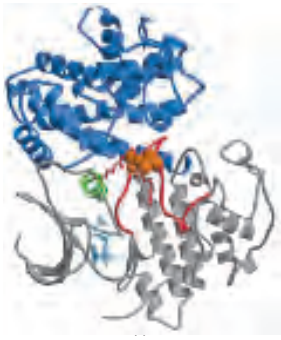


chapter 12

BIOSIGNALING

When I first entered the study of hormone action, some 25 years ago, there was a widespread feeling among biologists that hormone action could not be studied meaningfully in the absence of organized cell structure. However, as I reflected on the history of biochemistry, it seemed to me there was a real possibility that hormones might act at the molecular level.

—Earl W. Sutherland, Nobel Address, 1971

The ability of cells to receive and act on signals from beyond the plasma membrane is fundamental to life. Bacterial cells receive constant input from membrane proteins that act as information receptors, sampling the surrounding medium for pH, osmotic strength, the availability of food, oxygen, and light, and the presence of noxious chemicals, predators, or competitors for food. These signals elicit appropriate responses, such as motion toward food or away from toxic substances or the formation of dormant spores in a nutrient-depleted medium. In multicellular organisms, cells with different functions exchange a wide variety of signals. Plant cells respond to growth hormones and to variations in sunlight. Animal cells exchange information about the concentrations of ions and glucose in extracellular fluids, the interdependent metabolic activities taking place in different tissues, and, in an embryo, the correct placement of cells during development. In all these cases, the signal represents *information* that is detected by specific receptors and converted to a cellular response, which always involves a *chemical* process. This conversion of information into a chemical change, **signal transduction,** is a universal property of living cells.

The number of different biological signals is large (Table 12–1), as is the variety of biological responses to these signals, but organisms use just a few evolutionarily conserved mechanisms to detect extracellular signals and *transduce* them into intracellular changes. In this chapter we examine some examples of the major classes of signaling mechanisms, looking at how they are integrated in specific biological functions such as the transmission of nerve signals; responses to hormones and growth factors; the senses of sight, smell, and taste; and

TABLE 12–1
Some Signals to Which Cells Respond

Antigens	Light
Cell surface glycoproteins/ oligosaccharides	Mechanical touch
Developmental signals	Neurotransmitters
Extracellular matrix components	Nutrients
Growth factors	Odorants
Hormones	Pheromones
	Tastants

control of the cell cycle. Often, the end result of a signaling pathway is the phosphorylation of a few specific target-cell proteins, which changes their activities and thus the activities of the cell. Throughout our discussion we emphasize the conservation of fundamental mechanisms for the transduction of biological signals and the adaptation of these basic mechanisms to a wide range of signaling pathways.

12.1 Molecular Mechanisms of Signal Transduction

Signal transductions are remarkably specific and exquisitely sensitive. **Specificity** is achieved by precise molecular complementarity between the signal and receptor molecules (Fig. 12–1a), mediated by the same kinds of weak (noncovalent) forces that mediate enzyme-substrate and antigen-antibody interactions. Multicellular organisms have an additional level of specificity, because the receptors for a given signal, or the intracellular targets of a given signal pathway, are present only in certain cell types. Thyrotropin-releasing hormone, for example, triggers responses in the cells of the anterior pituitary but not in hepatocytes, which lack receptors for this hormone. Epinephrine alters glycogen metabolism in hepatocytes but not in erythrocytes; in this case, both cell types have receptors for the hormone, but whereas hepatocytes contain glycogen and the glycogen-metabolizing enzyme that is stimulated by epinephrine, erythrocytes contain neither.

Three factors account for the extraordinary sensitivity of signal transducers: the high affinity of receptors for signal molecules, cooperativity (often but not always) in the ligand-receptor interaction, and amplification of the signal by enzyme cascades. The **affinity** between signal (ligand) and receptor can be expressed as the dissociation constant K_d, usually 10^{-10} M or less—meaning that the receptor detects picomolar concentrations of a signal molecule. Receptor-ligand interactions are quantified by Scatchard analysis, which yields a quantitative measure of affinity (K_d) and the number of ligand-binding sites in a receptor sample (Box 12–1).

Cooperativity in receptor-ligand interactions results in large changes in receptor activation with small changes in ligand concentration (recall the effect of cooperativity on oxygen binding to hemoglobin; see Fig. 5–12). **Amplification** by **enzyme cascades** results when an enzyme associated with a signal receptor is activated and, in turn, catalyzes the activation of many molecules of a second enzyme, each of which activates many molecules of a third enzyme, and so on (Fig. 12–1b). Such cascades can produce amplifications of several orders of magnitude within milliseconds.

The sensitivity of receptor systems is subject to modification. When a signal is present continuously, **desensitization** of the receptor system results (Fig. 12–1c); when the stimulus falls below a certain threshold, the system again becomes sensitive. Think of what happens to your visual transduction system when you walk from bright sunlight into a darkened room or from darkness into the light.

A final noteworthy feature of signal-transducing systems is **integration** (Fig. 12–1d), the ability of the system to receive multiple signals and produce a unified response appropriate to the needs of the cell or organism. Different signaling pathways converse with

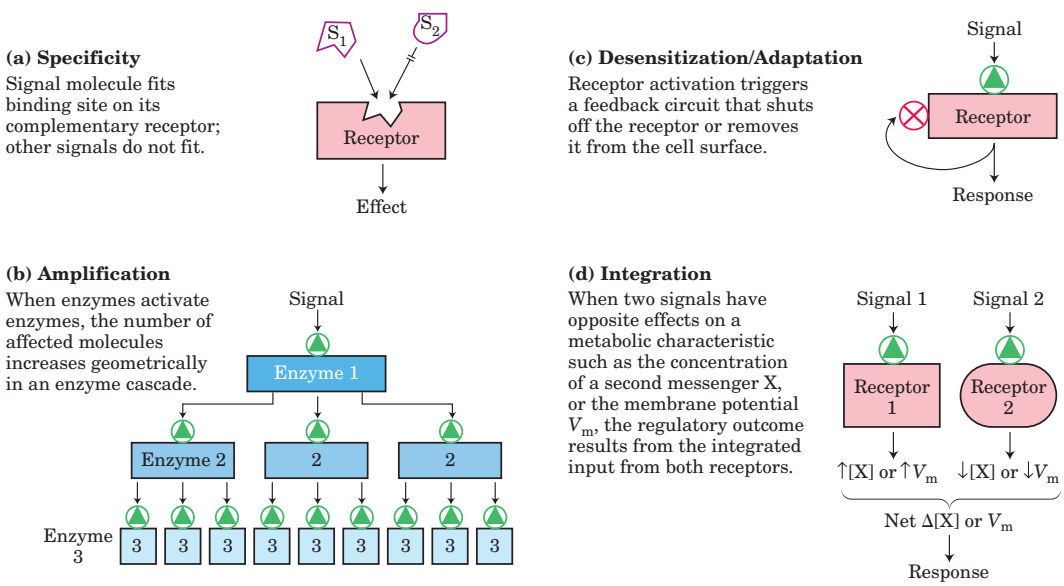


FIGURE 12–1 Four features of signal-transducing systems.

Scatchard Analysis Quantifies the Receptor-Ligand Interaction

The cellular actions of a hormone begin when the hormone (ligand, L) binds specifically and tightly to its protein receptor (R) on or in the target cell. Binding is mediated by noncovalent interactions (hydrogen-bonding, hydrophobic, and electrostatic) between the complementary surfaces of ligand and receptor. Receptor-ligand interaction brings about a conformational change that alters the biological activity of the receptor, which may be an enzyme, an enzyme regulator, an ion channel, or a regulator of gene expression.

Receptor-ligand binding is described by the equation

$$\underset{\text{Receptor}}{\text{R}} + \underset{\text{Ligand}}{\text{L}} \rightleftharpoons \underset{\text{Receptor-ligand complex}}{\text{RL}}$$

This binding, like that of an enzyme to its substrate, depends on the concentrations of the interacting components and can be described by an equilibrium constant:

$$\underset{\text{Receptor}}{\text{R}} + \underset{\text{Ligand}}{\text{L}} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \underset{\text{Receptor-ligand complex}}{\text{RL}}$$

$$K_a = \frac{[RL]}{[R][L]} = \frac{k_{+1}}{k_{-1}} = 1/K_d$$

where K_a is the association constant and K_d is the dissociation constant.

Like enzyme-substrate binding, receptor-ligand binding is saturable. As more ligand is added to a fixed amount of receptor, an increasing fraction of receptor molecules is occupied by ligand (Fig. 1a). A rough measure of receptor-ligand affinity is given by the concentration of ligand needed to give half-saturation of the receptor. Using **Scatchard analysis** of receptor-ligand binding, we can estimate both the dissociation constant K_d and the number of receptor-binding sites in a given preparation. When binding has reached equilibrium, the total number of possible binding sites, B_{max}, equals the number of unoccupied sites, represented by [R], plus the number of occupied or ligand-bound sites, [RL]; that is, $B_{max} = [R] + [RL]$. The number of unbound sites can be expressed in terms of total sites minus occupied sites: $[R] = B_{max} - [RL]$. The equilibrium expression can now be written

$$K_a = \frac{[RL]}{[L](B_{max} - [RL])}$$

Rearranging to obtain the ratio of receptor-bound ligand to free (unbound) ligand, we get

$$\frac{[\text{Bound}]}{[\text{Free}]} = \frac{[RL]}{[L]} = K_a(B_{max} - [RL])$$

$$= \frac{1}{K_d}(B_{max} - [RL])$$

From this slope-intercept form of the equation, we can see that a plot of [bound ligand]/[free ligand] versus [bound ligand] should give a straight line with a slope of $-K_a$ $(-1/K_d)$ and an intercept on the abscissa of B_{max}, the total number of binding sites (Fig. 1b). Hormone-ligand interactions typically have K_d values of 10^{-9} to 10^{-11} M, corresponding to very tight binding.

Scatchard analysis is reliable for the simplest cases, but as with Lineweaver-Burk plots for enzymes, when the receptor is an allosteric protein, the plots deviate from linearity.

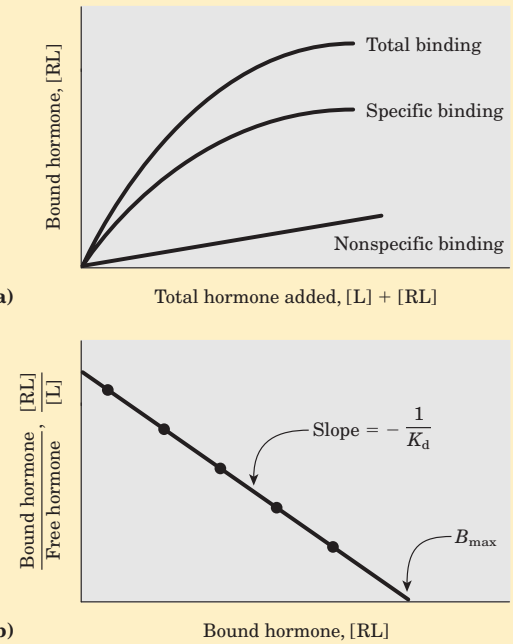


FIGURE 1 Scatchard analysis of a receptor-ligand interaction. A radiolabeled ligand (L)—a hormone, for example—is added at several concentrations to a fixed amount of receptor (R), and the fraction of the hormone bound to receptor is determined by separating the receptor-hormone complex (RL) from free hormone. **(a)** A plot of [RL] versus [L] + [RL] (total hormone added) is hyperbolic, rising toward a maximum for [RL] as the receptor sites become saturated. To control for nonsaturable, nonspecific binding sites (eicosanoid hormones bind nonspecifically to the lipid bilayer, for example), a separate series of binding experiments is also necessary. A large excess of unlabeled hormone is added along with the dilute solution of labeled hormone. The unlabeled molecules compete with the labeled molecules for specific binding to the saturable site on the receptor, but not for the nonspecific binding. The true value for specific binding is obtained by subtracting nonspecific binding from total binding. **(b)** A linear plot of [RL]/[L] versus [RL] gives K_d and B_{max} for the receptor-hormone complex. Compare these plots with those of V_0 versus [S] and $1/V_0$ versus 1/[S] for an enzyme-substrate complex (see Fig. 6–12, Box 6–1).

each other at several levels, generating a wealth of interactions that maintain homeostasis in the cell and the organism.

We consider here the molecular details of several representative signal-transduction systems. The trigger for each system is different, but the general features of signal transduction are common to all: a signal interacts with a receptor; the activated receptor interacts with cellular machinery, producing a second signal or a change in the activity of a cellular protein; the metabolic activity (broadly defined to include metabolism of RNA, DNA, and protein) of the target cell undergoes a change; and finally, the transduction event ends and the cell returns to its prestimulus state. To illustrate these general features of signaling systems, we provide examples of each of six basic signaling mechanisms (Fig. 12–2).

1. Gated ion channels of the plasma membrane that open and close (hence the term "gating") in response to the binding of chemical ligands or changes in transmembrane potential. These are the simplest signal transducers. The acetylcholine receptor ion channel is an example of this mechanism (Section 12.2).
2. Receptor enzymes, plasma membrane receptors that are also enzymes. When one of these receptors is activated by its extracellular ligand, it catalyzes the production of an intracellular second messenger. An example is the insulin receptor (Section 12.3).
3. Receptor proteins (serpentine receptors) that *indirectly* activate (through GTP-binding proteins, or G proteins) enzymes that generate intracellular second messengers. This is illustrated by the β-adrenergic receptor system that detects epinephrine (adrenaline) (Section 12.4).
4. Nuclear receptors (steroid receptors) that, when bound to their specific ligand (such as the hormone estrogen), alter the rate at which specific genes are transcribed and translated into cellular proteins. Because steroid hormones function through mechanisms intimately related to the regulation of gene expression, we consider them here only briefly (Section 12.8) and defer a detailed discussion of their action until Chapter 28.
5. Receptors that lack enzymatic activity but attract and activate cytoplasmic enzymes that act on downstream proteins, either by directly converting them to gene-regulating proteins or by activating a cascade of enzymes that finally activates a gene regulator. The JAK-STAT system exemplifies the first mechanism (Section 12.3); and the TLR4 (Toll) signaling system in humans, the second (Section 12.6).

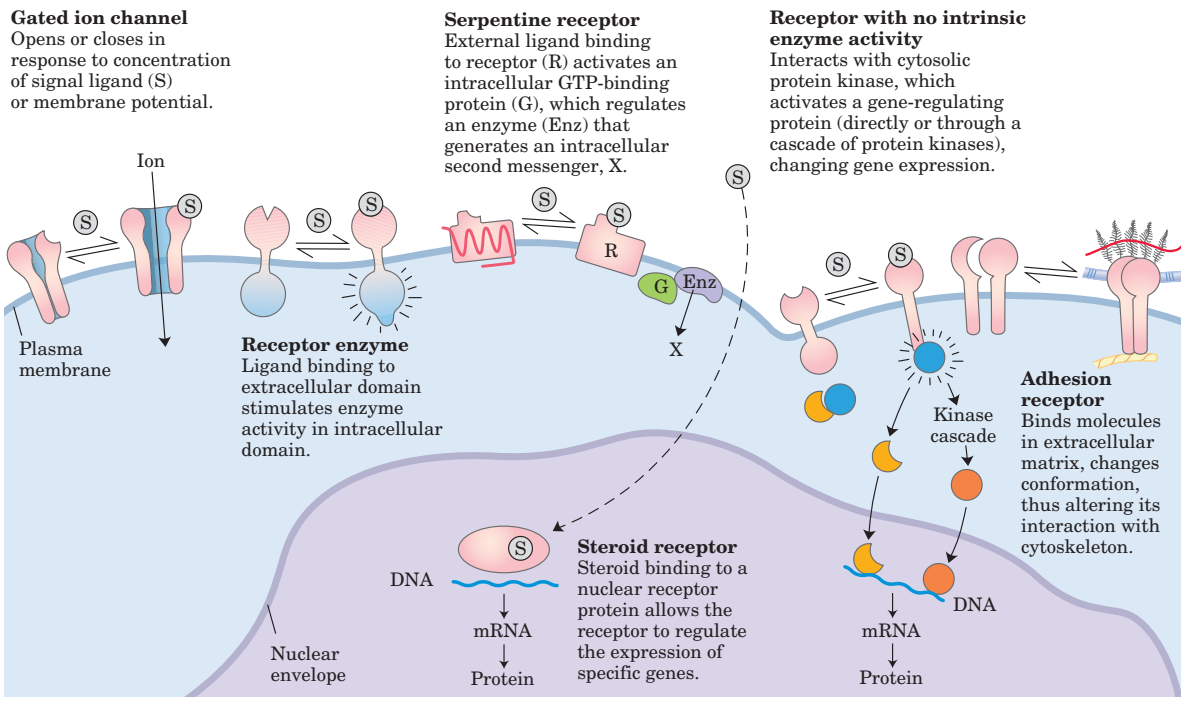


FIGURE 12–2 Six general types of signal transducers.

6. Receptors (adhesion receptors) that interact with macromolecular components of the extracellular matrix (such as collagen) and convey to the cytoskeletal system instructions on cell migration or adherence to the matrix. Integrins (discussed in Chapter 10) illustrate this general type of transduction mechanism.

As we shall see, transductions of all six types commonly require the activation of protein kinases, enzymes that transfer a phosphoryl group from ATP to a protein side chain.

SUMMARY 12.1 Molecular Mechanisms of Signal Transduction

- All cells have specific and highly sensitive signal-transducing mechanisms, which have been conserved during evolution.
- A wide variety of stimuli, including hormones, neurotransmitters, and growth factors, act through specific protein receptors in the plasma membrane.
- The receptors bind the signal molecule, amplify the signal, integrate it with input from other receptors, and transmit it into the cell. If the signal persists, receptor desensitization reduces or ends the response.
- Eukaryotic cells have six general types of signaling mechanisms: gated ion channels; receptor enzymes; membrane proteins that act through G proteins; nuclear proteins that bind steroids and act as transcription factors; membrane proteins that attract and activate soluble protein kinases; and adhesion receptors that carry information between the extracellular matrix and the cytoskeleton.

12.2 Gated Ion Channels

Ion Channels Underlie Electrical Signaling in Excitable Cells

The excitability of sensory cells, neurons, and myocytes depends on ion channels, signal transducers that provide a regulated path for the movement of inorganic ions such as Na^+, K^+, Ca^{2+}, and Cl^- across the plasma membrane in response to various stimuli. Recall from Chapter 11 that these ion channels are "gated"; they may be open or closed, depending on whether the associated receptor has been activated by the binding of its specific ligand (a neurotransmitter, for example) or by a change in the transmembrane electrical potential, V_m. The Na^+K^+ ATPase creates a charge imbalance across the plasma membrane by carrying 3 Na^+ out of the cell for every 2 K^+ carried in (Fig. 12–3a), making the inside negative relative to the outside. The membrane is said to be polarized. By convention, V_m is negative when the inside of the cell is negative relative to the outside. For a typical animal cell, $V_m = -60$ to -70 mV.

Because ion channels generally allow passage of either anions or cations but not both, ion flux through a channel causes a redistribution of charge on the two sides of the membrane, changing V_m. Influx of a positively charged ion such as Na^+, or efflux of a negatively charged ion such as Cl^-, depolarizes the membrane and brings V_m closer to zero. Conversely, efflux of K^+ hyperpolarizes the membrane and V_m becomes more negative. These ion fluxes through channels are passive, in contrast to active transport by the Na^+K^+ ATPase.

The direction of spontaneous ion flow across a polarized membrane is dictated by the electrochemical

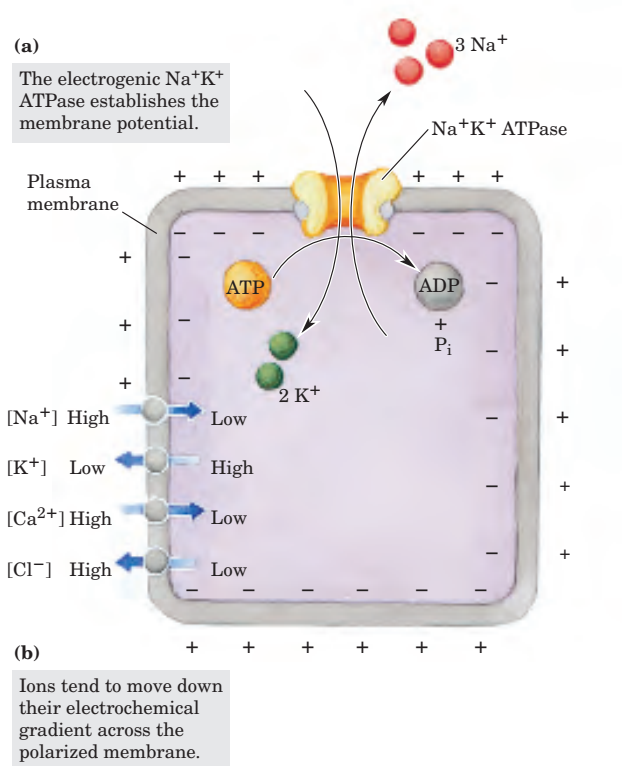


FIGURE 12–3 Transmembrane electrical potential. **(a)** The electrogenic Na^+K^+ ATPase produces a transmembrane electrical potential of -60 mV (inside negative). **(b)** Blue arrows show the direction in which ions tend to move spontaneously across the plasma membrane in an animal cell, driven by the combination of chemical and electrical gradients. The chemical gradient drives Na^+ and Ca^{2+} inward (producing depolarization) and K^+ outward (producing hyperpolarization). The electrical gradient drives Cl^- outward, against its concentration gradient (producing depolarization).

TABLE 12–2 Ion Concentrations in Cells and Extracellular Fluids (mM)

	K^+		Na^+		Ca^{2+}		Cl^-	
Cell type	*In*	*Out*	*In*	*Out*	*In*	*Out*	*In*	*Out*
Squid axon	400	20	50	440	≤0.4	10	40–150	560
Frog muscle	124	2.3	10.4	109	<0.1	2.1	1.5	78

potential of that ion across the membrane. The force (ΔG) that causes a cation (say, Na^+) to pass spontaneously inward through an ion channel is a function of the ratio of its concentrations on the two sides of the membrane (C_{in}/C_{out}) and of the difference in electrical potential ($\Delta\psi$ or V_m):

$$\Delta G = RT \ln \left(\frac{C_{in}}{C_{out}} \right) + Z \mathcal{F} V_m \qquad (12\text{–}1)$$

where R is the gas constant, T the absolute temperature, Z the charge on the ion, and $\mathcal{F}$ the Faraday constant. In a typical neuron or myocyte, the concentrations of Na^+, K^+, Ca^{2+}, and Cl^- in the cytosol are very different from those in the extracellular fluid (Table 12–2). Given these concentration differences, the resting V_m of −60 mV, and the relationship shown in Equation 12–1, opening of a Na^+ or Ca^{2+} channel will result in a spontaneous inward flow of Na^+ or Ca^{2+} (and depolarization), whereas opening of a K^+ channel will result in a spontaneous outward flux of K^+ (and hyperpolarization) (Fig. 12–3b).

A given ionic species continues to flow through a channel only as long as the combination of concentration gradient and electrical potential provides a driving force, according to Equation 12–1. For example, as Na^+ flows down its concentration gradient it depolarizes the membrane. When the membrane potential reaches +70 mV, the effect of this membrane potential (to resist further entry of Na^+) exactly equals the effect of the Na^+ concentration gradient (to cause more Na^+ to flow inward). At this equilibrium potential (E), the driving force (ΔG) tending to move an ion is zero. The equilibrium potential is different for each ionic species because the concentration gradients differ for each ion.

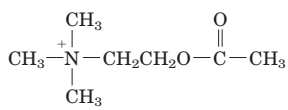
The number of ions that must flow to change the membrane potential significantly is negligible relative to the concentrations of Na^+, K^+, and Cl^- in cells and extracellular fluid, so the ion fluxes that occur during signaling in excitable cells have essentially no effect on the concentrations of those ions. However, because the intracellular concentration of Ca^{2+} is generally very low (~10^{-7} M), inward flow of Ca^{2+} can significantly alter the cytosolic [Ca^{2+}].

The membrane potential of a cell at a given time is the result of the types and numbers of ion channels open at that instant. In most cells at rest, more K^+ channels than Na^+, Cl^-, or Ca^{2+} channels are open and thus the resting potential is closer to the E for K^+ (−98 mV) than that for any other ion. When channels for Na^+, Ca^{2+}, or Cl^- open, the membrane potential moves toward the E for that ion. The precisely timed opening and closing of ion channels and the resulting transient changes in membrane potential underlie the electrical signaling by which the nervous system stimulates the skeletal muscles to contract, the heart to beat, or secretory cells to release their contents. Moreover, many hormones exert their effects by altering the membrane potentials of their target cells. These mechanisms are not limited to complex animals; ion channels play important roles in the responses of bacteria, protists, and plants to environmental signals.

To illustrate the action of ion channels in cell-to-cell signaling, we describe the mechanisms by which a neuron passes a signal along its length and across a synapse to the next neuron (or to a myocyte) in a cellular circuit, using acetylcholine as the neurotransmitter.

The Nicotinic Acetylcholine Receptor Is a Ligand-Gated Ion Channel

One of the best-understood examples of a **ligand-gated receptor channel** is the **nicotinic acetylcholine receptor** (see Fig. 11–51). The receptor channel opens in response to the neurotransmitter acetylcholine (and to nicotine, hence the name). This receptor is found in the postsynaptic membrane of neurons at certain synapses and in muscle fibers (myocytes) at neuromuscular junctions.



Acetylcholine (Ach)

Acetylcholine released by an excited neuron diffuses a few micrometers across the synaptic cleft or neuromuscular junction to the postsynaptic neuron or myocyte, where it interacts with the acetylcholine receptor and triggers electrical excitation (depolarization) of the receiving cell. The acetylcholine receptor is an allosteric protein with two high-affinity binding sites for acetylcholine, about 3.0 nm from the ion gate, on the two α

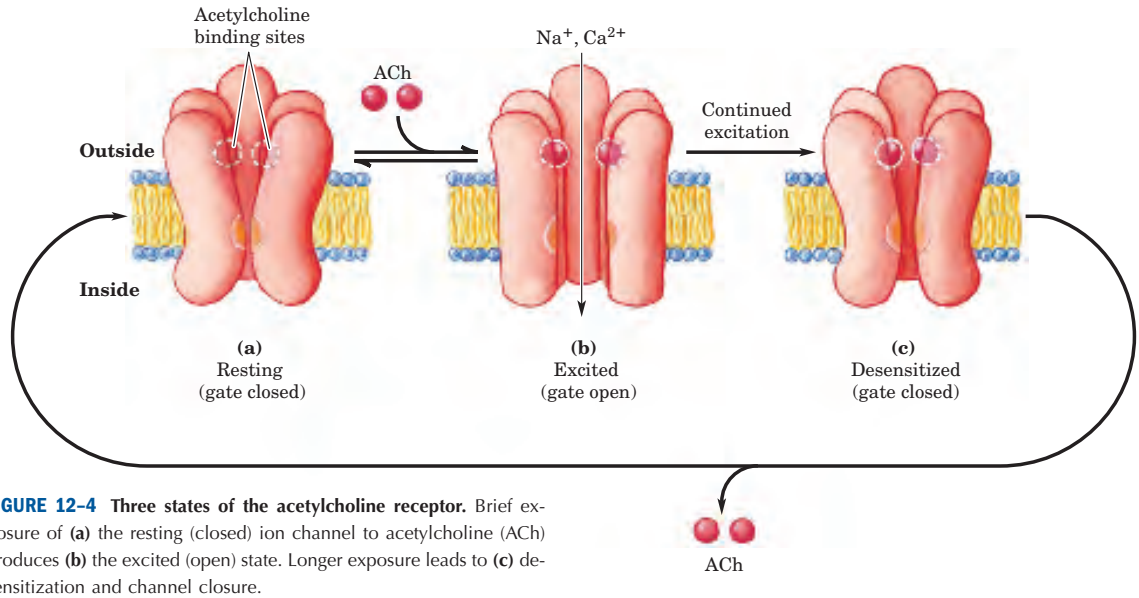


FIGURE 12–4 Three states of the acetylcholine receptor. Brief exposure of **(a)** the resting (closed) ion channel to acetylcholine (ACh) produces **(b)** the excited (open) state. Longer exposure leads to **(c)** desensitization and channel closure.

subunits. The binding of acetylcholine causes a change from the closed to the open conformation. The process is positively cooperative: binding of acetylcholine to the first site increases the acetylcholine-binding affinity of the second site. When the presynaptic cell releases a brief pulse of acetylcholine, both sites on the postsynaptic cell receptor are occupied briefly and the channel opens (Fig. 12–4). Either Na^+ or Ca^{2+} can now pass, and the inward flux of these ions depolarizes the plasma membrane, initiating subsequent events that vary with the type of tissue. In a postsynaptic neuron, depolarization initiates an action potential (see below); at a neuromuscular junction, depolarization of the muscle fiber triggers muscle contraction.

Normally, the acetylcholine concentration in the synaptic cleft is quickly lowered by the enzyme acetylcholinesterase, present in the cleft. When acetylcholine levels remain high for more than a few milliseconds, the receptor is desensitized (Fig. 12–1c). The receptor channel is converted to a third conformation (Fig. 12–4c) in which the channel is closed and the acetylcholine is very tightly bound. The slow release (in tens of milliseconds) of acetylcholine from its binding sites eventually allows the receptor to return to its resting state—closed and resensitized to acetylcholine levels.

Voltage-Gated Ion Channels Produce Neuronal Action Potentials

Signaling in the nervous system is accomplished by networks of neurons, specialized cells that carry an electrical impulse (action potential) from one end of the cell (the cell body) through an elongated cytoplasmic extension (the axon). The electrical signal triggers release of neurotransmitter molecules at the synapse, carrying the signal to the next cell in the circuit. Three types of **voltage-gated ion channels** are essential to this signaling mechanism. Along the entire length of the axon are **voltage-gated Na^+ channels** (Fig. 12–5; see also Fig. 11–50), which are closed when the membrane is at rest ($V_m = -60$ mV) but open briefly when the membrane is depolarized locally in response to acetylcholine (or some other neurotransmitter). The depolarization induced by the opening of Na^+ channels causes **voltage-gated K^+ channels** to open, and the resulting efflux of K^+ repolarizes the membrane locally. A brief pulse of depolarization traverses the axon as local depolarization triggers the brief opening of neighboring Na^+ channels, then K^+ channels. After each opening of a Na^+ channel, a short refractory period follows during which that channel cannot open again, and thus a unidirectional wave of depolarization sweeps from the nerve cell body toward the end of the axon. The voltage sensitivity of ion channels is due to the presence at critical positions in the channel protein of charged amino acid side chains that interact with the electric field across the membrane. Changes in transmembrane potential produce subtle conformational changes in the channel protein (see Fig. 11–50).

At the distal tip of the axon are **voltage-gated Ca^{2+} channels.** When the wave of depolarization reaches these channels, they open, and Ca^{2+} enters from the extracellular space. The rise in cytoplasmic $[Ca^{2+}]$ then triggers release of acetylcholine by exocytosis into the synaptic cleft (step ③ in Fig. 12–5). Acetylcholine diffuses to the postsynaptic cell (another

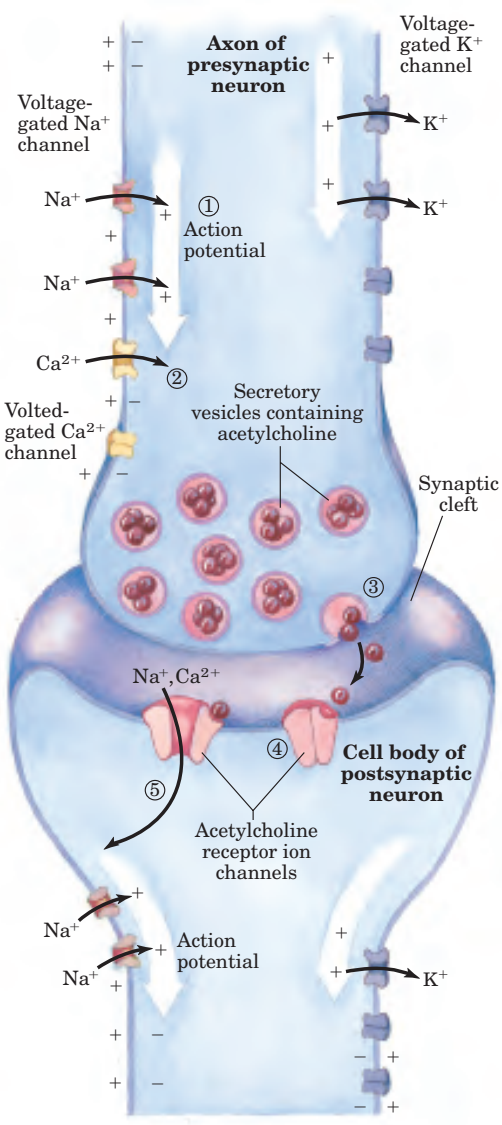


FIGURE 12–5 Role of voltage-gated and ligand-gated ion channels in neural transmission. Initially, the plasma membrane of the presynaptic neuron is polarized (inside negative) through the action of the electrogenic Na^+K^+ ATPase, which pumps 3 Na^+ out for every 2 K^+ pumped into the neuron (see Fig. 12–3). ① A stimulus to this neuron causes an action potential to move along the axon (white arrow), away from the cell body. The opening of one voltage-gated Na^+ channel allows Na^+ entry, and the resulting local depolarization causes the adjacent Na^+ channel to open, and so on. The directionality of movement of the action potential is ensured by the brief refractory period that follows the opening of each voltage-gated Na^+ channel. ② When the wave of depolarization reaches the axon tip, voltage-gated Ca^{2+} channels open, allowing Ca^{2+} entry into the presynaptic neuron. ③ The resulting increase in internal $[Ca^{2+}]$ triggers exocytic release of the neurotransmitter acetylcholine into the synaptic cleft. ④ Acetylcholine binds to a receptor on the postsynaptic neuron, causing its ligand-gated ion channel to open. ⑤ Extracellular Na^+ and Ca^{2+} enter through this channel, depolarizing the postsynaptic cell. The electrical signal has thus passed to the cell body of the postsynaptic neuron and will move along its axon to a third neuron by this same sequence of events.

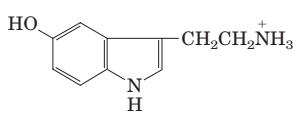
neuron or a myocyte), where it binds to acetylcholine receptors and triggers depolarization. Thus the message is passed to the next cell in the circuit.

We see, then, that gated ion channels convey signals in either of two ways: by changing the cytosolic concentration of an ion (such as Ca^{2+}), which then serves as an intracellular **second messenger** (the hormone or neurotransmitter is the first messenger), or by changing V_m and affecting other membrane proteins that are sensitive to V_m. The passage of an electrical signal through one neuron and on to the next illustrates both types of mechanism.

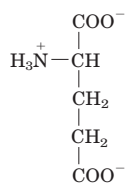
Neurons Have Receptor Channels That Respond to Different Neurotransmitters

Animal cells, especially those of the nervous system, contain a variety of ion channels gated by ligands, voltage, or both. The neurotransmitters 5-hydroxytryptamine (serotonin), glutamate, and glycine can all act through receptor channels that are structurally related to the acetylcholine receptor. Serotonin and glutamate trigger the opening of cation (K^+, Na^+, Ca^{2+}) channels, whereas glycine opens Cl^--specific channels. Cation and anion channels are distinguished by subtle differences in the amino acid residues that line the hydrophilic channel. Cation channels have negatively charged Glu and Asp side chains at crucial positions. When a few of these acidic residues are experimentally replaced with basic residues, the cation channel is converted to an anion channel.

Depending on which ion passes through a channel, the ligand (neurotransmitter) for that channel either depolarizes or hyperpolarizes the target cell. A single neuron normally receives input from several (or many) other neurons, each releasing its own characteristic neurotransmitter with its characteristic depolarizing or hyperpolarizing effect. The target cell's V_m therefore reflects the *integrated* input (Fig. 12–1d) from multi-



Serotonin
(5-hydroxytryptamine)



Glutamate