}$ -binding proteins; $\text{Ca}^{2+}$ -calmodulin activates calmodulin-dependent protein kinases.
Cyclic AMP (cAMP)	A G protein activates plasma membrane adenylyl cyclase, which catalyzes the formation of cAMP from ATP.	Activates cAMP-dependent protein kinase (protein kinase A).
Cyclic GMP (cGMP)	Generated from guanosine triphosphate in a reaction catalyzed by a plasma membrane receptor with guanylyl cyclase activity.	Activates cGMP-dependent protein kinase (protein kinase G).
Diacylglycerol (DAG)	A G protein activates plasma membrane phospholipase C, which catalyzes the generation of DAG and $\text{IP}_3$ from plasma membrane phosphatidylinositol bisphosphate ( $\text{PIP}_2$ ).	Activates protein kinase C.
Inositol trisphosphate ( $\text{IP}_3$ )	See DAG above.	Releases $\text{Ca}^{2+}$ from endoplasmic reticulum into the cytosol.



metabolized by local enzymes to inactive forms. The eicosanoids exert a wide array of effects, particularly on blood vessels and in inflammation. Many of these will be described in future chapters.

Certain drugs influence the eicosanoid pathway and are among the most commonly used in the world today. **Aspirin**, for example, inhibits cyclooxygenase and, therefore, blocks the synthesis of the endoperoxides, prostaglandins, and thromboxanes. It and other drugs that also block cyclooxygenase are collectively termed **nonsteroidal anti-inflammatory drugs (NSAIDs)**. Their major uses are to reduce pain, fever, and inflammation. The term *nonsteroidal* distinguishes them from synthetic glucocorticoids (analogs of steroid hormones made by the adrenal glands) that are used in large doses as anti-inflammatory drugs. These steroids induce expression of a protein that inhibits phospholipase A<sub>2</sub>. Therefore, these steroids block the production of all eicosanoids.

### Cessation of Activity in Signal Transduction Pathways

Responses to messengers are often transient events that persist only briefly and subside when the receptor is no longer bound to the first messenger. There are numerous ways in which this may occur. For example, the first messenger may be metabolized by enzymes in its vicinity, or be taken up by cells and destroyed, or it may simply diffuse away. When events such as these happen, the rate of second-messenger production decreases. The intracellular concentration of second messenger will then decrease due to the actions of cytosolic breakdown enzymes such as cAMP-phosphodiesterase, described earlier. The importance of these events is to prevent chronic overstimulation of a cell by a messenger, which can be very detrimental.

In addition to the removal of a first messenger, the receptors can be inactivated in at least three other ways: (1) The receptor becomes chemically altered (usually by phosphorylation),

**Figure 5.12** Pathways for eicosanoid synthesis and some of their major functions. Phospholipase A<sub>2</sub> is the one enzyme common to the formation of all the eicosanoids. Anti-inflammatory steroids induce expression of a protein that inhibits phospholipase A<sub>2</sub>. The pathway mediated by cyclooxygenase is inhibited by aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs). There are also drugs available that inhibit the lipoxygenase enzyme, thereby blocking the formation of leukotrienes. These drugs may be helpful in controlling asthma, in which excess leukotrienes have been implicated in the allergic and inflammatory components of the disease.

### PHYSIOLOGICAL INQUIRY

- Based on the pathways shown in this figure, why are people advised to avoid taking aspirin or other NSAIDs prior to a surgical procedure?

*Answer can be found at end of chapter.*

which may decrease its affinity for a first messenger, and so the messenger is released from its receptor; (2) phosphorylation of the receptor may prevent further G-protein binding to the receptor; and (3) plasma membrane receptors may be removed when the combination of first messenger and receptor is taken into the cell by endocytosis. The processes described here are physiologically controlled. For example, in many cases the inhibitory phosphorylation of a receptor is mediated by a protein kinase that was initially activated in response to the first messenger. This receptor inactivation constitutes negative feedback.

This concludes our description of the basic principles of signal transduction pathways. It is essential to recognize that the pathways do not exist in isolation but may be active simultaneously in a single cell, undergoing complex interactions. This is possible because a single first messenger may trigger changes in the activity of more than one pathway and, much more importantly, because many different first messengers may simultaneously influence a cell. Moreover, a great deal of “cross talk” can occur at one or more levels among the various signal transduction pathways. For example, active molecules generated in the cAMP pathway can alter the activity of receptors and signaling molecules generated by other pathways. ■

### SUMMARY

#### Receptors

- Receptors for chemical messengers are proteins or glycoproteins located either inside the cell or, much more commonly, in the plasma membrane. The binding of a messenger by a receptor manifests specificity, saturation, and competition.



- II. Receptors are subject to physiological regulation by their own messengers. This includes down- and up-regulation.
- III. Different cell types express different types of receptors; even a single cell may express multiple receptor types.

Signal Transduction Pathways

- I. Binding a chemical messenger activates a receptor, and this initiates one or more signal transduction pathways leading to the cell's response.
- II. Lipid-soluble messengers bind to receptors inside the target cell. The activated receptor acts in the nucleus as a transcription factor to alter the rate of transcription of specific genes, resulting in a change in the concentration or secretion of the proteins the genes encode.
- III. Water-soluble messengers bind to receptors on the plasma membrane. The pathways induced by activation of the receptor often involve second messengers and protein kinases.
  - a. The receptor may be a ligand-gated ion channel. The channel opens, resulting in an electrical signal in the membrane and, when  $\text{Ca}^{2+}$  channels are involved, an increase in the cytosolic  $\text{Ca}^{2+}$  concentration.
  - b. The receptor may itself be an enzyme. With one exception, the enzyme activity is that of a protein kinase, usually a tyrosine kinase. The exception is the receptor that functions as a guanylyl cyclase to generate cyclic GMP.
  - c. The receptor may activate a cytosolic janus kinase associated with it.
  - d. The receptor may interact with an associated plasma membrane G protein, which in turn interacts with plasma membrane effector proteins—ion channels or enzymes.
- IV. The membrane effector enzyme adenylyl cyclase catalyzes the conversion of cytosolic ATP to cyclic AMP. Cyclic AMP acts as a second messenger to activate intracellular cAMP-dependent protein kinase, which phosphorylates proteins that mediate the cell's ultimate responses to the first messenger.
- V. The plasma membrane enzyme phospholipase C catalyzes the formation of diacylglycerol (DAG) and inositol trisphosphate ( $\text{IP}_3$ ). DAG activates protein kinase C, and  $\text{IP}_3$  acts as a second messenger to release  $\text{Ca}^{2+}$  from the endoplasmic reticulum.
- VI. The calcium ion is one of the most widespread second messengers.
  - a. An activated receptor can increase cytosolic  $\text{Ca}^{2+}$  concentration by causing certain  $\text{Ca}^{2+}$  channels in the plasma membrane and/or endoplasmic reticulum to open.
  - b.  $\text{Ca}^{2+}$  binds to one of several intracellular proteins, most often calmodulin. Calcium-activated calmodulin activates or inhibits many proteins, including calmodulin-dependent protein kinases.
- VII. The signal transduction pathways triggered by activated plasma membrane receptors may influence genetic expression by activating transcription factors.
- VIII. Eicosanoids are derived from arachidonic acid, which is released from phospholipids in the plasma membrane. They exert widespread intracellular and extracellular effects on cell activity.
- IX. Cessation of receptor activity occurs when the first-messenger molecule concentration decreases or when the receptor is chemically altered or internalized, in the case of plasma membrane receptors.

REVIEW QUESTIONS

- What is the chemical nature of receptors? Where are they located?
- Explain why different types of cells may respond differently to the same chemical messenger.
- Describe the basis of down-regulation and up-regulation, and how these processes are related to homeostasis.
- What is the first step in the action of a messenger on a cell?
- Describe the signal transduction pathway that lipid-soluble messengers use.
- Classify plasma membrane receptors according to the signal transduction pathways they initiate.
- What is the result of opening a membrane ion channel?
- Contrast receptors that have intrinsic enzyme activity with those associated with cytoplasmic janus kinases.
- Describe the function of plasma membrane G proteins.
- Draw a diagram describing the adenylyl cyclase–cAMP system.
- Draw a diagram illustrating the phospholipase C/DAG/ $\text{IP}_3$  system.
- How does the  $\text{Ca}^{2+}$ –calmodulin system function?

KEY TERMS

5.1 Receptors

affinity	receptors
agonists	saturation
antagonist	signal transduction
competition	specificity
down-regulation	up-regulation
internalization	

5.2 Signal Transduction Pathways

adenylyl cyclase	guanylyl cyclase
calmodulin	inositol trisphosphate ( $\text{IP}_3$ )
calmodulin-dependent protein kinases	janus kinases (JAKs)
cAMP-dependent protein kinase	leukotrienes
cAMP phosphodiesterase	lipoxigenase
cGMP-dependent protein kinase	nuclear receptors
cyclic AMP (cAMP)	phospholipase $\text{A}_2$
cyclic endoperoxides	phospholipase C
cyclic GMP (cGMP)	prostaglandins
cyclooxygenase (COX)	protein kinase
diacylglycerol (DAG)	protein kinase C
eicosanoids	receptor activation
first messengers	receptor tyrosine kinases
G-protein-coupled receptors	second messengers
G proteins	signal transduction pathways
	thromboxanes

CLINICAL TERMS

5.1 Receptors

antihistamines	oxymetazoline
beta-adrenergic receptor blocker	phenylephrine

5.2 Signal Transduction Pathways

aspirin	nonsteroidal anti-inflammatory drugs (NSAIDs)
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# Clinical Case Study: A Child with Unexplained Weight Gain and Calcium Imbalance



A 3-year-old girl was seen by her pediatrician to determine the cause of a recent increase in the rate of her weight gain. Her height was normal (95 cm/37.4 inches) but she weighed 16.5 kg (36.3 pounds), which is in the 92nd percentile for her age. The girl's mother—who was very short and overweight—stated that the child seemed listless at times and was rarely very active. She was also prone to muscle cramps and complained to her mother that her fingers

and toes “felt funny,” which the pediatrician was able to interpret as tingling sensations. She had a good appetite but not one that appeared unusual or extreme. The doctor suspected that the child had developed a deficiency in the amount of thyroid hormone in her blood. This hormone is produced by the thyroid gland in the neck (look ahead to Figure 11.21) and is responsible in part for normal metabolism, that is, the rate at which calories are expended. Too little thyroid hormone typically results in weight gain and may also cause fatigue or lack of energy. A blood test was performed, and indeed the girl's thyroid hormone concentration was low. Because there are several conditions that may result in a deficiency of thyroid hormone, an additional exam was performed. During that exam, the physician noticed that the fourth metacarpals (the bones at the base of the ring fingers) on each of the girl's hands were shorter than normal, and he could feel hard bumps (nodules) just beneath the girl's skin at various sites on her body. He ordered a blood test for  $\text{Ca}^{2+}$  and for a hormone called parathyroid hormone (PTH).

PTH gets its name because the glands that produce it lie adjacent (*para*) to the thyroid gland. PTH normally acts on the kidneys and bones to maintain calcium ion homeostasis in the blood.

## Reflect and Review #1

- In what general ways is balance of  $\text{Ca}^{2+}$  achieved in the blood? (Refer back to Section 1.8 of Chapter 1 for help.)

Should the  $\text{Ca}^{2+}$  concentration in the blood decrease for any reason, PTH secretion will increase and stimulate the release of  $\text{Ca}^{2+}$  from bones into the blood. It also stimulates the retention of  $\text{Ca}^{2+}$  by the kidneys, such that less  $\text{Ca}^{2+}$  is lost in the urine. These two factors help to restore a normal blood  $\text{Ca}^{2+}$  concentration—a classic example of homeostasis. The doctor suspected that the nodules he felt were  $\text{Ca}^{2+}$  deposits and that the shortened fingers were the result of improper bone formation during development due to a  $\text{Ca}^{2+}$  imbalance. Abnormally low blood  $\text{Ca}^{2+}$  would also explain the muscle cramps and the tingling sensations. This is because a homeostatic extracellular  $\text{Ca}^{2+}$  concentration is also critical for normal function of muscles and nerves. The results of the blood test confirmed that the  $\text{Ca}^{2+}$  concentration was lower than normal. A logical explanation for why  $\text{Ca}^{2+}$  may be low would be because PTH concentrations were low. Paradoxically, however, the PTH concentration was increased in the girl's blood. This means that plenty of PTH was present but was somehow unable to act on its targets—the

bones and kidneys—to maintain  $\text{Ca}^{2+}$  balance in the blood. What could prevent PTH from doing its job? How might this be related to the thyroid hormone imbalance that was responsible for the weight gain?

A genetic condition in which the PTH concentration in the blood is high but  $\text{Ca}^{2+}$  is low is **pseudohypoparathyroidism**. The prefix *hypo* in this context refers to “less than normal amounts of” PTH in the blood. This girl's condition seemed to fit a diagnosis of hypoparathyroidism, because her  $\text{Ca}^{2+}$  concentration was low and she consequently demonstrated several symptoms characteristic of low  $\text{Ca}^{2+}$ . These findings would suggest that there was not enough PTH available. However, because her PTH concentration was *not* low—in fact, it was higher than normal—the condition is called *pseudo*, or “false,” hypoparathyroidism.

A blood sample was taken from the girl and the white blood cells were subjected to DNA analysis to test the possibility that a mutation might exist in a gene required for PTH signaling.

## Reflect and Review #2

- What is a mutation, and how might it result in a change in the primary structure of a protein? (Refer back to Figures 2.16 and 2.17 for help.)

That analysis revealed that the girl was heterozygous for a mutation in the *GNAS1* gene, which encodes the alpha subunit of the stimulatory G protein ( $G_s$  alpha). Recall from Figure 5.6 that  $G_s$  couples certain plasma membrane receptors to adenylyl cyclase and the production of cAMP, an important second messenger in many cells. PTH is known to act by binding to a plasma membrane receptor and activating adenylyl cyclase via this pathway. Because the girl had decreased expression of normal  $G_s$  alpha, her cells were unable to respond adequately to PTH, and consequently her blood concentration of  $\text{Ca}^{2+}$  could not be maintained within the normal range, *even though she was not deficient in PTH*.

PTH, however, is not the only messenger in the body that acts through a  $G_s$ -coupled receptor linked to cAMP production. As you have learned in this chapter, there are many other such molecules. One of them is a factor that stimulates thyroid hormone production by the thyroid gland. This explains why the young girl had a low thyroid hormone concentration in addition to her PTH/ $\text{Ca}^{2+}$  imbalance.

Pseudohypoparathyroidism is a very rare disorder, but it illustrates a larger and extremely important medical concern called target-organ resistance. Such diseases are characterized by normal or even increased blood concentrations of signaling molecules such as PTH, but insensitivity (that is, resistance) of a target organ (or organs) to the molecule (Table 5.5). In our patient, the cause of the resistance was insufficient  $G_s$ -alpha action due to an inherited mutation; in other cases, it may result from defects in other aspects of cell signaling pathways or in receptor structure. It is likely that the girl inherited the mutation from her mother, who showed some similar symptoms.

The girl was treated with a thyroid hormone pill each day, calcium tablets twice per day, and a derivative of vitamin D (which

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helps the intestines absorb  $\text{Ca}^{2+}$ ) twice per day. She will need to remain on this treatment plan for the rest of her life. In addition, it will be important for her physician to monitor other physiological functions mediated by other chemical messengers that are known to act via  $G_s$  alpha.

**Clinical term: pseudohypoparathyroidism**

**TABLE 5.5**

**Mechanisms leading to target-organ resistance to chemical messengers such as PTH.**

Messenger (e.g. PTH)	Receptor for messenger (e.g. PTH receptor)	Signaling pathway activated by messenger (e.g. cAMP)	Is there target-organ resistance?
Present	Present	Present	No
Present	Missing/Abnormal	Present	Yes
Present	Present	Missing/Abnormal	Yes (this case study)

See Chapter 19 for complete, integrative case studies.

**CHAPTER 5 TEST QUESTIONS Recall and Comprehend**

Answers appear in Appendix A.

These questions test your recall of important details covered in this chapter. They also help prepare you for the type of questions encountered in standardized exams. Many additional questions of this type are available on Connect and LearnSmart.

1–3: Match a receptor feature (a–e) with each choice.

- Defines the situation when all receptor binding sites are occupied by a messenger
- Defines the strength of receptor binding to a messenger
- Reflects the fact that a receptor normally binds only to a single messenger

**Receptor feature:**

- affinity
  - saturation
  - competition
  - down-regulation
  - specificity
4. Which of the following intracellular or plasma membrane proteins requires  $\text{Ca}^{2+}$  for full activity?
- calmodulin
  - janus kinase (JAK)
  - cAMP-dependent protein kinase
  - guanylyl cyclase
5. Which is correct?
- cAMP-dependent protein kinase phosphorylates tyrosine residues.
  - Protein kinase C is activated by cAMP.
  - The subunit of  $G_s$  proteins that activates adenylyl cyclase is the beta subunit.
  - Lipid-soluble messengers typically act on receptors in the cell cytosol or nucleus.
  - The binding site of a typical plasma membrane receptor for its messenger is located on the cytosolic surface of the receptor.

6. Inhibition of which enzyme/enzymes would inhibit the conversion of arachidonic acid to leukotrienes?

- cyclooxygenase
- lipoygenase
- phospholipase  $A_2$
- adenylyl cyclase
- both b and c

7–10: Match each type of molecule with the correct choice (a–e); a given choice may be used once, more than once, or not at all.

**Molecule:**

- second messenger
- example of a first messenger
- part of a trimeric protein in membranes
- enzyme

**Choices:**

- neurotransmitter or hormone
- cAMP-dependent protein kinase
- calmodulin
- $\text{Ca}^{2+}$
- alpha subunit of G proteins

**CHAPTER 5 TEST QUESTIONS Apply, Analyze, and Evaluate**

Answers appear in Appendix A.

These questions, which are designed to be challenging, require you to integrate concepts covered in the chapter to draw your own conclusions. See if you can first answer the questions without using the hints that are provided; then, if you are having difficulty, refer back to the figures or sections indicated in the hints.

- Patient A is given a drug that blocks the synthesis of all eicosanoids, whereas patient B is given a drug that blocks the synthesis of leukotrienes but none of the other eicosanoids. What enzymes do these drugs most likely block? *Hint:* Refer back to the pathways covered in Figure 5.12.

- Certain nerves to the heart release the neurotransmitter norepinephrine. If these nerves are removed in experimental animals, the heart becomes extremely sensitive to the administration of a drug that is an agonist



of norepinephrine. Explain why this may happen, in terms of receptor physiology. *Hint:* See “Regulation of Receptors” in Section 5.1.

3. A particular hormone is known to elicit—completely by way of the cyclic AMP system—six different responses in its target cell. A drug is found that eliminates one of these responses but not the other five. Which of the following, if any, could the drug be blocking: the hormone’s receptors,  $G_s$

protein, adenylyl cyclase, or cyclic AMP? *Hint:* The cAMP pathway is covered in Figure 5.6.

4. If a drug were found that blocked all  $Ca^{2+}$  channels that were directly linked to G proteins, would this eliminate the function of  $Ca^{2+}$  as a second messenger? Why or why not? *Hint:* Refer to Table 5.3 for help.

## CHAPTER 5 TEST QUESTIONS *General Principles Assessment*

*Answers appear in Appendix A.*

*These questions reinforce the key theme first introduced in Chapter 1, that general principles of physiology can be applied across all levels of organization and across all organ systems.*

1. What examples from this chapter demonstrate the general principle of physiology that *controlled exchange of materials occurs between compartments and across cell membranes*? Specifically, how is this related to another general principle of physiology, namely, *information flow between cells, tissues, and organs is an essential feature of homeostasis and allows for integration of physiological processes*?
2. Another general principle of physiology states that *physiological processes require the transfer and balance of matter and energy*. How is energy balance related to intracellular signaling?

## CHAPTER 5 ANSWERS TO PHYSIOLOGICAL INQUIRY QUESTIONS

**Figure 5.3** The structures of both the messenger and its receptors determine their ability to bind to each other with specificity. It is the binding of a messenger to a receptor that causes the activation (function) of the receptor. In addition, any molecule with a structure that is sufficiently similar to that of the messenger may also bind that receptor; in the case of competitors, this may decrease the function of the messenger-receptor system. The specificity of the messenger-receptor interaction allows each messenger to exert a discrete action. This is the basis of many therapeutic drugs that are used to block the deleterious effects of an excess of naturally occurring messengers.

**Figure 5.4** The lipid nature of certain messengers makes it possible for them to diffuse through the lipid bilayer of a plasma membrane. Consequently, the receptors for such messengers exist inside the cell. By contrast, hydrophilic messengers cannot penetrate a lipid bilayer, and as a result their receptors are located within plasma membranes with an extracellular component that can detect specific ligands. Therefore, the cellular location of receptors for chemical messengers depends upon the chemical characteristics of the messengers, which, in turn, determines their permeability through cell membranes.

**Figure 5.5** Expressing more than one type of receptor allows a cell to respond to more than one type of first messenger. For example, one first messenger might activate a particular biochemical pathway in a cell by activating one type of receptor and signaling pathway. By contrast, another first messenger acting on a different receptor and activating a

different signaling pathway might inhibit the same biochemical process. In this way, the biochemical process can be tightly regulated.

**Figure 5.8** Enzymes can generate large amounts of product without being consumed; a single enzyme molecule can continue to function repeatedly on fresh substrate. This is an extremely efficient way to generate a second messenger like cAMP. Enzymes have many other advantages (see Table 3.4), including the ability to have their activities fine-tuned by other inputs (see Figures 3.36 to 3.38). This enables the cell to adjust its response to a first messenger depending on the other conditions present.

**Figure 5.9** Not necessarily. In some cases, a kinase may phosphorylate the same protein in many different types of cells. However, many cells also express certain cell-specific proteins that are not found in all tissues, and some of these proteins may be substrates for cAMP-dependent protein kinase. Thus, the proteins that are phosphorylated by a given kinase depend upon the cell type, which makes the cellular response tissue-specific. As an example, in the kidneys, cAMP-dependent protein kinase phosphorylates proteins that insert water channels in cell membranes and thereby decrease urine volume, whereas in heart muscle the same kinase phosphorylates  $Ca^{2+}$  channels that increase the strength of muscle contraction.

**Figure 5.12** Aspirin and NSAIDs block the cyclooxygenase pathway. This includes the pathway to the production of thromboxanes, which as shown in the figure are important for blood clotting. Because of the risk of bleeding that occurs with any type of surgery, the use of such drugs prior to the surgery may increase the likelihood of excessive bleeding.

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