Cell Signaling 2.8

Learning Objectives

- Distinguish among autocrine, paracrine, endocrine, neural, and neuroendocrine signaling
- Explain why Ca^{2+} is a special ion with respect to signaling
- Describe in general terms what ligand-gated ion channels do
- List the major steps in turning on and off of heterotrimeric G-protein-coupled receptors
- Distinguish among G_s, G_i, G_q, and $G_{12/13}$ mechanisms
- Describe the steps in a G_s mechanism's activation of PKA and how they differ among tissues
- Describe the steps in a G_q mechanism's activation of CAM kinase
- List the four types of catalytic receptors
- Describe the steps in a JAK—STAT pathway
- Describe in general terms lipophilic signaling molecules' effects on gene transcription

SIGNALING *TRANSDUCES* ONE EVENT INTO ANOTHER

In its broadest context, cell signaling involves the **transduction** of some event into another event. In sensory transduction, a sensory cell is exposed to some external signal that is *transduced* to produce a nervous signal, the action potential. As we will see later in Chapter 3.2, this action potential can move along cell membranes to rapidly convey the signal, the action potential, to remote parts of the sensory neuron. The action potential is then *transduced* to release neurotransmitter at the synapse—the gap between one neuron and another. The neurotransmitter is then *transduced* to form the response of the postsynaptic cell, the one on the other side of the synapse. In the case of cutaneous (skin) senses, the original sensory signal is mechanical—a push or a pull on the nerves in the skin. The mechanical signal is transduced to an electrical signal, and the electrical signal is then transduced to a chemical signal. This simple series of events illustrates the use of **mechanical**, **electrical**, and **chemical** signals in the body (see Figure 2.8.1).

CELL-TO-CELL COMMUNICATION CAN ALSO USE DIRECT MECHANICAL, ELECTRICAL, OR CHEMICAL SIGNALS

Mechanical signals can originate in the external environment, as in the case of sensory transduction, or they can be the signals from another cell. Mechanical signaling requires close contact of cells and generally occurs through cell junctions as discussed in Chapter 2.2. Mechanical force originates on filaments within cells that eventually connect to the extracellular matrix through cytoskeletal elements. Transmission of these forces occurs through the extracellular matrix, but it is also sensed by neighboring cells. All of the pressure sensors in the body are really stretch receptors, in which mechanical stretch is transduced into electrical signals or chemical signals.

Electrical signals are usually used within the cell, as part of a signaling pathway to communicate intracellularly, and most often to move the signal rapidly from one place in the cell to another. Less frequently, direct electrical coupling occurs between cells. Such electrical coupling uses gap junctions, whose structure was discussed in Chapter 2.1. This mechanism is vitally important in coordinating some smooth muscle contraction and cardiac contraction.

Chemical signals do not require close contact and can be classified according to the distances involved, the mechanism of transmission, and the target of the chemical signals. These various classes of inter- and intracellular communication are shown in Figure 2.8.2. In some cases the signaling molecule remains bound to the cell and so transmission of this signal requires contact between the signaling cell and its target. This **contact-dependent signaling** is important in development and in immune responses. In other cases, the signaling cell releases a chemical that either acts locally (a **paracrine** or **autocrine** signal) or travels through the blood to act on remote targets (an **endocrine** signal). Autocrine signals have receptors on the signaling cell itself or others like it. Paracrine signals affect other types of cells located in the neighborhood of the signaling cell. Neurons also release signaling molecules, usually at the end of a long extension of the cell, the axon. When the neural chemical signal enters the blood and acts on distant targets, it is called a **neuroendocrine** signal. When it acts in the vicinity of its release site, it is a **neurotransmitter**.
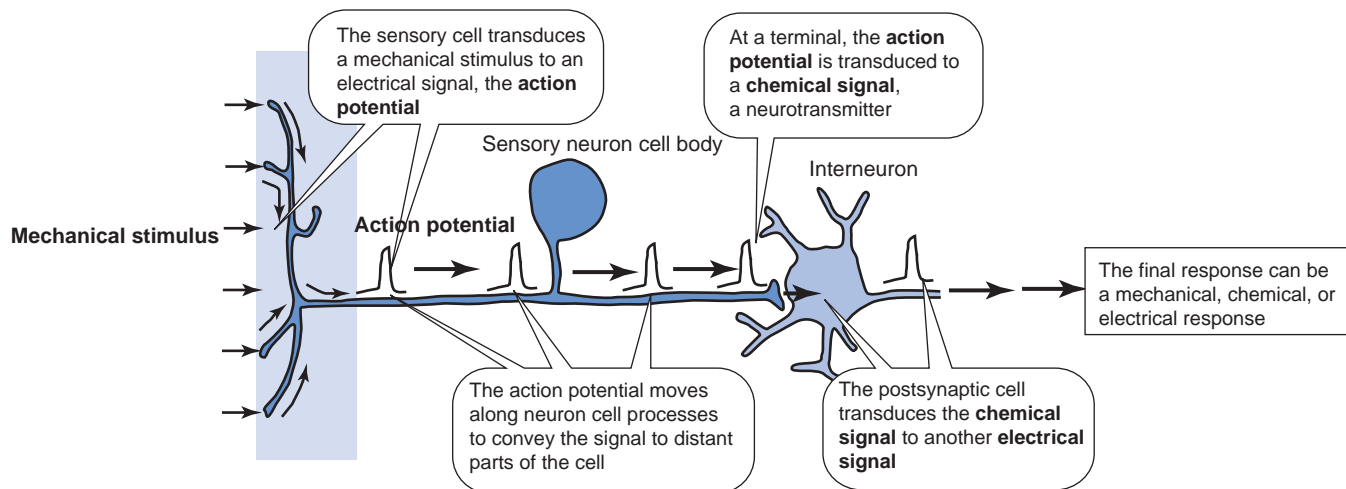
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FIGURE 2.8.1 Transduction of signals. Some kinds of sensory cells can transduce mechanical stimuli to electrical signals which can be conveyed along their surface for rapid spatial relay of the signal. At the end of the cell, the electrical signal is transduced to a chemical signal to convey the signal across the gap between the cells. The postsynaptic cell transduces this chemical signal back to an electrical signal.

SIGNALS ELICIT A VARIETY OF CLASSES OF CELLULAR RESPONSES

Intra- and intercellular signals begin a cascade of events that eventually changes cell behavior. The response of cells to signaling events includes altered:

- ion transport;
- metabolism;
- gene expression or differentiation;
- shape, movement, or force production;
- cell growth or cell division;
- apoptosis or programmed cell death.

ELECTRICAL SIGNALS AND NEUROTRANSMITTERS ARE FASTEST; ENDOCRINE SIGNALS ARE SLOWEST

The speed of response to an initial stimulus depends on the mode of delivery of the signal and the mechanism of the response in the target cells. Electrical signals are the fastest way to transmit a signal from one place in the body to another, in milliseconds, but the overall response depends on what happens in the target cell. If the response involves changes in activity of proteins already present in the target cell, the response can be rapid. If the response involves altered gene expression that requires synthesis of new protein, response can take hours. If it involves altered cell growth, it can take days to complete. Neurotransmitter signaling is the fastest response, followed by changes in cell shape or the development of force. Endocrine signals are slowest but last longer.

VOLTAGE-GATED ION CHANNELS CONVEY ELECTRICAL SIGNALS

Ion channels allow ions to cross biological membranes that they otherwise cannot penetrate. Because they are electrically charged, movement of ions makes a current, and the current either charges or discharges the membrane—the current alters the membrane potential. Thus the voltage-gated ion channels are largely responsible for alterations in the membrane potential that is rapidly conveyed through the cell.

VOLTAGE-GATED Ca²⁺ CHANNELS TRANSDUCE AN ELECTRICAL SIGNAL TO AN INTRACELLULAR Ca²⁺ SIGNAL

Cells maintain a very low intracellular $[Ca^{2+}]$ (<100 nM) to avoid Ca^{2+} precipitation with phosphate and organic phosphates (ATP, etc.) present in high concentrations in the cytoplasm. The low cytoplasmic $[Ca^{2+}]$ allows increases in cytoplasmic $[Ca^{2+}]$ to be used as a signal. Multiple types of voltage-gated Ca^{2+} channels (**v**oltage-**d**ependent **c**alcium **c**hannel, VDCCs) reside on the surface membrane of many cells. Depolarization of the cell membrane opens these channels, causing Ca^{2+} to move from the ECF, with 1.2 mM $[Ca^{2+}]$, to the intracellular compartment. The cytoplasm contains a number of proteins that bind Ca^{2+} with high affinity and that change shape or activity upon Ca^{2+} binding. The effects of increasing cytoplasmic $[Ca^{2+}]$ include the following:

1. **Stimulus−secretion coupling**: The increased $[Ca^{2+}]$ binds to Ca^{2+} sensors on vesicles, causing the fusion of the secretory vesicles with the plasma membrane and release of secreted products into the ECF.
2. **Excitation−contraction coupling**: The increased $[Ca^{2+}]$ binds to Ca-sensitive elements on contractile filaments or cytoskeletal elements, causing either force development or shortening by muscle cells.
3. **Calmodulin-dependent activation of enzymes**: Calmodulin is a small cytosolic protein that binds four Ca^{2+} molecules and then activates many enzymes such as myosin light chain kinase in smooth muscle.
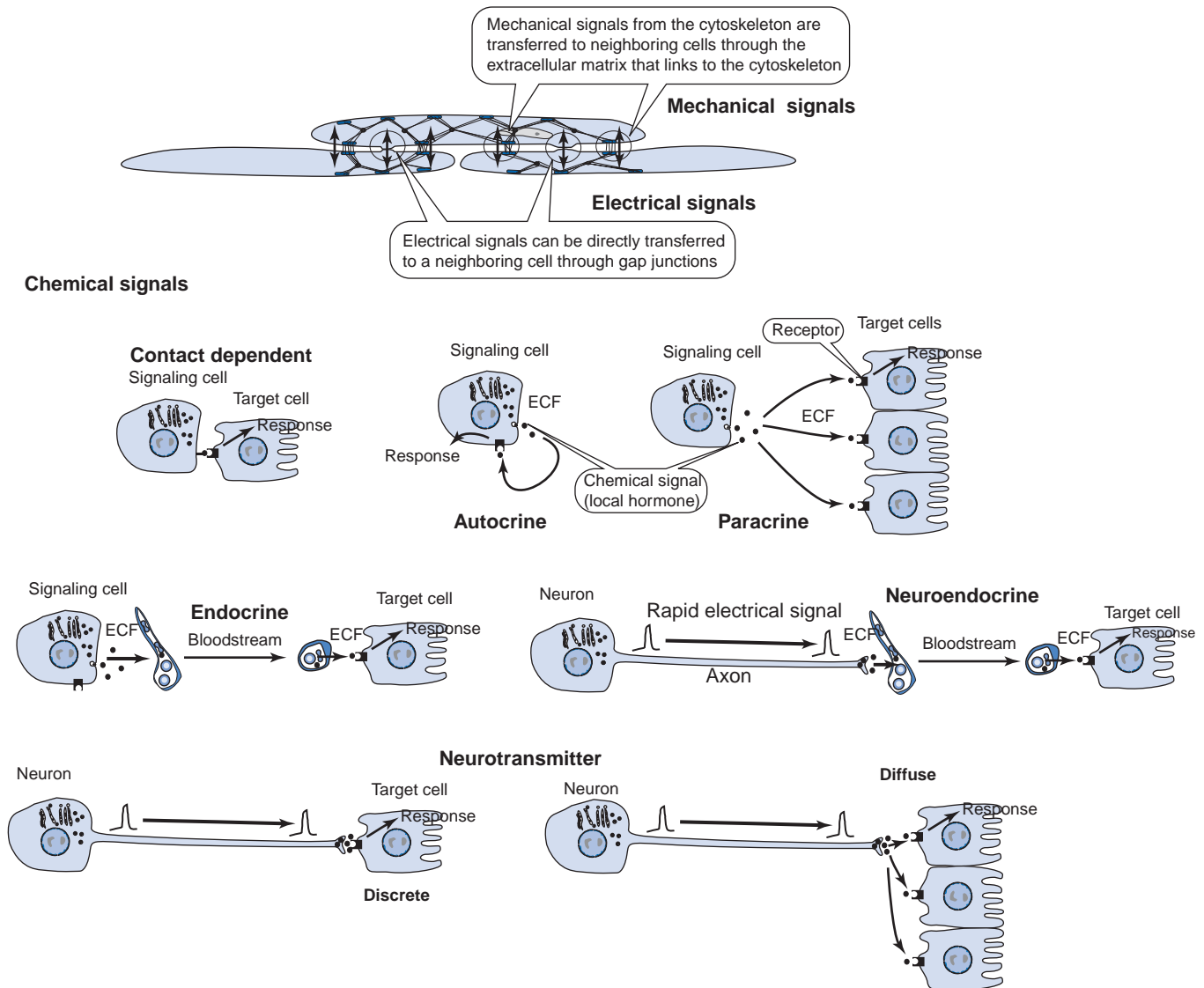
FIGURE 2.8.2 Main classes of signaling. Mechanical signals can pass from cell to cell through filaments in the extracellular matrix attached to membrane-bound proteins in the surfaces of cells, particularly at cell junctions such as desmosomes. Electrical signals can also pass directly from one cell to another through gap junctions. Some signaling molecules remain bound to the surface and so the signal affects the target cell only by direct contact of the signaling cell with the target cell. Cells can release chemical signals that act locally. When they affect the signaling cell, or others like it, they are autocrine signals. When they affect other nearby cells, they are paracrine signals. Signals that are released into the bloodstream to affect distant target cells are endocrine signals. If they are released from long processes by neurons, they are neuroendocrine signals. Nerve cells release a variety of chemical signals at terminals near target cells. These are neurotransmitters. If they are released very close to clustered receptors on target cells, they are discrete neurotransmitters. If they are released into a general area to affect multiple cells, they are diffuse neurotransmitters. Electrical signals are most often used intracellularly to rapidly convey the action potential from one part of the cell to another. This is the fastest movement of a signal in the body.

4. **Direct activation of enzymes**: Ca^{2+} can directly bind to some enzymes, such as PKC, and activate them. Figure 2.8.3 illustrates these aspects of Ca^{2+} signaling in cells.

LIGAND-GATED ION CHANNELS OPEN UPON BINDING WITH CHEMICAL SIGNALS

Fast release of chemical signals by electrical signals in nerve terminals, followed by an electrical signal in the target cell, is the fastest mechanism of signaling used in the body. This is the classic neurotransmitter mechanism: the electrical signal (the action potential) on the presynaptic cell propagates down the axon to the nerve terminal where it causes a local intracellular Ca^{2+} signal that releases chemical neurotransmitter into the ECF immediately adjacent to the postsynaptic cell. The chemical signal then binds to a receptor which is also an ion channel, causing a flux of ions across the postsynaptic cell membrane and a resulting electrical signal in the postsynaptic cell. Three major classes of surface membrane, ligand-gated ion channels (LGICs) have been identified, as shown in Figure 2.8.4, and many of these have multiple subtypes. The major types are distinguished by their structures. Receptors for acetylcholine, serotonin (=5 hydroxy tryptamine), gamma amino butyric acid (GABA), and glycine all consist of five subunits. Each family, such as **nicotinic ACh receptor**, has
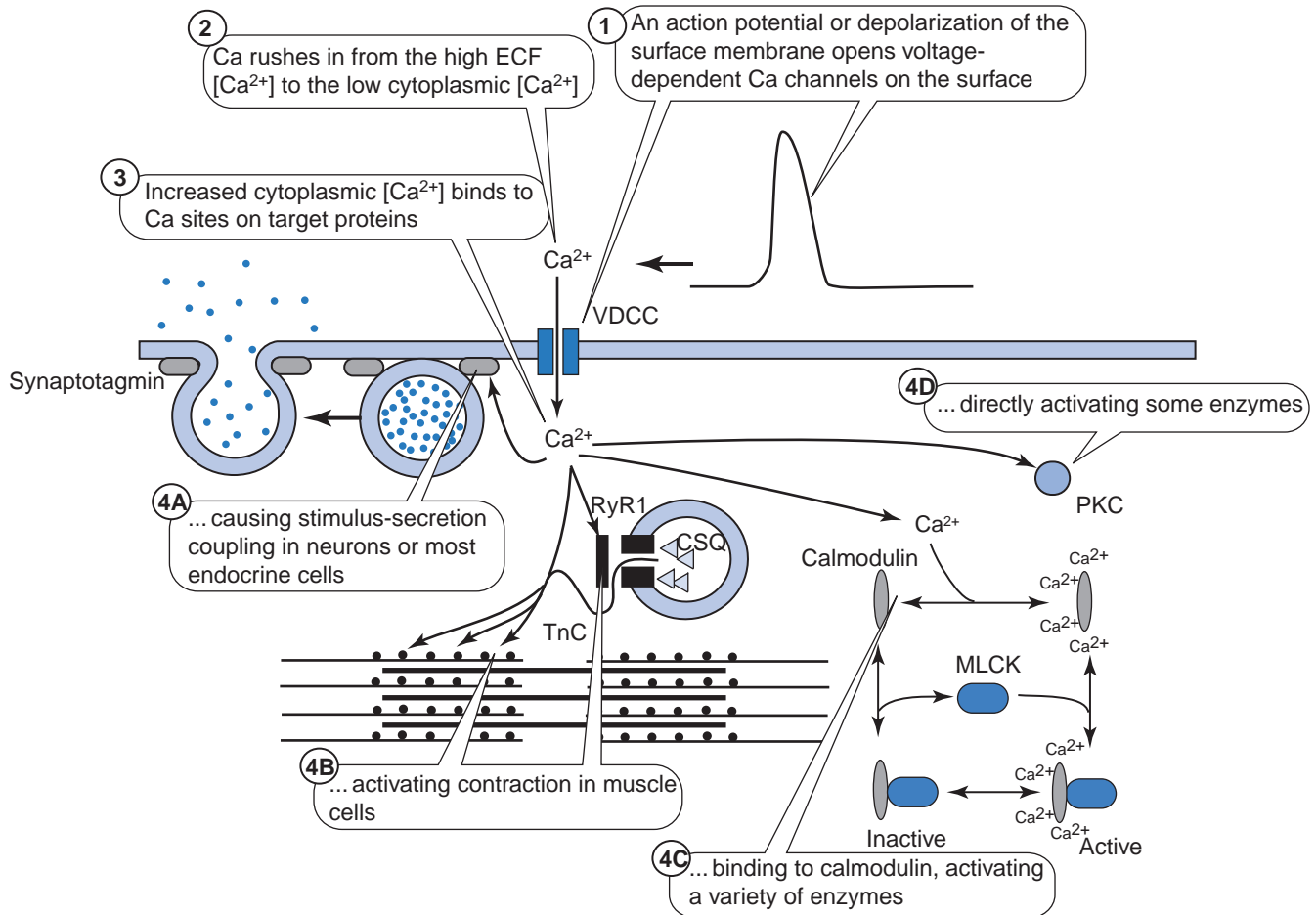
FIGURE 2.8.3 Electrically coupled calcium signaling. Depolarization of the cell membrane opens a calcium channel that lets Ca^{2+} into the cell. At rest, the cytoplasmic $[Ca^{2+}]$ is very low. Upon stimulation, influx of Ca^{2+} raises the $[Ca^{2+}]$ enough for Ca^{2+} to bind to Ca^{2+}-binding sites on specific proteins. Ca^{2+} binding to synaptotagmin causes fusion of secretory vesicles with the plasma membrane. Binding to troponin C (TnC) activates force in skeletal or cardiac muscle. Ca^{2+} binding to calmodulin activates a number of enzymes such as MLCK (myosin light chain kinase) involved in smooth muscle contraction. In other cases, Ca^{2+} directly activates some enzymes; PKC is shown. RyR1 is the ryanodine receptor on the endoplasmic reticulum membrane; CSQ is calsequestrin, a calcium-binding protein in the lumen of some ER membranes.
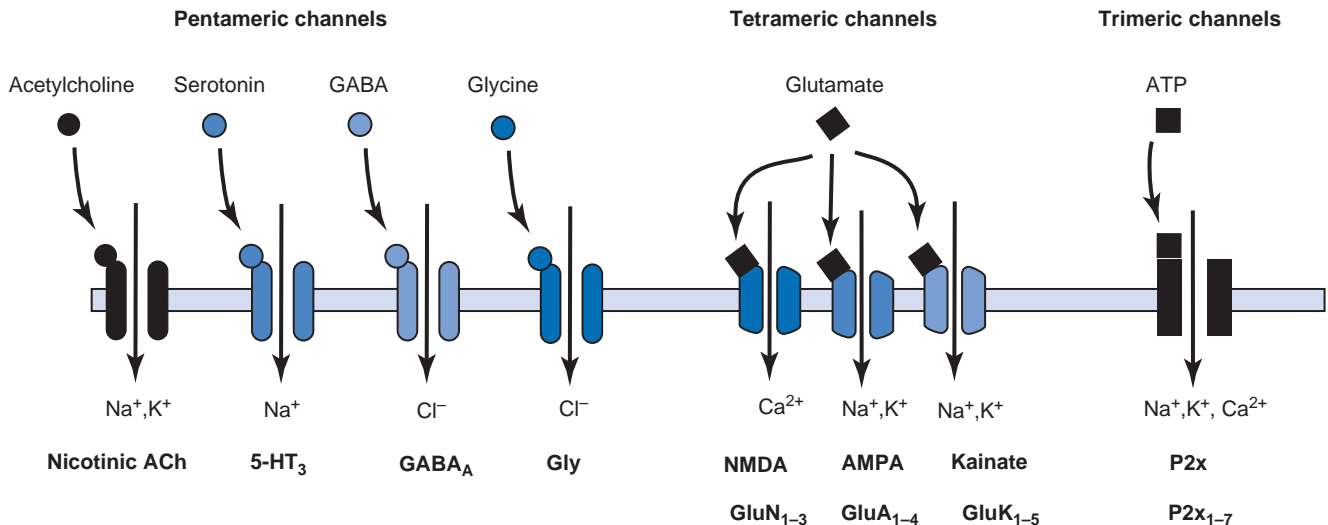

FIGURE 2.8.4 Ligand-gated ion channels. These channels reside in the plasma membrane and respond to specific ligands by allowing specific ions to cross the membrane. The channels are classified according to their structure and agonist or chemical signal that opens the channel. The names of the channels are at the bottom of the figure, and alternate naming conventions have been proposed. Each family of channels has multiple isoforms that depend on the subunit make-up of the channels.
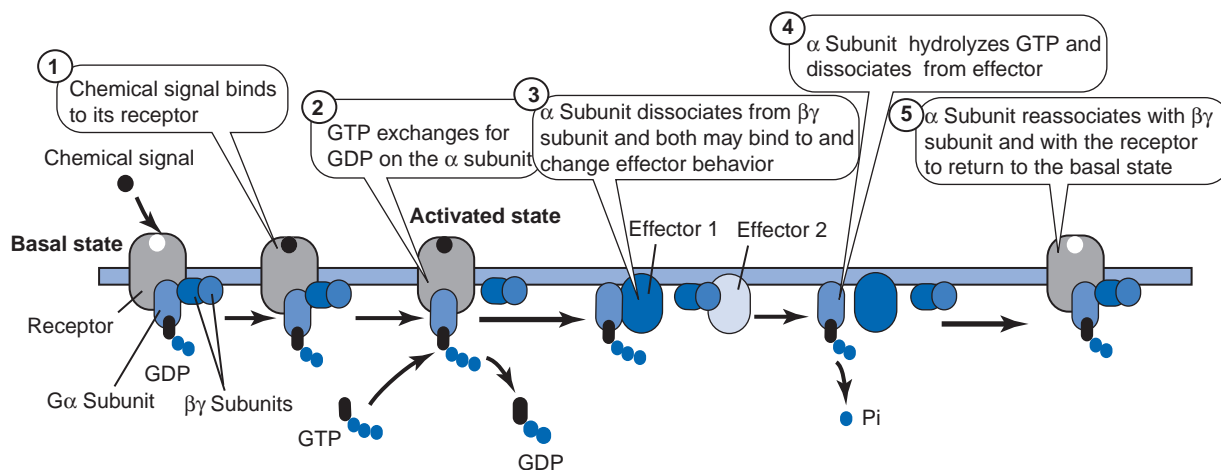
FIGURE 2.8.5 General scheme for heterotrimeric GPCRs. The receptors are membrane-bound proteins that bind chemical signals. The heterotrimeric G-protein consists of an α subunit that binds and hydrolyzes GTP and a βγ subunit that does not dissociate. Binding of the ligand to its receptor triggers the exchange of GTP for GDP and subsequent dissociation of the α subunit and βγ subunit and both are then able to alter the behavior of effector targets in the cell. The α subunit spontaneously hydrolyzes its GTP, and the α subunit reassociates with the βγ subunit to return to the basal, unstimulated state.

multiple subtypes consisting of different subunits, but each member of the subtype responds to one chemical signal, acetylcholine in this case. Receptors for glutamate each have four subunits, and this family of LGIC has further subtypes distinguished by artificial agonists (stimulators of the receptor), NMDA (N-methyl D-aspartic acid), AMPA (α-amino-3-hydroxy-5-methyl-4 isoxazole propionic acid), and kainate.

HETEROTRIMERIC G-PROTEIN-COUPLED RECEPTORS (GPCRS) ARE VERSATILE

G-protein-coupled signaling pathways are versatile because of their modular structure: they consist of **receptors**, **heterotrimeric G-proteins**, and **effectors**. Receptors are membrane-bound proteins that bind signaling molecules on the external surface of cells. Binding alters the conformation of the receptor, and this change is transferred to a heterotrimeric G-protein, consisting of α, β, and γ subunits. In the unstimulated state, the Gα subunit binds GDP. Upon ligand binding to its receptor, the receptor causes the Gα·GDP to exchange GTP for the bound GDP, and the Gα·GTP dissociates from the βγ subunits. The dissociated subunits can then bind to effector molecules, exerting some change in their behavior. The Gα·GTP has inherent GTPase activity, so it reverts back to Gα·GDP and reassociates with the βγ subunit. The overall plan of the G-protein signaling pathway is shown in Figure 2.8.5.

THERE ARE FOUR CLASSES OF G-PROTEINS: Gα_S, Gα_I/Gα_O, Gα_Q, AND Gα_{12}/Gα_{13}

β ADRENERGIC STIMULATION IS AN EXAMPLE OF A Gα_S MECHANISM

A number of signaling molecules can bind to a receptor that is coupled to a Gα$_s$-protein. All Gα$_s$-protein's α subunit binds to a membrane-bound enzyme,

adenylyl cyclase, which converts ATP into 3′,5′ cyclic AMP, or **cAMP**. Increasing the concentration of cAMP activates **protein kinase A (PKA)**, which phosphorylates (adds a phosphate to) specific target proteins. Phosphorylation of these target proteins alters cell behavior, the final consequence of exposure to the chemical signal. The basic pathway for this signaling mechanism is shown in Figure 2.8.6. This signal is turned on by activation of adenylyl cyclase and then PK and then phosphorylation of proteins. It is turned off by the simultaneous inactivation of all three of these. The α subunit of the G-protein spontaneously hydrolyzes its GTP and reassociates with the βγ subunit. This inactivates adenylyl cyclase and stops its synthesis of cAMP. The cAMP is also broken down by **phosphodiesterase (PDE)** so that the increased [cAMP] is removed. The cAMP dissociates from the regulatory subunits of the PKA, causing it to inactivate so that target proteins are no longer phosphorylated. However, this does not reverse phosphorylation of proteins. The phosphorylated proteins are dephosphorylated by specific enzymes called protein phosphatases. There are four classes of serine/threonine **phosphoprotein phosphatases**: **PP1**, **PP2a**, **PP2b**, and **PP2c**, which dephosphorylate proteins phosphorylated at serine and threonine residues. Fine control of this system is provided by the regulation of both adenylyl cyclase, as described, and by the regulation of both PDE and the protein phosphatases.

α$_2$ ADRENERGIC STIMULATION IS AN EXAMPLE OF A Gα_I/Gα_O MECHANISM

Other molecules bind to their G-protein-coupled receptor (GPCR) and release a Gα subunit that inhibits adenylyl cyclase. These are referred to as G$_i$ mechanisms. An example is epinephrine binding to α$_2$ receptors on neurons and is illustrated in Figure 2.8.7. Other members of this class achieve the same end—reduction in cAMP levels—by activating PDE. Retinal photoreceptor
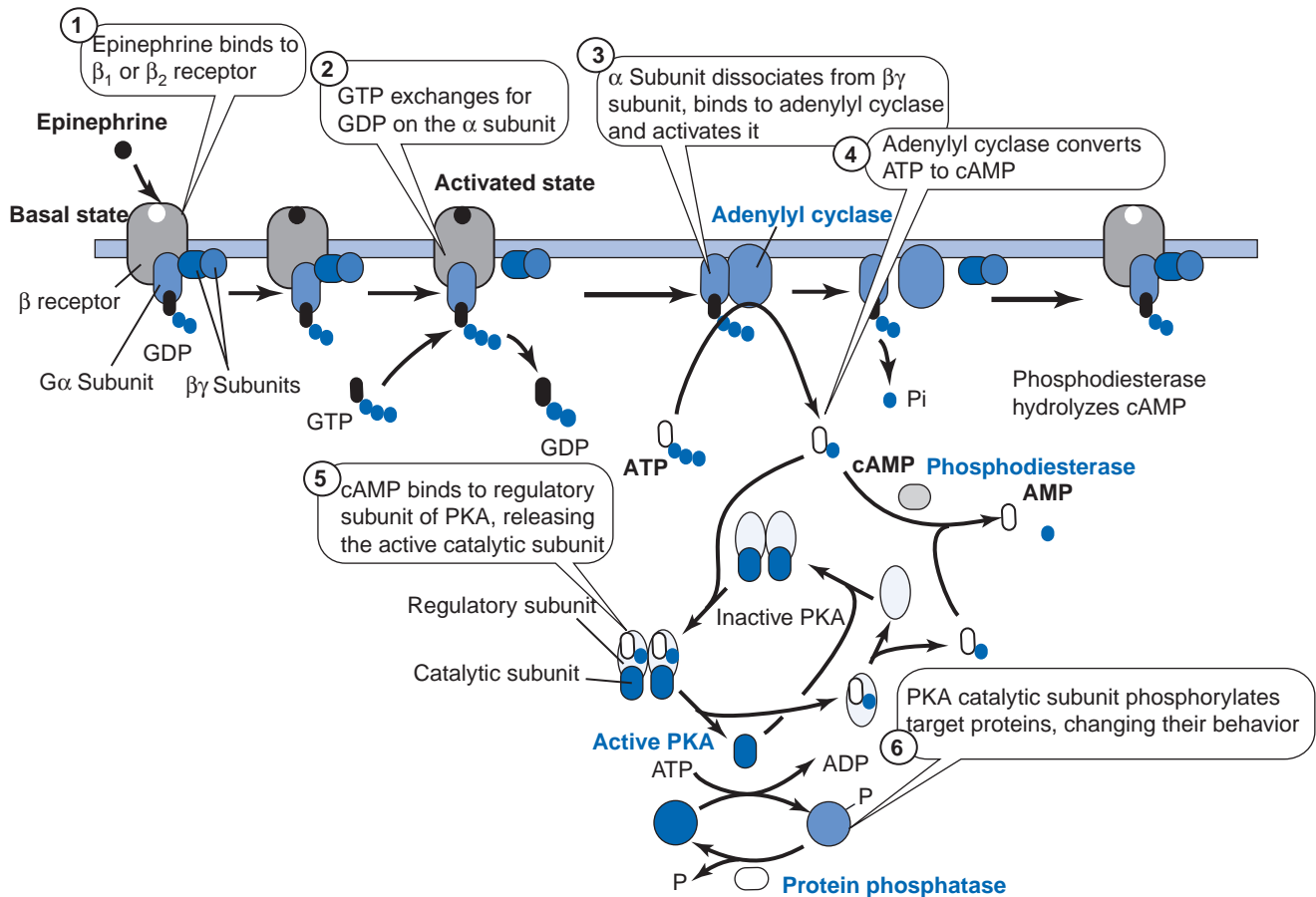
FIGURE 2.8.6 Mechanism of G_s-coupled receptors. Many different kinds of ligands bind to G_s-coupled receptors. Epinephrine is shown, which binds to β_1 and β_2 receptors that are coupled to G_s proteins. Activation follows the general scheme shown in Figure 2.8.5. Here the $G\alpha_s$ subunit binds to adenylyl cyclase, activating it and increasing the cytoplasmic concentration of cAMP. This activates PKA that phosphorylates a variety of target proteins. The signal is turned off by (1) hydrolysis of GTP by the $G\alpha$ subunit and dissociation and removal of activation of adenylyl cyclase; (2) hydrolysis of cAMP by PDE and removal of activation of PKA; (3) dephosphorylation of target proteins by protein phosphatases.
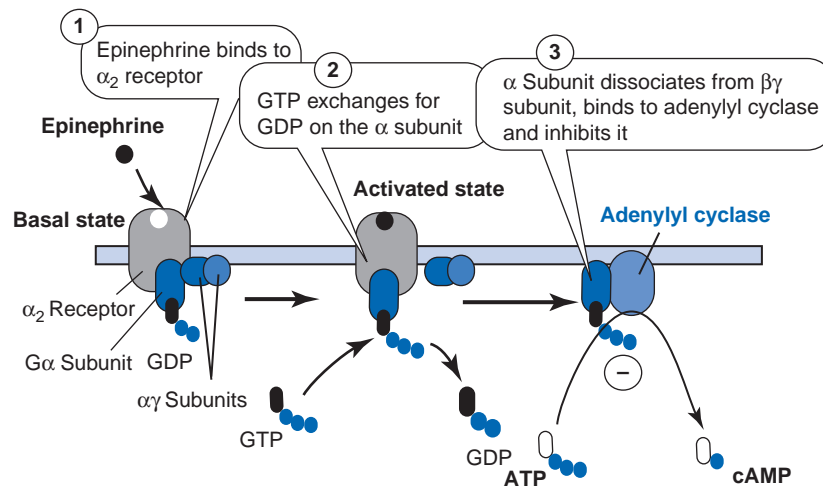

FIGURE 2.8.7 Mechanism of G_i-coupled receptors. Here epinephrine binds to α_2 receptors, which is followed by the inhibition of adenylyl cyclase and a reduction in cytoplasmic [cAMP].

cells, for example, activate cGMP PDE. This mechanism is illustrated in Figure 2.8.8. Still other ligands, such as acetylcholine, bind to M2 receptors that cause inhibition of adenylyl cyclase, and the $\beta\gamma$ subunit activates a K^+ channel (see Figure 2.8.9). Thus this class of GPCR exerts a variety of effects including direct inhibition of adenylyl cyclase, activation of PDE, and direct activation of K^+ channels.
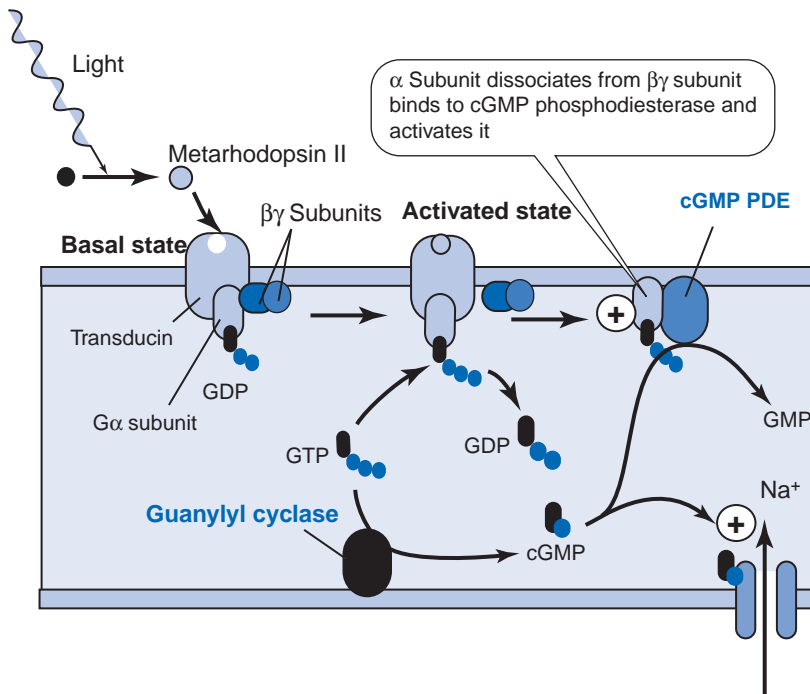
FIGURE 2.8.8 GPCR involved in retinal signal transduction. Through a series of steps, light converts rhodopsin to metarhodopsin II, which binds to a heterotrimeric G-protein called transducin. The $G\alpha$ subunit exchanges GTP for GDP, dissociates from the $\beta\gamma$ subunit, and activates cGMP PDE. Guanylyl cyclase in these cells makes cGMP continuously, and cGMP opens a Na^+ channel. Degrading the cGMP reduces [cGMP] and therefore regulates the open state of the channel, producing an electrical signal.
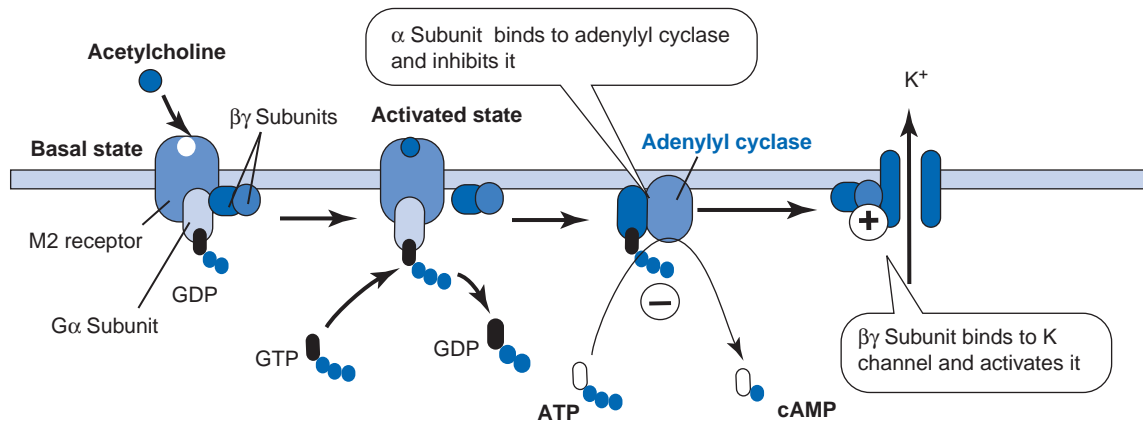

FIGURE 2.8.9 G_i mechanism involved in M2 GPCR. Acetylcholine binds to a variety of receptors. The M2 receptor, present in heart, couples binding of acetylcholine to a $G\alpha_i$ subunit that inhibits adenylyl cyclase. The $\beta\gamma$ subunit released by acetylcholine binding directly activates a K^+ channel.

$G\alpha_Q/G\alpha_{11}$ GPCR ACTIVATES PHOSPHOLIPASE C AND RELEASES CA FROM INTRACELLULAR STORES

A third class of GPCR activates **phospholipase C** on the surface membrane, which cleaves phosphatidyl inositol bisphosphate to produce **diacylglycerol (DAG)** and **inositol triphosphate (IP3)**. The IP3 releases Ca^{2+} from the endoplasmic reticulum, while the DAG activates **protein kinase C (PKC)** (see Figure 2.8.10).

$G\alpha_{12}/G\alpha_{13}$-COUPLED RECEPTORS ACTIVATE SMALL MONOMERIC GTPASES

The $G\alpha_{12}$ is the last of the four major families of heterotrimeric G-proteins (G_s, G_i, G_q, and G_{12}) that we will discuss. $G\alpha_{12}$ and $G\alpha_{13}$ are linked to **GTP exchange factors (GEFs)** that activate small monomeric G-proteins by exchanging their bound GDP with GTP. A second set of

modulatory proteins, called **GAPs**, for **GTPase Activating Proteins**, facilitate the inactivation of these small monomeric GTPases. The overall plan is shown in Figure 2.8.11.

The superfamily of small monomeric GTPases is divided into several major subfamilies, including:

Ras, **Rho**, **Rab**, **Ran**, and **Arf**

These are all small ($20-40$ kDa) proteins that are membrane bound due to covalent attachment of lipids (such as *N*-myristoylation on Arf proteins). The Rho GTPases regulate the cytoskeleton and play a role in the regulation of smooth muscle contraction. One of its effectors is Rho Kinase, a serine−threonine protein kinase that phosphorylates myosin light chain phosphatase and inactivates it (see Chapter 3.8). Rho is also involved in cell cycle progression and gene expression. The Rab family of monomeric GTPases regulates vesicular traffic as well as modulation of actin
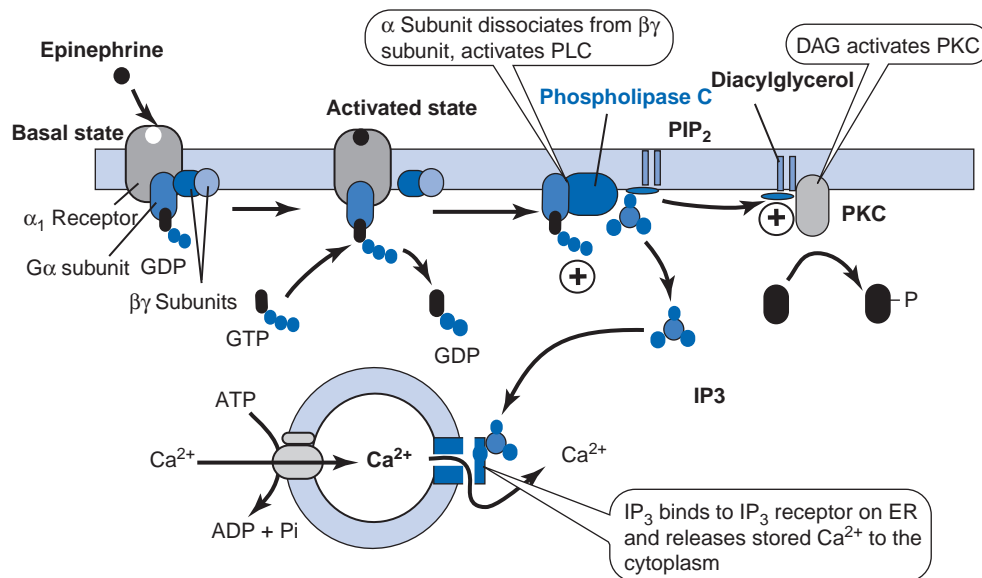
FIGURE 2.8.10 Mechanism of $G\alpha_q$ signaling. Binding of ligand to its GPCR results in release of the $G\alpha_q$ and $\beta\gamma$ subunits. The $G\alpha_q$ subunit activates phospholipase C, which cleaves phosphatidyl inositol bisphosphate in the surface membrane, liberating DAG and IP3. IP3 releases Ca^{2+} stored in the ER and DAG activates PKC that phosphorylates sets of target proteins.
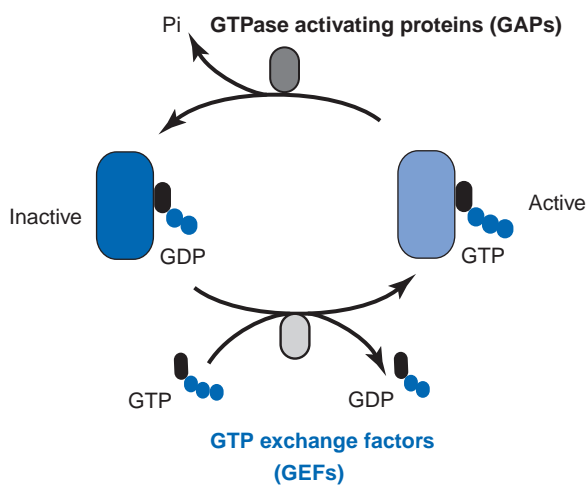

FIGURE 2.8.11 Heterotrimeric G-proteins with $G\alpha_{12}$-activated GEFs that promote the exchange of GTP for GDP on small monomeric G-proteins. This converts the small monomeric G-proteins into an active form. Reversion to the inactive form is catalyzed by GAPs, GTPase activating proteins. The GEF binds to the $G\alpha_{12}$ subunit.

dynamics. Members of the Ran family regulate transport of materials between the cytoplasm and nucleus. Arf stands for "ADP-ribosylation factor." All six mammalian Arfs are located in the Golgi and regulate vesicular transport there.

THE RESPONSE OF A CELL TO A CHEMICAL SIGNAL DEPENDS ON THE RECEPTOR AND ITS EFFECTORS

According to Figures 2.8.6, 2.8.7, and 2.8.10, epinephrine can bind to β GPCRs, with the final activation of adenylyl cyclase, increased [cAMP], and activation of PKA; or it can bind to α_2 receptors with the inhibition of adenylyl cyclase, decreased [cAMP], and no activation of PKA; or it can bind to α_1 receptors with subsequent activation of PLC, release of Ca^{2+} from intracellular stores, and activation of PKC. These different effects typically occur in separate cells. Thus the response of the cell depends on the receptor for the chemical signal and not on the chemical signal alone. Further, some cells respond differently than others because they express entirely different sets of target proteins. In the liver, for example, the primary target for PKA from the stimulation of β adrenergic receptors is phosphorylase kinase, which then phosphorylates phosphorylase, activating it, and glycogen synthetase inactivating it. In the heart, the primary targets of β adrenergic stimulation are the voltage-dependent Ca^{2+} channels in the surface membrane, phospholamban on the sarcoplasmic reticulum, ryanodine receptors (RyR2) on the sarcoplasmic reticulum, and troponin I (TnI). These are illustrated in Figure 2.8.12. Thus the effects of epinephrine using the same type of receptor are glycogenolysis in the liver and increased contractile strength in the heart. These differences indicate that the final effect is a function of (1) the chemical signal, (2) its receptor, (3) the effector, and (4) the targets within the cell.

CHEMICAL SIGNALS CAN BIND TO AND DIRECTLY ACTIVATE MEMBRANE-BOUND ENZYMES

A variety of extracellular chemical signals can bind to receptors on the surface membrane; these are amplifying enzymes or directly activate an amplifying enzyme. They are of four main types: **receptor guanylyl cyclase**, **receptor serine/threonine kinase**, **receptor tyrosine kinase**, and **receptor-associated tyrosine kinase**. These are illustrated in Figure 2.8.13.
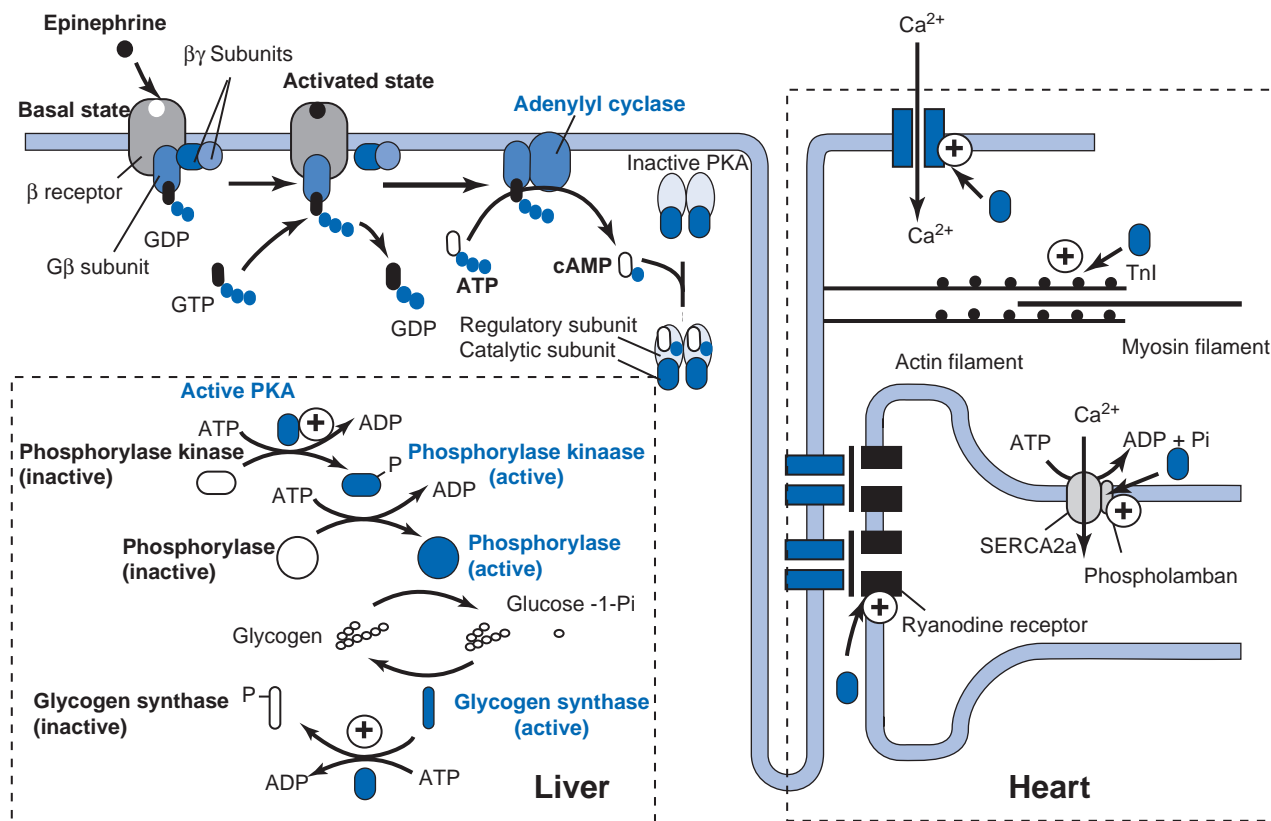
FIGURE 2.8.12 Different effects of beta adrenergic stimulation of liver cells versus heart cells. In the liver, the primary response of beta adrenergic stimulation is activation of glycogenolysis through activation of glycogen phosphorylase through a cascade of protein phosphorylation reactions. In the heart, the primary response of beta adrenergic stimulation is faster activation and relaxation of the muscle through control of cytoplasmic $[Ca^{2+}]$ by phosphorylation of voltage-dependent Ca^{2+} channels on the surface of the cell, phosphorylation of the ryanodine receptor on the surface of the sarcoplasmic reticulum, phosphorylation of TnI on the contractile filaments, and phosphorylation of phospholamban to relieve inhibition of the SERCA2a Ca^{2+} pump on the surface of the SR.

MANY SIGNALS ALTER GENE EXPRESSION

So far we have discussed signaling molecules that cannot penetrate the cell membrane. These bind to receptors on the surface of the cell, and the binding is transduced into a second messenger such as Ca^{2+}, cAMP, cGMP, IP3, and DAG, one of a number of small monomeric GTPases, or causes phosphorylation of intracellular proteins. A number of lipophilic signaling molecules penetrate the cell membrane and bind to receptors either in the cytoplasm or in the nucleus and alter the expression of specific genes in the cell. Hormones that alter gene expression this way include the sex hormones **testosterone**, **estrogen**, and **progesterone**; **corticosteroids**, including **glucocorticoids**, produced by the adrenal cortex; **mineralocorticoids**, produced by the adrenal gland that regulate electrolyte and water balance; **vitamin D**, which regulates calcium and phosphate balance, among other effects; and **thyroid hormone** and **retinoic acid**, which have effects in almost all cells.

NUCLEAR RECEPTORS ALTER GENE TRANSCRIPTION

The nuclear receptors constitute a **superfamily** of proteins that are structurally related and perform similar functions, but they exhibit specificity for binding ligands and specificity of action. These nuclear receptors include the following: estrogen receptors α and β (ERα and ERβ); androgen receptor (AR); progesterone receptor (PR); glucocorticoid receptor (GR); mineralocorticoid receptor (MR); vitamin D receptor (VDR); thyroid receptors α and β (TRα and TRβ); retinoic acid receptor types α, β, and γ (RARα, RARβ, and RARγ); and 9-*cis*-retinoic acid receptors (RXRα, RXRβ, and RXRγ). These nuclear receptors are restricted to the nucleus with the exception of the mineralocorticoid receptor (MR) and the GR which reside in the cytoplasm. Upon binding with its ligand, MR and GR move into the nucleus where they bind to the regulatory region of a modulated gene. Binding of the lipophilic ligand with its receptor changes the conformation of the receptor–ligand complex, allowing the receptor to bind to specific nucleotide sequences on the DNA, called **response elements**. Binding of these receptors begins a cascade of events that eventually activates the transcription of specific genes. The newly transcribed mRNA is transferred to the cytoplasm where it is translated into a protein. The set of proteins regulated by the hormones confers specific capabilities on the cells in which they are expressed. Thus cell function is regulated by controlling cell concentration of specific active proteins.
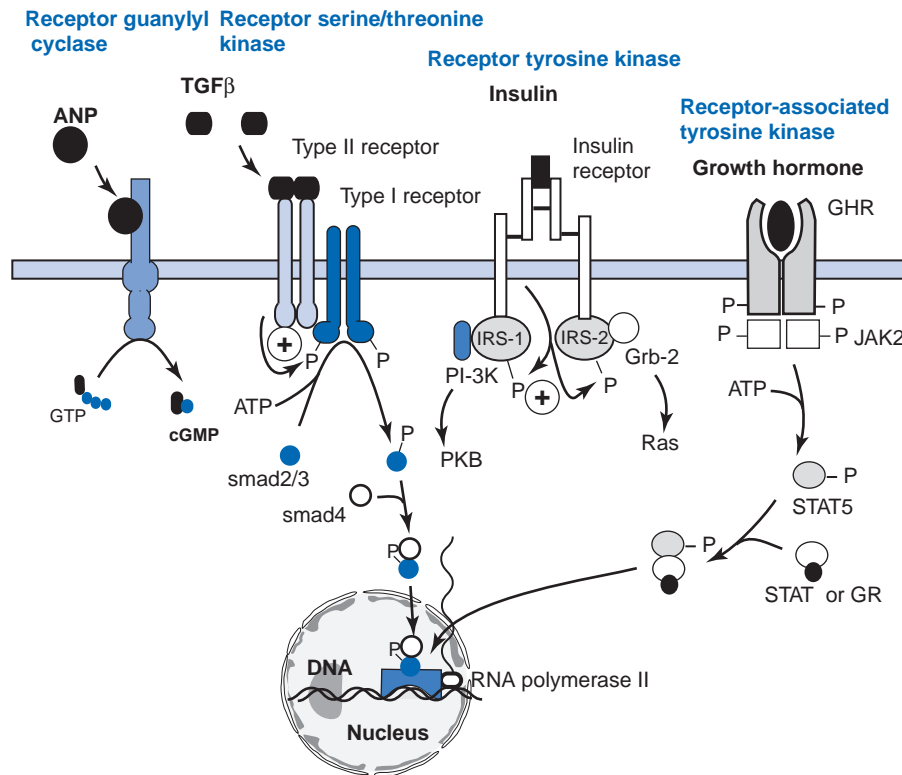
FIGURE 2.8.13 Catalytic receptors. Chemical signals binding to the extracellular surface of the cell are coupled to enzymes that alter intracellular components. **Receptor guanylate cyclases** respond to atrial natriuretic peptide or brain natriuretic peptide, and binding increases the concentration of cGMP in the cell, which increases activity of cGMP-dependent protein kinase. **Receptor serine/threonine kinases**: dimers of a variety of growth factors including transforming growth factor beta (TGF-β), myostatin, and bone morphogenic protein (BMP) bind to an activin type II receptor that recruits an activin type I receptor and phosphorylates it. This active serine/threonine kinase then phosphorylates one of a family of proteins called smads (smad2 or smad3 is shown), which binds to smad4. The complex enters the nucleus and regulates gene expression. **Receptor tyrosine kinases**: Insulin or insulin-like growth factor (e.g., IGF-1 and IGF-2) binds to the insulin receptor. The activated receptor phosphorylates several intracellular substrates. Insulin receptor substrate-1 (IRS-1) is shown. The phosphorylated proteins can activate two main pathways: the PI-3K (phosphatidyl inositol 3 kinase) pathway activates PKB and PKC downstream. The Ras pathway is activated by Grb-2 that binds to IRS and then activates a Ras GTP exchange protein leading eventually to transcription factors. **Receptor-associated tyrosine kinases** are used by growth hormone (shown), prolactin and erythropoietin, and most interleukins. Their binding induces close proximity of two receptors that bind members of the Janus family of tyrosine kinases (JAKs: JAK1-3 and TYK2). These transphosphorylate themselves and their receptor and phosphorylate proteins called STAT (for **s**ignal **t**ransduction and **a**ctivation of **t**ranscription). These form dimers with other transcription factors and regulate gene transcription.

NUCLEAR RECEPTORS RECRUIT HISTONE ACETYLASE TO UNWRAP THE DNA FROM THE HISTONES

Heterochromatin is highly condensed DNA that cannot be transcribed. **Euchromatin** is more easily accessible for the assembly of transcriptional subunits, and DNA in this configuration has a higher rate of transcription. The configuration of chromatin is regulated by the **acetylation** of **histones**. Histones are a family of proteins, described in Chapter 2.2, that form a complex with DNA called a **nucleosome** that is stabilized by the attraction of the negatively charged DNA to the positively charged histones. In this form, the DNA cannot be transcribed. Acetylation reduces the association of the DNA with the histones by reducing the positive charge on the histones. Deacetylation promotes condensation of the DNA into heterochromatin. The enzyme **histone acetyl transferase (HAT)** sticks acetyl groups on the histones, and **histone deacetylase** removes them. Both of these enzymes associate with coactivators and repressors of transcription. A variety of proteins associated with nuclear receptors possess HAT activity.

NUCLEAR RECEPTORS RECRUIT TRANSCRIPTION FACTORS

Once acetylation of the histones has allowed the reorganization of chromatin, several other complexes of proteins bind to the DNA to initiate transcription. The transcription factor TFIID (for transcription factor polymerase II) is a complex of proteins that binds to a TATAA sequence on the DNA some 25−30 nucleotides upstream of the initiation site (see Chapter 1.3). TFIID contains a **TATAA-binding protein (TBP)**, which binds directly to the TATAA sequence, and a series of other factors called **TBP-associated factors (TAFs)**. TBP then binds a second basal transcription factor, **TFIIB**. This allows the binding of RNA polymerase II to the complex, which is then fully activated by the binding of assorted other transcription factors, **TFIIF**, **TFIIE**, and **TFIIH**. The nuclear receptor influences transcription through specific proteins that interact both with the nuclear receptor on its recognition site and with the RNA polymerase complex on its initiation site. This interaction is possible because the DNA can form loops that closely appose the nuclear receptors and the
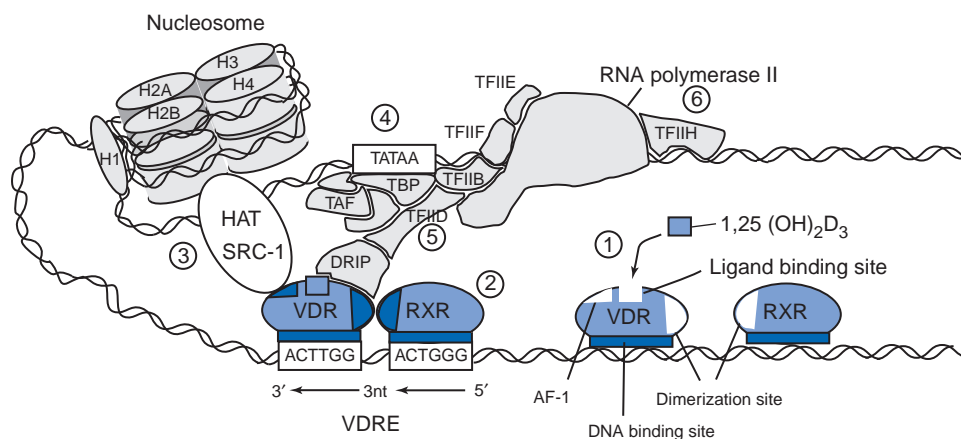
FIGURE 2.8.14 Simplified model of activation of transcription of DNA by vitamin D_3. Not all steps in this process are established, and the figure is meant to convey some of the players and their postulated roles. It is not to be taken too seriously. The active form of vitamin D_3, $1,25(OH)_2D_3$, binds to its receptor, VDR (vitamin D receptor), which is nonspecifically bound to DNA. Binding of the ligand (1) results in dimerization of the VDR with RXR (9-*cis*-retinoic acid receptor) and binding of the dimer to specific vitamin D-responsive elements on the DNA (VDRE) (2). These are similar repeat motifs on the DNA as indicated by the sequences ACTTGG and ACTGGG. Transactivation begins with the recruitment of coactivators with HAT activity (3). One of these is SRC-1 for the steroid receptor coactivator. Acetylation of histones causes chromatin remodeling that facilitates transcription. Actual initiation of transcription requires the binding of TBP to the TATAA box, along with several TAFs (4). This complex is TFIID, for transcription factor for RNA polymerase II. TFIID then binds TFIIB, which forms a bridge to RNA polymerase II. Several proteins, called vitamin D-receptor interacting proteins, or DRIPs, form a bridge to RNA polymerase II, stabilizing the preinitiation complex (5). Transcription is then initiated (6).

preinitiation complex. This process is illustrated for VDR in Figure 2.8.14. In other cases, nuclear receptors can also regulate gene expression by suppressing transcription. For example, glucocorticoids suppress the effects of transcription factor **nuclear factor** κB (**NF-**κ**B**), which stimulates genes as part of the inflammatory response. Glucocorticoids reduce inflammation by this effect (see Chapter 9.5).

OTHER SIGNALING PATHWAYS ALSO REGULATE GENE EXPRESSION

The binding of signaling molecules to nuclear receptors is only one of many routes for the regulation of gene expression. As noted earlier, receptor serine/threonine kinases can alter gene expression through phosphorylation of smads; receptor tyrosine kinases alter gene expression through Ras, and receptor-associated tyrosine kinase alters gene expression through phosphorylation of STATs. Signals that affect 3′,5′ cyclic AMP levels also regulate gene expression. Specific genes possess a regulatory sequence called the cAMP response element or **CRE**. PKA phosphorylates **CREB** (CRE-binding protein), a transcription factor that binds to the CRE. CREB can also be phosphorylated by CaM kinase II and CaM kinase IV. Transcriptional activation by CREB requires coactivators including CREB binding protein (**CBP**) with a molecular weight of 300 kDa. CREB forms homodimers to activate transcription, but it can also form heterodimers with CREM (CRE modifier) that either activate or inhibit transcription. A schematic of CREB's involvement in the regulation of transcription is shown in Figure 2.8.15.
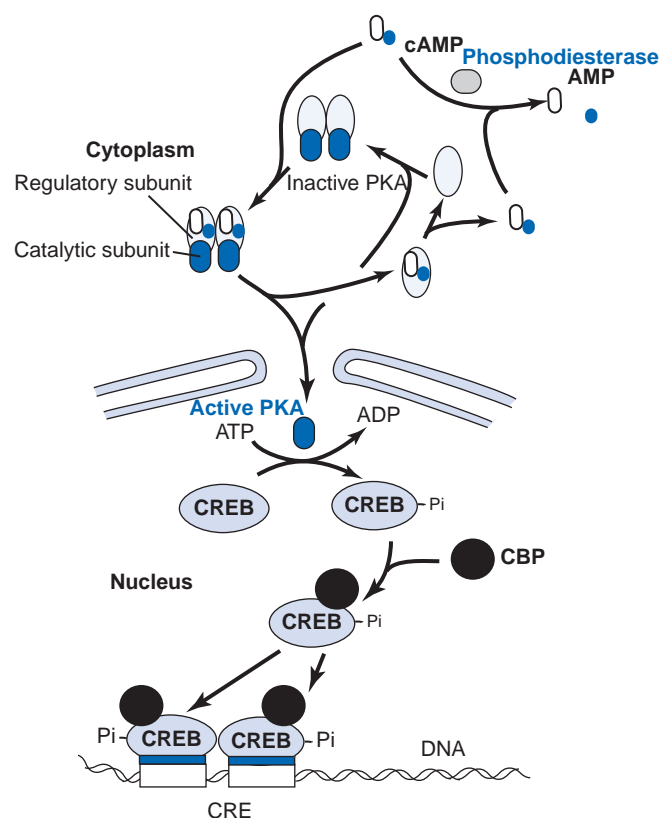

FIGURE 2.8.15 Modulation of gene transcription by cyclic AMP-dependent PKA. Activation of adenylyl cyclase occurs on the surface membrane of the cell through a G_s-coupled receptor for a hormone. The increased cAMP activates PKA by dissociating the regulatory subunit from the catalytic subunit. The catalytic subunit translocates to the nucleus where it phosphorylates CREB, a protein that binds to cyclic AMP responsive elements (CRE) on the DNA strand. CREB binds to CBP and may form homodimers or heterodimers and may activate or inhibit transcription. A variety of cofactors are recruited to complete the mechanism.
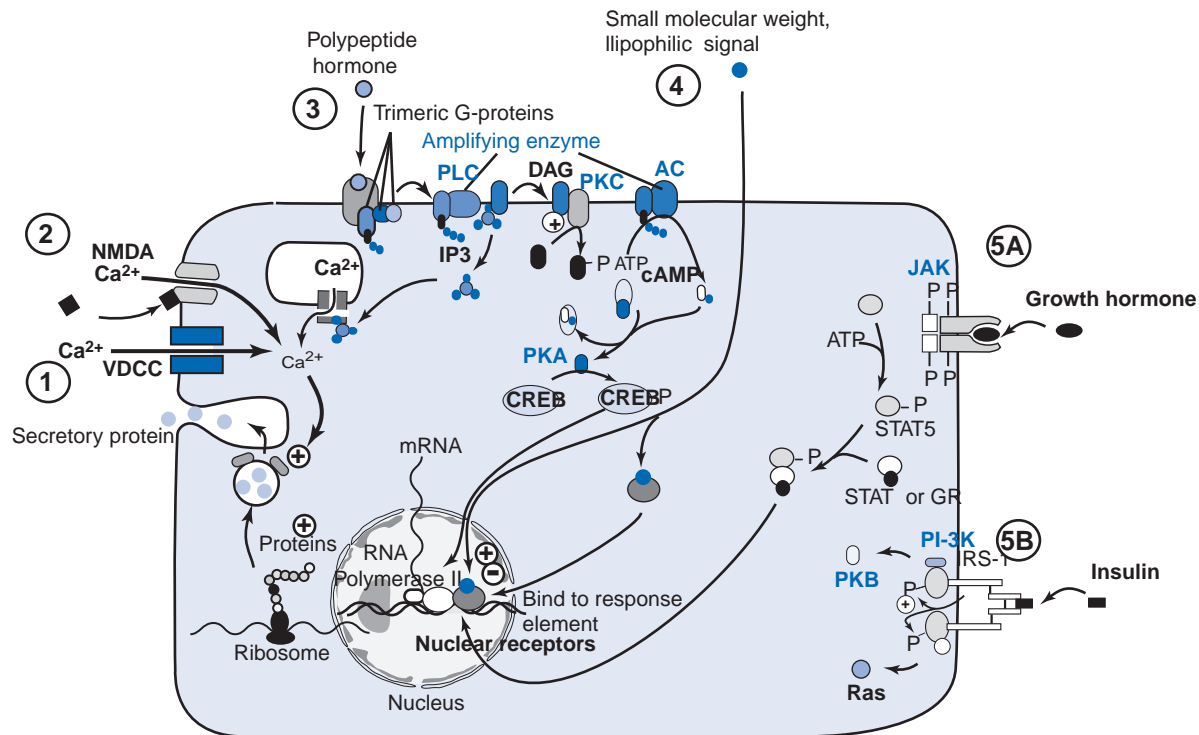
FIGURE 2.8.16 Summary of major signaling pathways. (1) Voltage-dependent channels open to convey electrical responses. (2) LGICs convert chemical signals into electrical signals. (3) Four different classes of heterotrimeric GPCRs convert extracellular chemical signals into intracellular chemical signals. The GPCR coupled to phospholipase C (G_q) and to adenylyl cyclase (G_s) is shown, but other responses also occur. (4) Small lipophilic signaling molecules enter the cell and affect gene transcription and other processes. (5) Extracellular chemical signals, larger proteins, activate enzymes that produce intracellular signals. Growth hormone and insulin receptor types are shown.

SUMMARY OF SIGNALING MECHANISMS

A synopsis of the signaling mechanisms discussed in this chapter is shown in Figure 2.8.16. The main classes of signaling include the following: (1) voltage-gated ion channels, including the fast Na^+ channel and K^+ channels involved in action potential origination and propagation. These channels maintain electrical signaling that is rapidly conveyed over the surface of the cell. (2) LGICs, the mainstay of synaptic transmission between neurons and between nerve and muscle. (3) Heterotrimeric GPCR, including four main subtypes: those that excite adenylyl cyclase (G_s mechanisms); those that inhibit adenylyl cyclase (G_I mechanisms); those that activate phospholipase C, releasing IP3 and DAG (G_q mechanisms); and those that stimulate GEFs to activate one of a family of small GTPase proteins. (4) Extracellular signals, which directly activate enzymes such as guanylyl cyclase, receptor serine/threonine kinase, receptor tyrosine kinase, and receptor-associated tyrosine kinase. (5) Signaling molecules that bind cytoplasmic or nuclear receptors. Other signaling mechanisms, such as those involving sphingosine phosphate, are not shown here.

SUMMARY

Mechanical, electrical, or chemical signals are used by cells to communicate. Mechanical signaling and some chemical signaling require close contact. Electrical signaling is the fastest way to move a signal from one part of a cell to another and to adjacent cells. Chemical signals can be used by a cell to regulate itself (autocrine), its near neighbors (paracrine) or distant cells through the medium of the blood (endocrine). Nerve cells use electrical signals over long cell processes to cause release of chemical signals near their target cells. Neuroendocrine signals are chemical signals released by neurons into the blood.

Voltage-gated ion channels make electrical signals possible. The influx of Ca^{2+} ions carries an electrical and a chemical signal because Ca^{2+} binds to specific receptors inside the cell to initiate secretion, to activate enzymes indirectly through CAM kinase or directly through activation of other enzymes, or to activate contraction.

LGICs convert chemical into electrical signals. This is used in neurotransmission: an action potential on one cell is converted to an electrical signal on another. Many chemical signals have multiple types of receptors, so that the effect in the postsynaptic cell depends on the chemical released by the presynaptic cell and the receptor expressed by the postsynaptic cell.

Many chemical signals bind to receptors on the surface membrane that are linked to heterotrimeric G-proteins (GPCRs). These dissociate upon ligand binding and generally the α subunit activates an amplifying enzyme such as adenylyl cyclase (for G_s mechanisms) or phospholipase C (for G_q mechanisms), which increase the

formation of 3′,5′ cyclic AMP or IP3, respectively. Other GPCRs inhibit adenylyl cyclase (G_i) or recruit a number of small monomeric G-proteins such as Ras and Rho ($G_{11/12}$). The $\beta\gamma$ subunit can also affect intracellular targets. Increased cytosolic cAMP activates PKA that phosphorylates specific target proteins. IP3 released by G_q-coupled receptors causes Ca^{2+} release from ER stores and activation of CaM kinase.

Other chemical signals bind to surface receptors that are catalytic. The four classes are receptor guanylyl cyclase, receptor serine/threonine kinase, receptor tyrosine kinase, and receptor-associated tyrosine kinase. Examples of these signals include insulin and growth hormone. Insulin binding activates an intrinsic tyrosine kinase that phosphorylates insulin receptor substrates that bind phosphatidyl inositol 3 kinase, PI-3K. This forms PIP3, which activates a phosphoinositide-dependent protein kinase (PDK). Growth hormone activates a receptor that in turn activates a member of the Janus Kinase family of proteins, which then phosphorylates STAT5 (signal transduction and activation of transcription). The phosphorylated STAT molecule turns on specific genes.

Small lipophilic chemical signals such as thyroxine, vitamin D, and the steroid hormones penetrate the cell membrane and bind to receptors either in the cytosol or in the nucleus. These receptors bind to specific regions of DNA called response elements. The receptors recruit a large number of accessory proteins that unravel the DNA and direct the synthesis of mRNA that codes for specific proteins.

The phosphorylation state of a set of regulated proteins depends not only on the activity of the protein kinase but also on the activity of the protein phosphatases. Both the kinases and the phosphatases may be regulated to alter the phosphorylation state of cellular proteins.

REVIEW QUESTIONS

1. What is an autocrine hormone? Paracrine hormone? Endocrine hormone?
2. What is the fastest way to convey a signal from one part of the body to another?
3. How does an electrical signal on the surface of a cell become a Ca^{2+} signal in its interior? List four distinct ways that Ca^{2+} can affect cell function.
4. What is a ligand-gated ion channel? What is the source of the extracellular ligand? Is the ligand a chemical, electrical, or mechanical signal?
5. What is meant by "G-protein-coupled receptor"? A $G\alpha_s$ mechanism couples to what amplifying enzyme? What is the product of this enzyme? What does this product do? What is a $G\alpha_i$ mechanism?
6. What amplifying enzyme is activated by a $G\alpha_q$ mechanism? What are its products? What do these products do?
7. Name the four classes of catalytic receptors.
8. Why do peptide hormones have receptors facing the extracellular space?
9. How do small lipophilic signals affect cell function? What is meant by "nuclear receptor"? What do these receptors do? What is meant by the term "response element"?
10. How does cAMP alter gene expression?