11
Modulation of Synaptic Transmission: Second Messengers

An Overall View

THE BINDING OF NEUROTRANSMITTER to postsynaptic receptors produces a postsynaptic potential either directly, by opening ion channels, or indirectly, by altering ion channel activity through changes in the postsynaptic cell's biochemical state. As we saw in Chapter 8, the type of action depends on the type of receptor. Activation of *ionotropic receptors* directly opens ion channels that are part of the receptor macro-molecule itself. In contrast, activation of *metabotropic receptors* regulates the opening of ion channels indirectly through biochemical signaling pathways. The receptor and ion channels that are affected are distinct macromolecules (Figure 11-1).

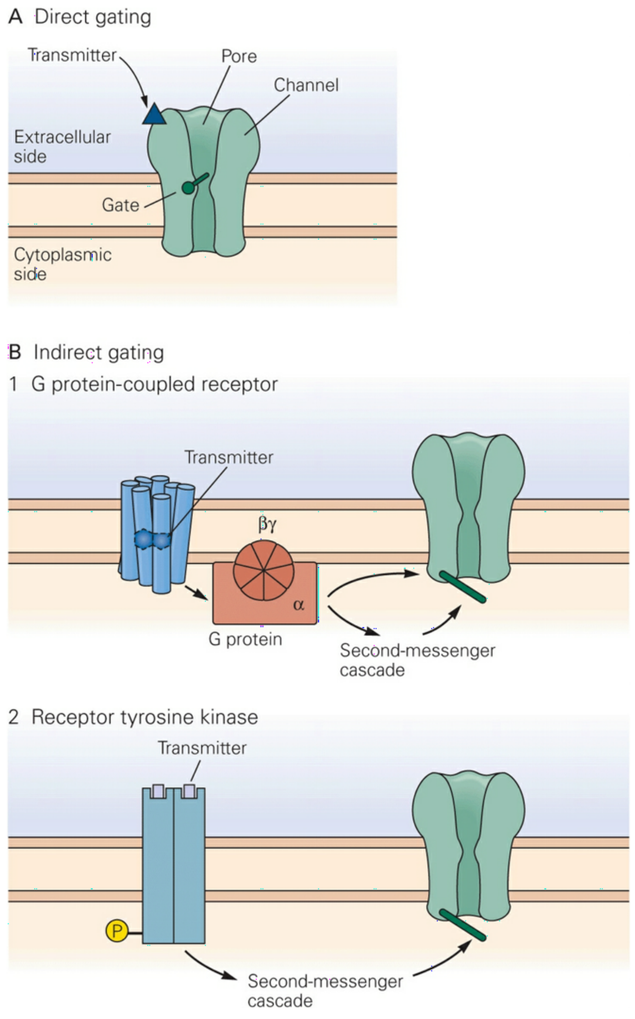


Figure 11-1 Neurotransmitter actions can be divided into two groups according to the way in which receptor and effector functions are coupled.

A. Direct transmitter actions are produced by ionotropic receptors, ligand-gated channels in which the receptor and ion channel are domains formed by a single macromolecule. The binding of transmitter to the receptor on the extracellular aspect of the protein directly opens the ion channel embedded in the cell membrane.

B. Indirect transmitter actions are caused by binding of transmitter to metabotropic receptors that are separate macromolecules from the ion channels that they regulate. There are two families of these receptors. **1**. G protein-coupled receptors activate guanosine triphosphate (GTP)-binding proteins that engage a second-messenger cascade or act directly on ion channels. **2**. Receptor tyrosine kinases initiate a cascade of protein phosphorylation reactions, beginning with autophosphorylation of the kinase itself on tyrosine residues.

Whereas the action of ionotropic receptors is fast and brief, metabotropic receptors produce effects that begin slowly and persist for long periods, ranging from hundreds of milliseconds to many minutes. The two types of receptors also differ in their functions. Ionotropic receptors *mediate* behaviors, from simple reflexes to complex cognitive processes. Metabotropic receptors *modulate* behaviors; they modify reflex strength, help focus attention, set emotional states, and contribute to long-lasting changes in neural circuits that underlie learning and memory. Metabotropic receptors are responsible for many of the actions of transmitters, hormones, and growth factors.

Ionotropic receptors change the balance of charge across the neuron's membrane quickly. As we have seen, this change is local at first but is propagated as an action potential along the axon if the change in membrane potential is suprathreshold. Activation of metabotropic receptors also begins as a local action that can spread to a wider region of the cell. A neurotransmitter reacting with a metabotropic receptor activates proteins that in turn activate effector enzymes. The effector enzymes then often produce second-messenger molecules that can diffuse within a cell to activate still other enzymes that catalyze modifications of a variety of target proteins, greatly changing their activities.

There are two major families of metabotropic receptors: G protein-coupled receptors and receptor tyrosine kinases. We first describe the G protein-coupled receptor family and later discuss the receptor tyrosine kinase family.

The G protein-coupled receptors are coupled to an effector by a trimeric guanine nucleotide-binding protein, or G protein (Figure 11-1B). This receptor family contains α- and β-adrenergic receptors for norepinephrine, muscarinic acetylcholine (ACh) receptors, γ-aminobutyric acid B ($GABA_B$) receptors, certain glutamate and serotonin receptors, all receptors for dopamine, receptors for neuropeptides, odorant receptors, rhodopsin (the protein that reacts to light, initiating visual signals, see Chapter 26), and many others. Many of these receptors are thought to be involved in neurological and psychiatric disease and are key targets for the actions of important classes of therapeutic drugs.

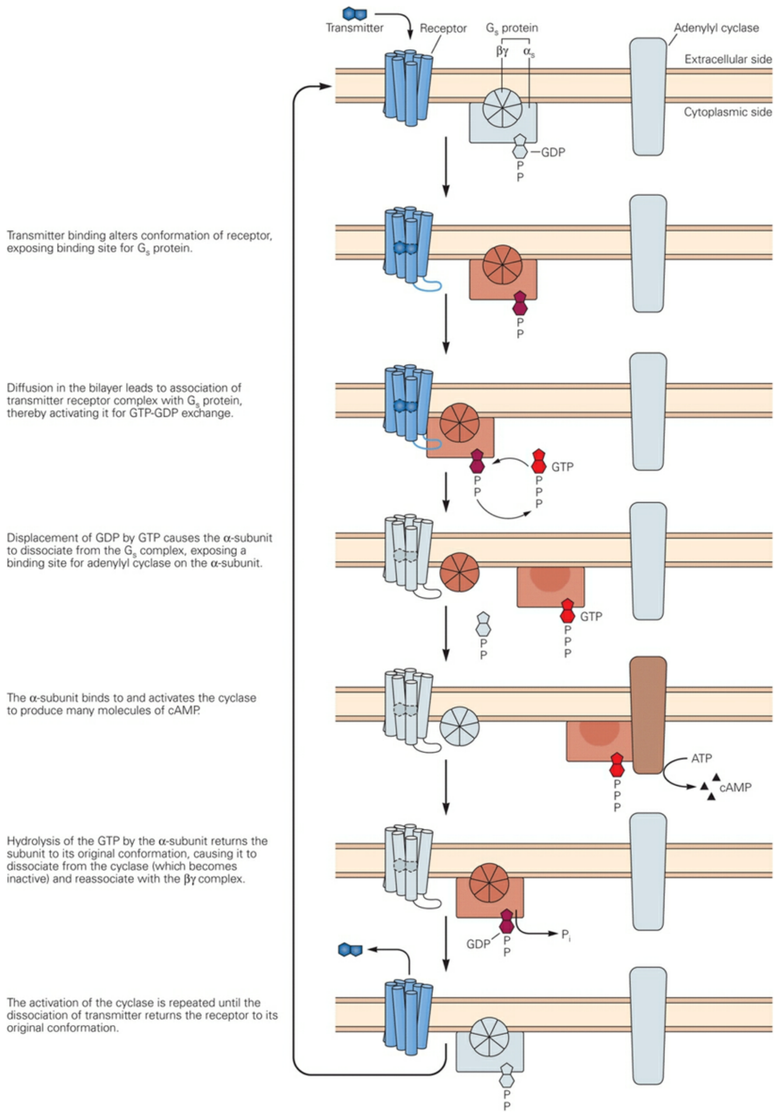
G protein-coupled receptors activate a variety of effectors. Typically the effector is an enzyme that produces a diffusible second messenger. These second messengers in turn trigger a biochemical cascade, either by activating specific protein kinases that phos-phorylate the hydroxyl group of specific serine or threonine residues in various proteins or by mobilizing Ca^{2+} ions from intracellular stores, thus initiating reactions that change the cell's biochemical state. In some instances the G protein or the second messenger act directly on an ion channel.

The Cyclic AMP Pathway Is the Best Understood Second-Messenger Signaling Cascade Initiated by G Protein-Coupled Receptors

The adenosine 3′,5′-cyclic monophosphate (cyclic AMP or cAMP) pathway is a prototypic example of a second-messenger cascade. It was the first second-messenger pathway to be discovered, and our conception of other second-messenger pathways is based on it.

The binding of transmitter to receptors linked to the cAMP cascade first activates a specific G protein, G_s (named for its action to *stimulate* cAMP synthesis). In its resting state $G_{s'}$ like all G proteins, binds

a molecule of guanosine diphosphate (GDP). The interaction of G_s with a ligand-bound receptor promotes the exchange of the bound GDP for guanosine triphosphate (GTP), leading to a conformational change that activates the G protein. In its activated state G_s stimulates the integral membrane protein adenylyl cyclase to catalyze the conversion of adenosine triphosphate (ATP) to cAMP. When associated with the cyclase, G_s also acts as a GTPase, hydrolyzing its bound GTP to GDP. When GTP is hydrolyzed, the G protein becomes inactive and dissociates from the cyclase, thereby stopping the synthesis of cAMP (Figure 11–2). Typically, a G_s protein remains active for a few seconds before its bound GTP is hydrolyzed.



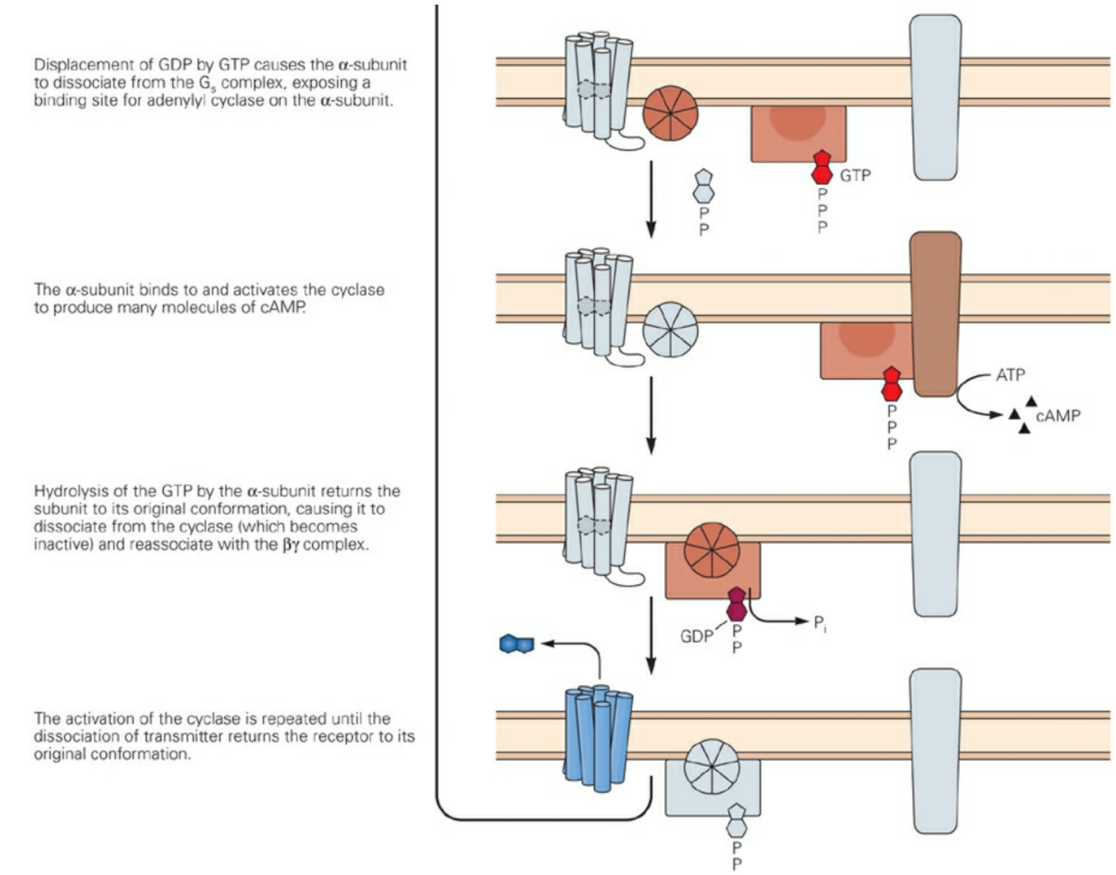


Figure 11-2 The cAMP cycle. (Opposite) The binding of a transmitter to certain metabotropic receptors activates the stimulatory G protein (G_s). The G protein is a heterotrimer consisting of α-, β-, and γ-subunits. The β- and γ-subunits form a unit that binds tightly to the membrane. In its resting state the α-subunit of G_s, termed $\alpha_{s'}$, binds a molecule of guanosine diphosphate (GDP). When activated by its interaction with a ligand-bound receptor, the GDP bound to α_s is exchanged for guanosine triphosphate (GTP), causing α_s to functionally dissociate from the βγ complex. Next α_s associates with an intracellular domain of adenylyl cyclase, thereby activating the enzyme to produce cAMP from adenosine triphosphate (ATP). When bound to the cyclase, α_s is a GTPase. The hydrolysis of GTP to GDP and inorganic phosphate (P_i) leads to the dissociation of α_s from the cyclase and its reassociation with the βγ complex. The cyclase then stops producing the second messenger. At some point during this cycle the transmitter dissociates from the receptor. The system returns to an inactive state when the transmitter-binding site on the receptor is

empty, the three subunits of the G protein reassociate, and the guanine nucleotide-binding site on the a-subunit is occupied by GDP (Adapted, with permission, from Alberts et al. 1994.)

Once a G protein-coupled receptor binds a ligand, it can interact sequentially with more than one G protein macromolecule. As a result, the sequential binding of relatively few molecules of transmitter to a small number of receptors can activate a large number of cyclase complexes. The signal is further amplified in the next step in the cAMP cascade, the activation of the protein kinase.

The major target of cAMP in most cells is the cAMP-dependent protein kinase (also called protein kinase A or PKA). This kinase, identified and characterized by Edward Krebs and colleagues, is a het-erotetrameric enzyme consisting of a dimer of two regulatory (R) subunits and two catalytic (C) subunits. In the absence of cAMP the R subunits bind to and inhibit the C subunits. In the presence of cAMP each R subunit binds two molecules of cAMP, leading to a conformational change that causes the R and C sub-units to dissociate (Figure 11–3). Dissociation frees the C subunits to transfer the γ-phosphoryl group of ATP to the hydroxyl groups of specific serine and threonine residues in substrate proteins.

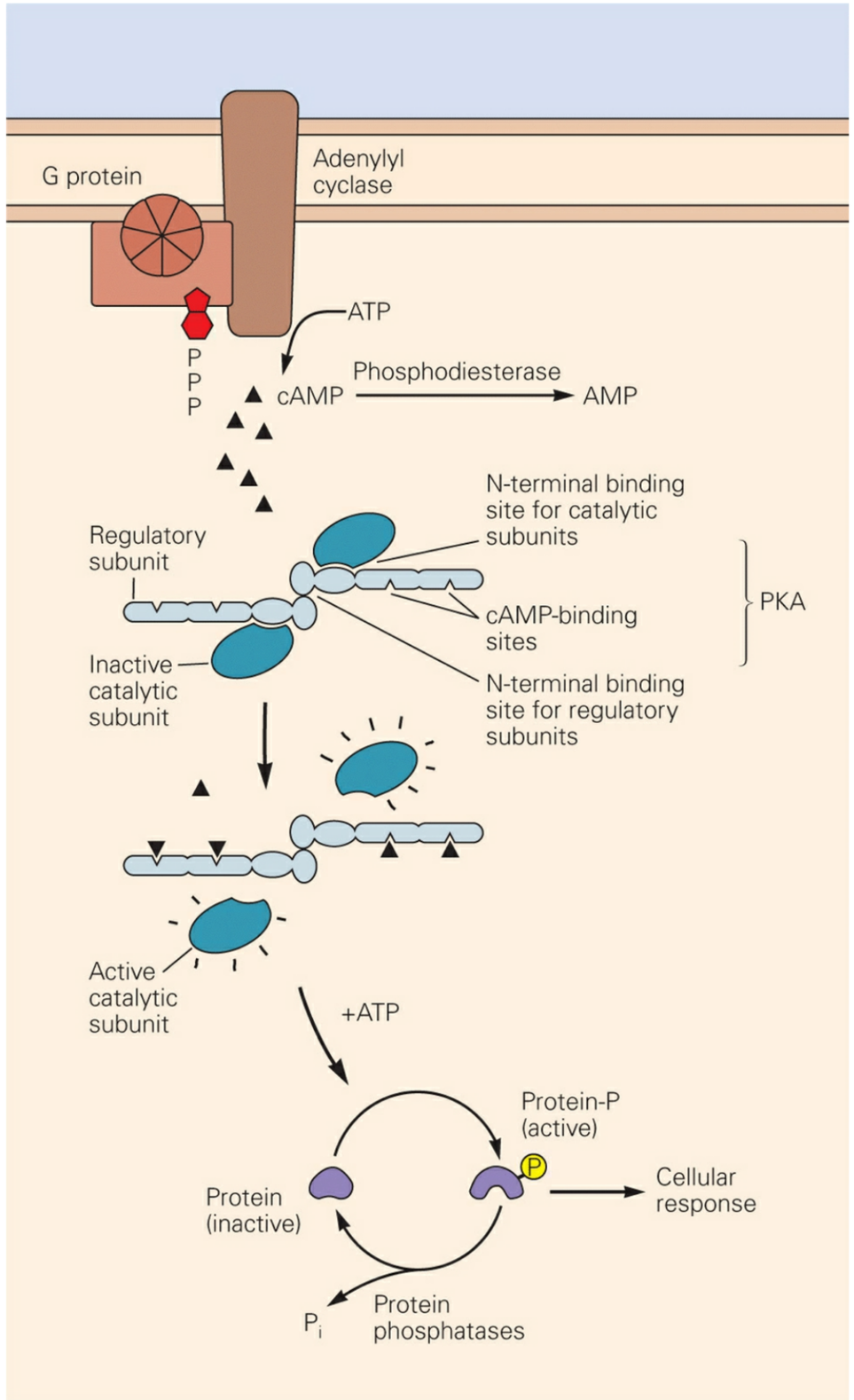


Figure 11-3 The cAMP pathway activates protein kinase A. Adenylyl cyclase converts adenosine triphosphate (ATP) into cAMP. Four cAMP molecules bind to the two regulatory sub-units of the protein kinase A (PKA), liberating the two catalytic subunits, which are then free to phosphorylate specific substrate proteins on certain serine or threonine residues, thereby producing a cellular response. Two kinds of enzymes regulate this pathway. Phosphodiesterases convert cAMP to adenosine monophosphate (which is inactive), and protein phosphatases remove phosphate groups (P) from the substrate proteins, releasing inorganic phosphate, P_i (see also Figure 11-14).

Protein kinase A is distantly related through evolution to other serine and threonine protein kinases that we shall consider: the Ca^{2+}/calmodulin-dependent protein kinases and protein kinase C. These kinases also have regulatory and catalytic domains, but both domains are within the same polypeptide molecule (see Figure 11-6).

In addition to blocking enzymatic activity, the R subunits of PKA also target the C subunits to distinct sites within cells. Human PKA has two types of R sub-units, each with two subtypes: $R_{I\alpha}$, RIβ, $R_{II\alpha}$, and RIIβ. The genes for each are distinct but derive from a common ancestor. The two R subunits in a molecule of PKA are present as a dimer, formed when the R subunits are synthesized. Because the dimer never separates, hybrid types of the kinase do not exist in the cell. This is functionally important because the types have different properties. For example, type II PKA (containing R_{II}-type subunits) is targeted to the membrane by A kinase attachment proteins (AKAPs). One AKAP targets PKA to the N-methyl-D-aspartate (NMDA)-type glutamate receptor by binding both PKA and the postsynaptic density protein PSD-95, which binds to the cytoplasmic tail of the NMDA receptor (see Chapter 10). In addition, this AKAP also binds a protein phos-phatase, which removes the phosphate group from substrate proteins (see Figure 11-14). By localizing PKA and other signaling components near their substrate, AKAPs form local signaling complexes that increase the specificity, speed, and efficiency of second-messenger cascades. Because AKAPs have only a weak affinity for R_I subunits, most type I PKA is free in the cytoplasm.

Kinases can only phosphorylate proteins on serine and threonine residues that are embedded within a context of specific *phosphorylation consensus sequences* of amino acids. For example, phosphorylation by PKA usually requires a sequence of two contiguous basic amino acids—either lysine or arginine—followed by any amino acid, and then by the serine or threonine residue that is phosphorylated (for example, Arg-Arg-Ala-Thr).

Several important protein substrates for PKA have been identified in neurons. These include voltage-gated and ligand-gated ion channels, synaptic vesicle proteins, enzymes involved in transmitter biosynthesis, and proteins that regulate gene transcription. As a result, the cAMP pathway has widespread effects on the electro-physiological and biochemical properties of neurons. We shall consider some of these actions later in this chapter.

The Second-Messenger Pathways Initiated by G Protein-Coupled Receptors Share a Common Molecular Logic

Approximately 800 of the roughly 23,000 genes thought to comprise the human genome code for G protein-coupled receptors. Although many of these are odorant receptors in olfactory neurons (see Chapter 32), many others are receptors for well-characterized neurotrans-mitters used throughout the nervous system. Despite their enormous diversity, all G protein-coupled receptors consist of a single polypeptide with seven characteristic membrane-spanning regions (serpentine receptors) (Figure 11–4).

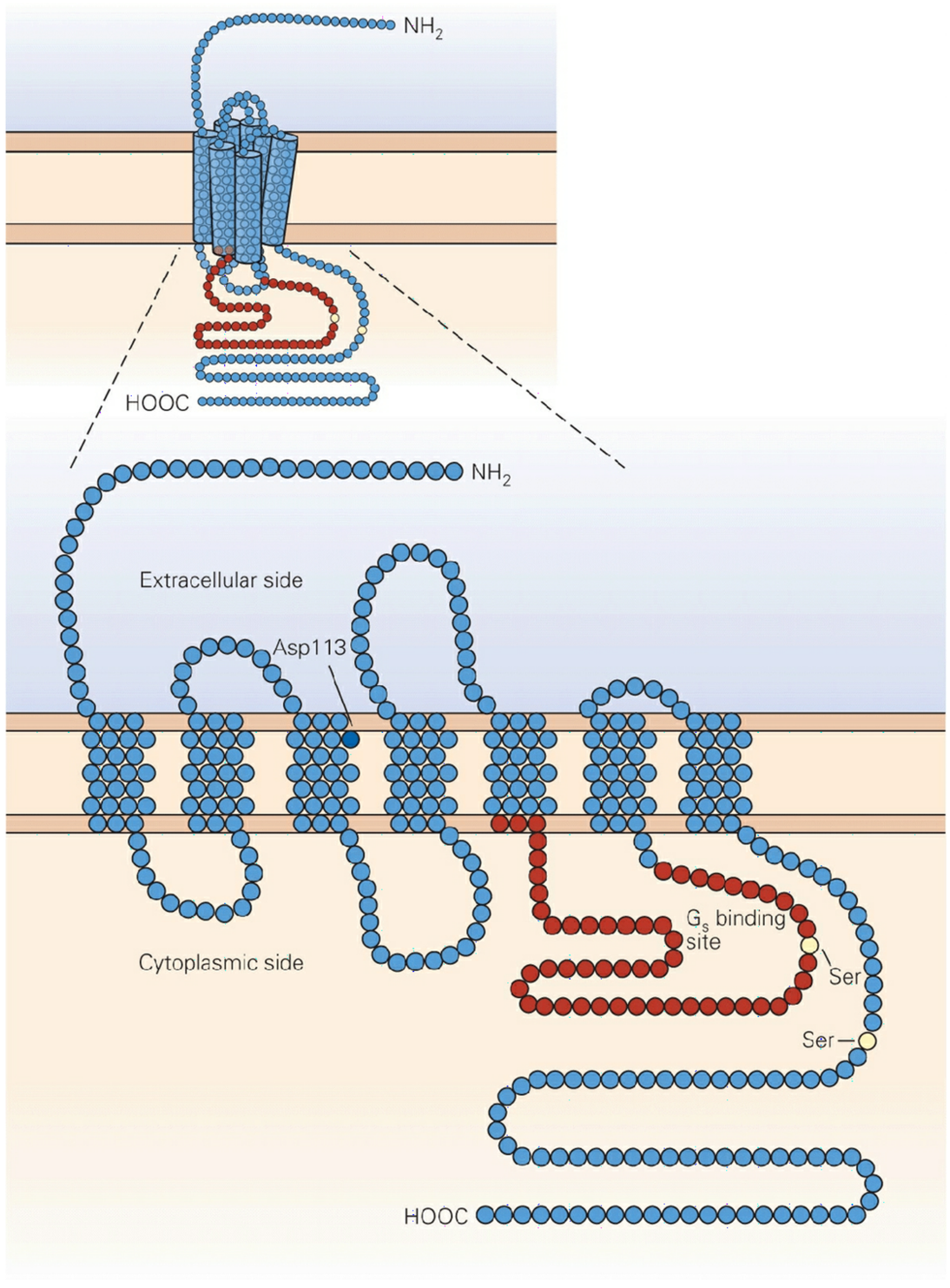


Figure 11-4 G protein-coupled receptors contain seven membrane-spanning domains. The β_2-adrenergin receptor shown here is representative of G protein-coupled receptors, including the β_1-adrenergic and muscarinic acetylcholine (ACh) receptors and rhodopsin. It consists of a single subunit with an extracellular amino terminus, intracellular carboxy terminus, and seven membrane-spanning α-helixes. The binding site for the neurotransmitter lies in a cleft in the receptor formed by the transmembrane helixes. The amino acid residue aspartic acid (Asp)-113 participates in binding. The part of the receptor indicated in **brown** associates with G protein α-subunits. Two serine (Ser) residues in the intracellular carboxy-terminal tail are sites for phosphorylation by specific receptor kinases, which helps inactivate the receptor. (Adapted, with permission, from Frielle et al. 1989.)

The number of substances that act as second messengers in synaptic transmission is much fewer than the number of transmitters. Approximately 100 substances serve as transmitters; each can activate several types of receptors on the cell surface. The few second messengers that have been well characterized fall into two categories, intracellular and transcellular. Intra-cellular messengers are molecules whose actions are confined to the cell in which they are produced. Trans-cellular messengers are molecules that can readily cross the cell membrane and thus can leave the cell in which they are produced to act as intercellular signals, or first messengers, on neighboring cells.

A Family of G Proteins Activates Distinct Second-Messenger Pathways

The first G protein, G_s (where "s" stands for stimulatory), was identified more than 30 years ago by Martin Rodbell, Al Gilman, and their colleagues. Since that time a large family of G proteins has been identified. The G proteins are associated with the inner leaflet of the plasma membrane, where they interact with G protein-coupled receptors.

The G proteins that couple receptor activation to intracellular effectors are trimers that consist of three subunits: α, β, and γ (Figure 11–2). The α-subunit is only loosely associated with the membrane and is usually the agent that couples the receptor to its primary effector enzyme. The β- and γ-subunits form a strongly bound complex that is more tightly associated

with the membrane. As we shall learn later in this chapter, the βγ complex of G proteins can also regulate the activity of certain ion channels directly.

Approximately 20 types of α-subunits have been identified, 5 types of α-subunits, and 12 types of γ-subunits. G proteins with different α-subunits couple different classes of receptors and effectors, and therefore have different physiological actions. The β-adrenergic receptor activates adenylyl cyclase by acting on G_s proteins; these contain the α_s type of α-subunit. Some mus-carinic ACh receptors inhibit the cyclase by acting on G_i proteins (where "i" stands for inhibitory); these contain the α_i type subunit. Still other G proteins ($G_q/_{11}$ proteins, which contain α_q - or α_{11}-subunits) activate phospholipase C and probably other signal transduction mechanisms not yet identified. The G_o protein, which contains the α_o-subunit, is expressed at particularly high levels in the brain. Compared with other organs of the body, the brain contains an exceptionally large variety of G proteins. Even so, because of the limited number of classes of G proteins, one type of G protein can often be activated by different classes of receptors.

The known effector targets for G proteins are more limited than the types of G proteins. Important effectors include certain ion channels that are activated by the βγ complex, adenylyl cyclase in the cAMP pathway, phospholipase C in the diacylglycerol-inositol polyphosphate pathway, and phospholipase A_2 in the arachidonic acid pathway. Each of these effectors (except for the ion channels) initiates changes in specific target proteins within the cell, either by generating second messengers that bind to the target protein or by activating a protein kinase that phosphorylates it. Despite their differences, second-messenger pathways activated by G protein signaling share a common design (Figure 11–5).

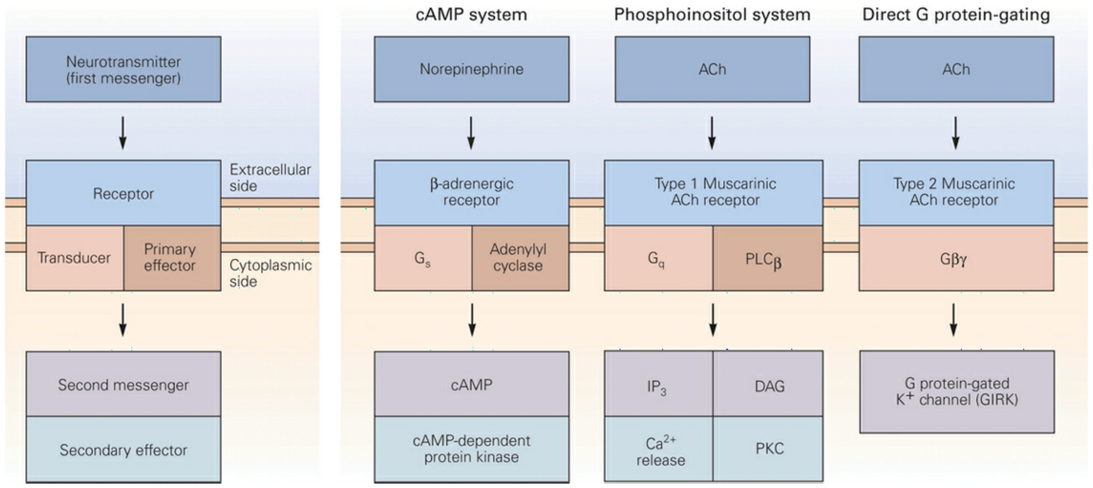


Figure 11-5 Synaptic second-messenger systems involving G protein coupling follow a common sequence. The signal transduction pathways illustrated here involve similar steps (left). Chemical transmitters arriving at receptor molecules in the plasma membrane activate a closely related family of G proteins (the transducers) that activate different enzymes or channels (the primary effectors). The activated enzymes produce a second messenger that activates a secondary effector or acts directly on a target (or regulatory) protein.

cAMP system. This pathway can be activated by a transmitter-bound β-adrenergic receptor, which acts through the G_s protein α_s-subunit to activate adenylyl cyclase. Adenylyl cyclase produces the second messenger cAMP, which activates PKA. The G protein here is termed G_s because it stimulates the cyclase. Some receptors activate a G! protein that inhibits the cyclase.

Phosphoinositol system. This pathway, activated by a type 1 muscarinic acetylcholine (ACh) receptor, uses the G_q or G_{11} type of G protein (with α or α_{11}-subunits, respectively) to activate a primary effector, phospholipase Cβ (PLC_β). This enzyme hydrolyzes the phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP_2), yielding a pair of second messengers: diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). In turn, IP_3 releases Ca^{2+} from internal stores, whereas DAG activates protein kinase C (PKC). The drop in membrane PIP_2 levels can directly alter the activity of some ion channels.

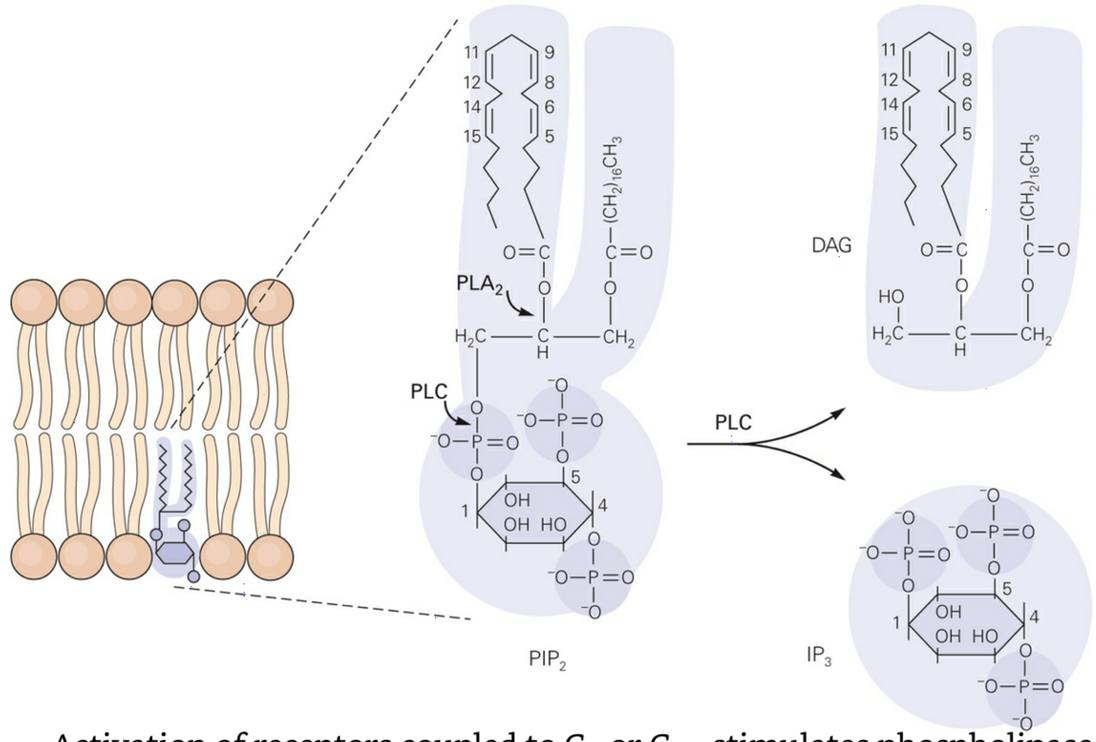
Direct G protein-gating. This pathway represents the simplest syn-

aptic mechanism for G protein-coupled receptor action. Acetylcholine (ACh) acting on type 2 muscarinic receptors activates the G_i protein, leading to functional dissociation of the α-subunit and βγ complex. The βγ complex interacts directly with a G protein-gated inward-rectifying K+ channel (GIRK), leading to channel opening and membrane hyperpolarization.

Hydrolysis of Phospholipids by Phospholipase C Produces Two Important Second Messengers, IP_3 and Diacylglycerol

Many important second messengers are generated through the hydrolysis of phospholipids in the inner leaflet of the plasma membrane. This hydrolysis is catalyzed by three enzymes—phospholipase C, phospholipase D, and phospholipase A_2—named for the ester bonds they hydrolyze in the phospholipid. The phospholipases each can be activated by different G proteins coupled to different receptors.

The most commonly hydrolyzed phospholipid is phosphatidylinositol 4,5-bisphosphate (PIP_2), which typically contains the fatty acid stearate esterified to the glycerol backbone in the first position and the unsaturated fatty acid arachidonate in the second:



Activation of receptors coupled to G_q or G_{11} stimulates phospholipase C, which leads to the hydrolysis of PIP_2 (specifically the phosphodiester bond that links the glycerol backbone to the polar head group) and production of two second messengers, diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). DAG, which is hydrophobic, remains in the membrane when formed, where it recruits the cytoplasmic protein kinase C (PKC). PKC and DAG together with certain membrane phospholipids form an active complex that can phosphorylate many protein substrates in the cell, both membrane-associated and cytoplasmic (Figure 11–6A). Activation of some isoforms of PKC requires elevated levels of cytoplasmic Ca^{2+} in addition to DAG (Box 11-1).

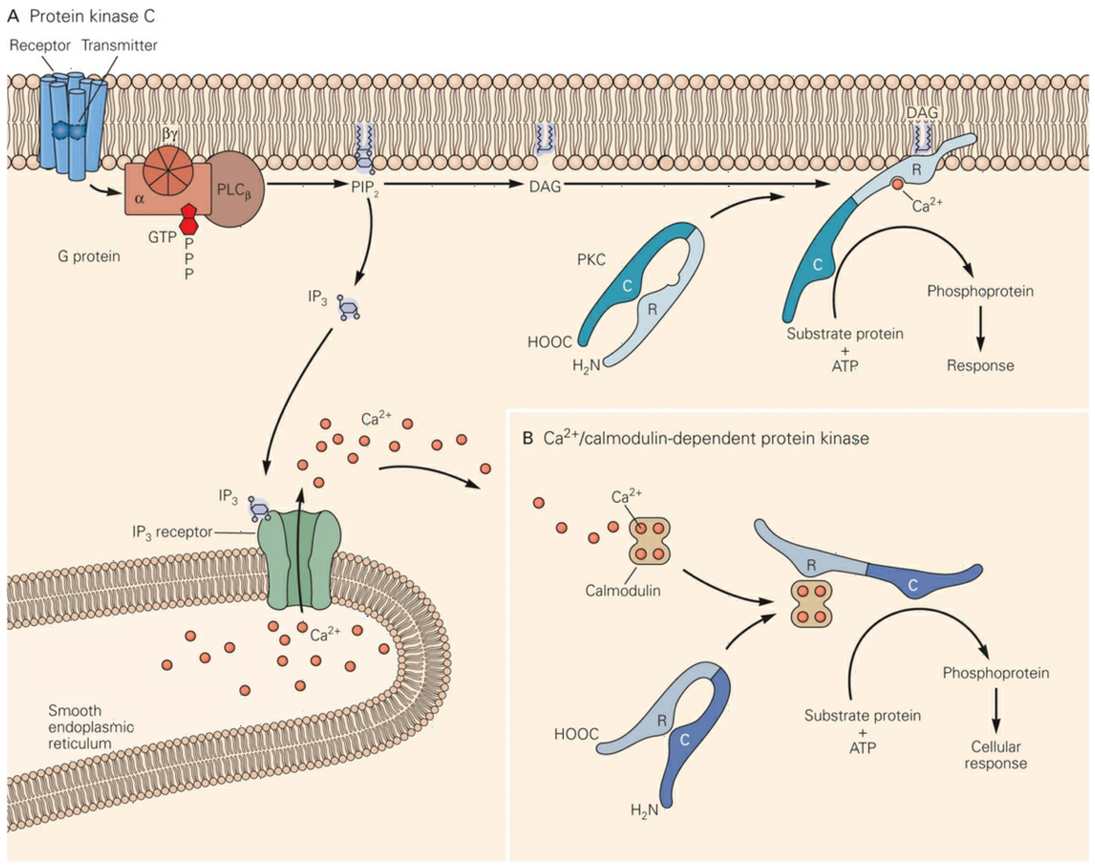


Figure 11-6 Hydrolysis of phospholipids in the cell membrane activates three major second-messenger cascades.

A. The binding of transmitter to a receptor activates a G protein that activates phospholipase Cβ (PLC_β). This enzyme cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2) into the second messengers, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 is water-soluble and diffuses into the cytoplasm, where it binds to a receptor-channel on the smooth endoplasmic reticulum, the IP_3 receptor, to release Ca^{2+} from internal stores. DAG remains in the membrane, where it activates protein kinase C (PKC). Membrane phospholipid is also a necessary cofactor for PKC activation. Some isoforms of PKC also require Ca^{2+} for activation. PKC is composed of a single protein molecule that has both a regulatory domain that binds DAG and a catalytic domain that phosphorylates proteins on serine or threonine residues.

B. The Ca^{2+}/calmodulin-dependent protein kinase is activated when Ca^{2+} binds to calmodulin. The Ca^{2+}/calmodulin complex then binds to a regulatory domain of the kinase, causing its activation. The kinase is composed of many similar subunits (only one of which is shown here), each having both regulatory and catalytic functions. The catalytic domain phosphorylates proteins on serine or threonine residues. (ATP, adenosine triphosphate; C, catalytic subunit; COOH, carboxy terminus; H_2N, amino terminus; R, regulatory subunit.)

The second product of the phospholipase C pathway, IP_3, stimulates the release of Ca^{2+} from intracellular membrane stores in the lumen of the smooth endoplasmic reticulum. The membrane of the reticu-lum contains a large integral membrane macromol-ecule, the IP_3 receptor, which forms both a receptor for IP_3 on its cytoplasmic surface and a Ca^{2+}-permeant channel that spans the membrane of the reticulum. When this macromolecule binds IP_3 the channel opens, releasing Ca^{2+} into the cytoplasm (Figure 11-6A).

The increase in intracellular Ca^{2+} triggers many biochemical reactions and opens calcium-gated channels in the plasma membrane. Calcium can also act as a second messenger to trigger the release of additional Ca^{2+} from internal stores by binding to another integral protein in the membrane of the smooth endoplasmic reticu-lum, the ryanodine receptor (so called because it binds the plant alkaloid ryanodine, which inhibits the receptor; in contrast, caffeine opens the ryanodine receptor). Like the IP_3 receptor to which it is distantly related, the ryanodine receptor forms a Ca^{2+}-permeant channel that spans the reticulum membrane; however, cytoplasmic Ca^{2+}, not IP_3, gates the ryanodine receptor-channel.

Calcium often acts by binding to the small cytoplas-mic protein calmodulin. An important function of the calcium/calmodulin complex is to activate the Ca^{2+}/calmodulin-dependent protein kinase (CaM kinase). This enzyme is a complex of many similar subunits, each containing both regulatory and catalytic domains within the same polypeptide chain. When the Ca^{2+}/calmodulin complex is absent, the C-terminal regulatory domain of the kinase binds and inactivates the catalytic portion. Binding to the Ca^{2+}/calmodulin complex causes conformational changes of the kinase molecule that unfetter the catalytic domain for action (Figure 11-6B). Once activated, CaM kinase can phosphorylate itself through intramolecular reactions at many sites in the molecule. Autophosphory-

lation has an important functional effect: It converts the enzyme into a form that is independent of Ca^{2+}/calmodulin and therefore persistently active even in the absence of Ca^{2+}.

Persistent activation of protein kinases is a general and important mechanism for maintaining biochemical processes underlying long-term changes in synaptic function associated with certain forms of memory. In addition to the persistent activation of Ca^{2+}/calmodu-lin-dependent protein kinase, PKA can also become persistently active following a transient increase in cAMP, because of the enzymatic degradation of its regulatory subunits through the ubiquitin pathway. The decline in regulatory subunit concentration results in the long-lasting presence of free catalytic subunits, even after cAMP levels have declined, leading to the continued phosphorylation of substrate proteins. PKC can also become persistently active through proteolytic cleavage of its regulatory and catalytic domains or expression of a PKC isoform that lacks a regulatory domain.

Hydrolysis of Phospholipids by Phospholipase A_2 Liberates Arachidonic Acid to Produce Other Second Messengers

Phospholipase A_2 hydrolyzes phospholipids distinct from PIP_2 by cleaving the fatty acyl bond between the 2' position of the glycerol backbone and arachidonic acid. This releases arachidonic acid, which is then converted through enzymatic action to one of a family of active metabolites called *eicosanoids*, so called because of their 20 (Greek *eicosa)* carbon atoms.

Three types of enzymes metabolize arachidonic acid: (1) cyclooxygenases, which produce prostaglandins and thromboxanes; (2) several lipoxygenases, which produce a variety of metabolites to be discussed below; and (3) the cytochrome P450 complex, which oxidizes arachidonic acid itself as well as cyclooxygen-ase and lipoxygenase metabolites (Figure 11–7).

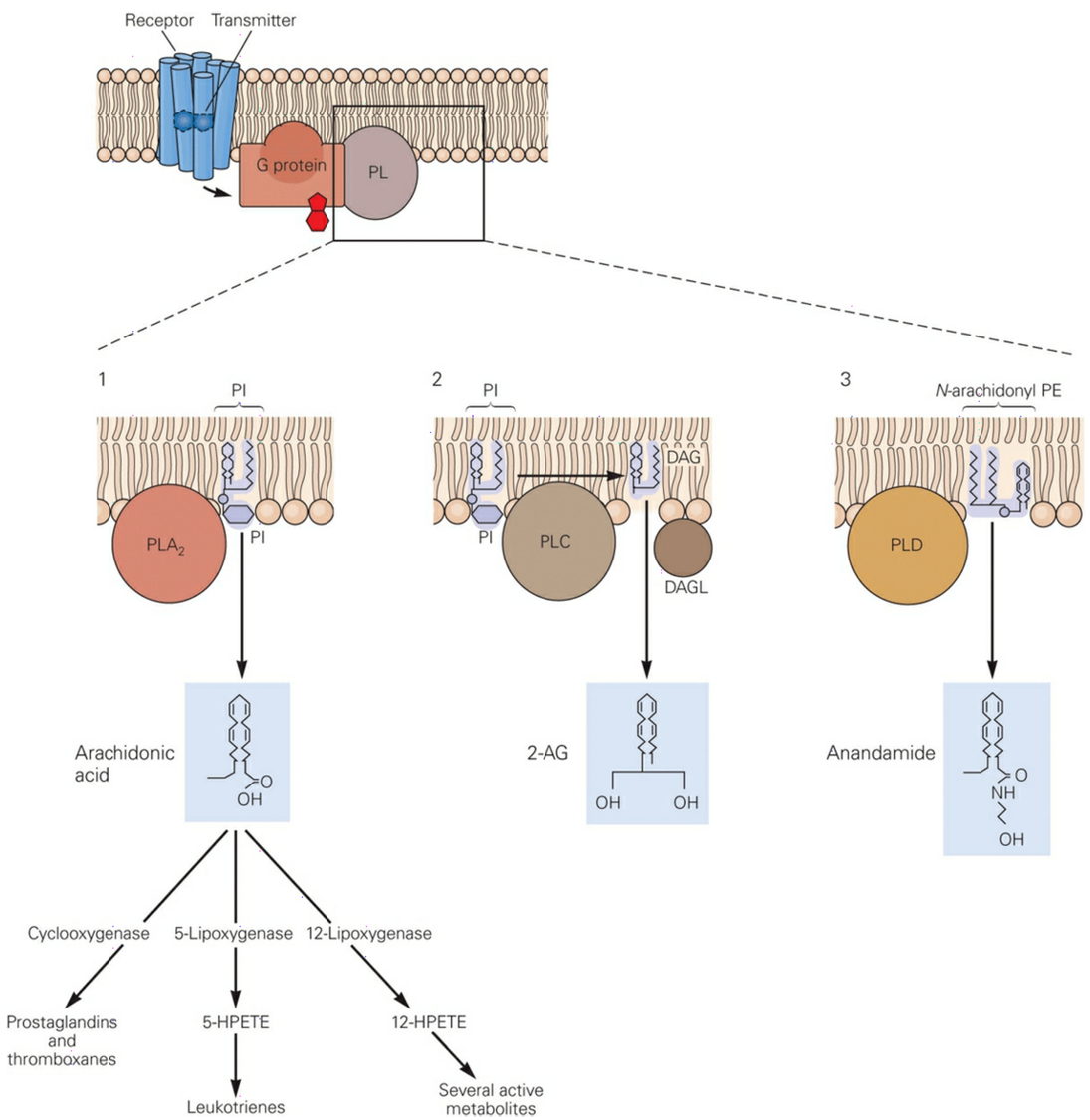


Figure 11-7 Three phospholipases generate distinct second messengers by hydrolysis of phospholipids containing arachidonic acid.

Pathway 1. Stimulation of G protein-coupled receptors leads to activation of phospholipase A_2 (PLA_2) by the free βγ subunit complex. Phospholipase A_2 hydrolyzes phosphatidylinositol (PI) in the plasma membrane, leading to the release of arachidonic acid, a 20-carbon fatty acid that is a component of many phospholipids. Once released, arachidonic acid is metabolized through several pathways, three of which are shown. The 12- and 5-lipoxygenase pathways both produce several active metabolites; the cyclooxygenase pathway produces prostaglandins and throm-

boxanes. Cyclooxygenase is inhibited by indomethacin, aspirin, and other nonsteroidal anti-inflammatory drugs. Arachidonic acid and many of its metabolites modulate the activity of certain ion channels.

Pathway 2. Other G proteins activate phospholipase C (PLC), which hydrolyzes PI in the membrane to generate DAG (see Figure 11-6). Hydrolysis of DAG by a second enzyme, diacyl-glycerol lipase (DAGL), leads to production of 2-arachidonyl-glycerol (2-AG), an endocannabinoid that is released from neuronal membranes and then activates G protein-coupled endocannabinoid receptors in the plasma membrane of other neighboring neurons.

Pathway 3. Elevation of intracellular Ca^{2+} activates phospholipase D (PLD), which hydrolyzes phospholipids that have an unusual polar head group containing arachidonic acid (*N*-arachidonylphosphatidylethanolamine [*N*-arachidonyl PE]). This action generates a second endocannabinoid termed anan-damide (arachidonylethanolamide). (HPETE, hydroperoxyeicosa-tetraenoic acid.)

Arachidonic acid and its metabolites are soluble in lipids and thus readily diffuse through membranes. Therefore, in addition to acting within the cell in which they are produced, these substances can act on neighboring cells, including a presynaptic neuron. In this way they act as transcellular synaptic messengers (discussed in the next section).

Synthesis of prostaglandins and thromboxanes in the brain is dramatically increased by nonspecific stimulation such as electroconvulsive shock, trauma, or acute cerebral ischemia (localized absence of blood). Many of the actions of prostaglandins are mediated in the plasma membrane by a family of G protein-coupled receptors. The members of this receptor family can activate or inhibit adenylyl cyclase or activate phospholipase C.

Box 11-1 Isoforms of Protein Kinase C

At least nine isoforms of protein kinase C (PKC) have been found in nervous tissue. Rather than having regulatory and catalytic functions in different subunits, like PKA, most PKC isoforms contain regulatory and catalytic domains in a single continuous polypeptide chain (see Figure 11-6A).

Two functionally interesting differences have thus far been found among these isoforms. The so-called major forms (α, β_I, β_{II}, and γ) all have a calcium-binding site and are activated by Ca^{2+} ions together with dia-cylglycerol. The minor forms (eg, Δ, ε, and ζ) lack the calcium-binding domain, and therefore their activity is independent of Ca^{2+}.

The second interesting difference is that, of the major forms, only PKCγ is activated by low concentrations of arachidonic acid, a membrane fatty acid, although all the isoforms respond to diacylglycerol or phorbol esters (plant toxins that bind to PKC and promote tumors).

With one exception, PKC isoforms also contain a site between the regulatory and catalytic domains that is sensitive to proteolysis. High levels of cytoplasmic Ca^{2+} can activate proteases that cleave PKC at this site, releasing a cytoplasmic form of PKC called protein kinase M (PKM). This protein fragment is constitutively active because it lacks the regulatory domain. As a result, elevations in Ca^{2+} can lead to prolonged activation of the kinase.

Long-lasting activation of PKC can also be produced through expression of the gene encoding PKCζ. This isoform is unique in that it lacks a regulatory domain and is therefore constitutively active. Expression of PKCζ produces persistent PKC activity in hippocampal neurons during the induction of long-term potentiation, which is thought to underlie certain forms of learning and memory in the hippocampus (see Chapter 67).

Lipoxygenases introduce an oxygen molecule into the arachidonic acid molecule, generating hydroper-oxyeicosatetraenoic acids (HPETEs). These metabolites are synthesized in response to depolarization of brain slices with high concentrations of extracellular K+, glutamate, or NMDA. The compounds 5-HPETE, 12-HPETE, and some of their downstream metabolites modulate certain ion channels. These metabolites may also be important in mediating pain sensation by activating transient receptor potential (TRP) ion channels in certain sensory neurons (see Chapter 5). They also can act as transcellular second messengers, a function that appears to be important for long-term synaptic changes in the hippocampus.

Transcellular Messengers Are Important for Regulating Presynaptic Function

Our understanding of the physiological importance of transcellular messengers is continuing to evolve. In addition to the lipoxygenase metabolites of arachi-donic acid, two other important classes of transcellular messengers are the endocannabinoids and gases, both of which readily diffuse through the membrane into the extracellular space.

Endocannabinoids Are Derived from Arachidonic Acid

In the early 1990s researchers identified two types of G protein-coupled receptors, CB1 and CB2, which bind with high affinity the active compound in marijuana, A^9-tetrahydrocannabinol (THC). Both classes of receptors are coupled to G_i and G_o types of G proteins.

The CB1 receptors are the most abundant type of G protein-coupled receptor in the brain and are found predominantly on axons and presynaptic terminals in the central and peripheral nervous systems. Activation of these receptors inhibits release of several types of neurotransmitters, including the inhibitory neurotransmitter GABA and the excitatory transmitter glutamate. The CB2 receptors are found mainly on lymphocytes, where they modulate the immune response.

The identification of the cannabinoid receptors led to the purification of their endogenous ligands, the endo-cannabinoids. Two major endocannabinoids have been identified. Anandamide (Sanskrit *ananda*, bliss) consists of arachidonic acid coupled to ethanolamine (arachidonyl-ethanolamide); 2-arachidonylglycerol (2-AG) consists of arachidonic acid esterified at the 2 position of glycerol. Both are produced by the enzymatic hydrolysis of phos-pholipids containing arachidonic acid, a process that is initiated either when certain G protein-coupled receptors are stimulated or the internal Ca^{2+} concentration is elevated (Figure 11–7). The two endocannabinoids bind to both CB1 and CB2 receptors.

Because the endocannabinoids are lipid metabolites that can diffuse through the membrane, they also can act as transcellular retrograde signals (Figure 11–8). Production of these metabolites is often stimulated in postsynaptic neurons by the increase in intracellular Ca^{2+} that results from postsynaptic excitation. Once produced, the endocannabinoids diffuse through the cell membrane to nearby presynaptic terminals, where they bind to CB1 receptors and inhibit transmitter release. In this manner the postsynaptic cell can control activity of the presynaptic neuron. There is now intense interest in understanding how the activation of these receptors in the brain leads to the various behavioral effects of marijuana.

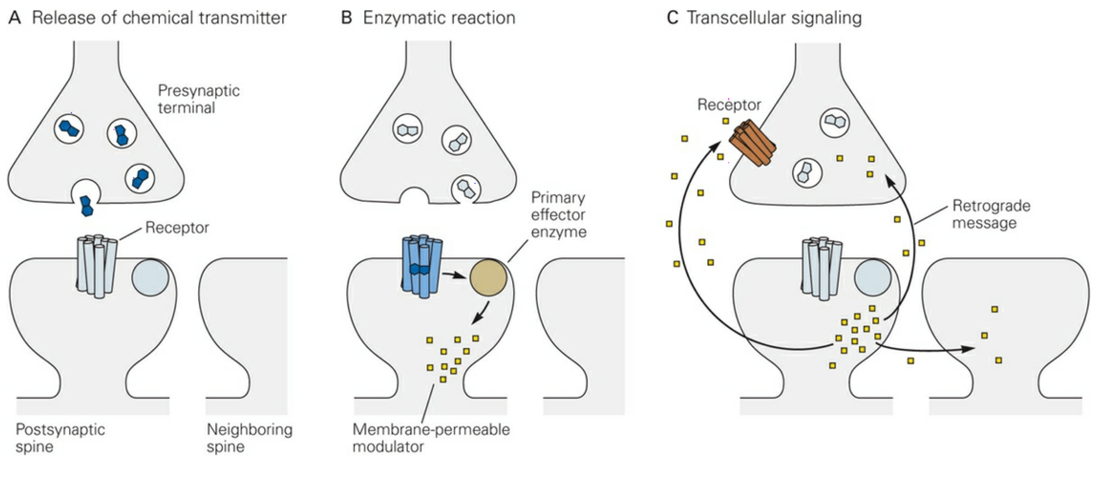


Figure 11-8 Transcellular signaling can occur from the postsynaptic neuron to the presynaptic neuron (retrograde transmission) and between postsynaptic cells. Until recently, synaptic signaling was thought to occur only from the presynaptic neuron to the postsynaptic cell. Transcellular signaling is initiated by a presynaptic signal. A presynaptic terminal releases a neurotransmitter at the synapse and that transmitter reacts with a G protein-coupled receptor in a postsynaptic dendritic spine (A). The receptor activates enzymes that produce a membrane-permeable modulator (B). The modulator is released from the postsynaptic spine and diffuses to neighboring postsy-naptic spines as well as presynaptic terminals (C). There it can produce either first-messenger effects, by acting on G protein-coupled receptors in the surface membrane, or second-messenger-like effects, by entering the cell to act within. This kind of modulator of the presynaptic terminal is called a *retrograde messenger* rather than a second messenger, and its action is called *transcellular signaling*.

The Gaseous Second Messengers, Nitric Oxide and Carbon Monoxide, Stimulate Cyclic GMP Synthesis

The two best studied gaseous transcellular messengers are nitric oxide (NO) and carbon monoxide (CO). Like other second messengers, NO and CO are not unique to neurons but operate in other cells of the body. For example, NO is a local hormone released from the endothelial cells of blood vessels, causing relaxation of the smooth muscle of vessel walls. Like the metabolites of arachidonic acid, NO and CO readily pass through cell membranes. They affect nearby cells without acting on a surface receptor, and they are extremely short-lived.

How do NO and CO produce their actions? Both gases stimulate the synthesis of guanosine 3′,5′-cyclic monophosphate (cyclic GMP or cGMP), which like cAMP is a cytoplasmic second messenger that activates a protein kinase. Specifically, NO and CO activate guanylyl cyclase, the enzyme that converts GTP to cGMP. There are two types of guanylyl cyclase. One is an integral membrane protein with an extracellular receptor domain and an intracellular catalytic domain that synthesizes cGMP. The other is cytoplasmic (soluble guanylyl cyclase) and is the isoform that is activated by NO.

Cyclic GMP has two major actions. It acts directly to open cyclic nucleotide-gated channels (important for phototransduction and olfactory signaling, as described in Chapters 26 and 32, respectively), and it activates the cGMP-dependent protein kinase (PKG). PKG differs from the cAMP-dependent protein kinase in that it is a single polypeptide with both regulatory (cGMP-binding) and catalytic domains, which are homologous to regulatory and catalytic domains in other protein kinases.

Cyclic GMP-dependent phosphorylation of proteins is prominent in Purkinje cells of the cerebellum, large neurons with copiously branching dendrites. There the cGMP cascade is activated by NO produced and released from the presynaptic terminals of granule cell axons (the parallel fibers) that make excitatory synapses onto the Purkinje cells. This increase in cGMP in the Purkinje neuron reduces the response of the AMPA receptors to glutamate, thereby depressing fast excitatory transmission at the parallel fiber synapse.

A Family of Receptor Tyrosine Kinases Mediates Some Metabotropic Receptor Effects

The receptor tyrosine kinases comprise the second major family of receptors that gate ion channels indirectly. These receptors are integral membrane proteins composed of a single subunit with an extracellular ligand-binding domain connected to a cytoplasmic region by a single transmembrane segment. The cyto-plasmic region contains a protein kinase domain that phosphorylates both itself (autophosphorylation) and other proteins on tyrosine residues (Figure 11–9). This phosphorylation results in the activation of a large number of proteins, including other kinases that are capable of acting on ion channels.

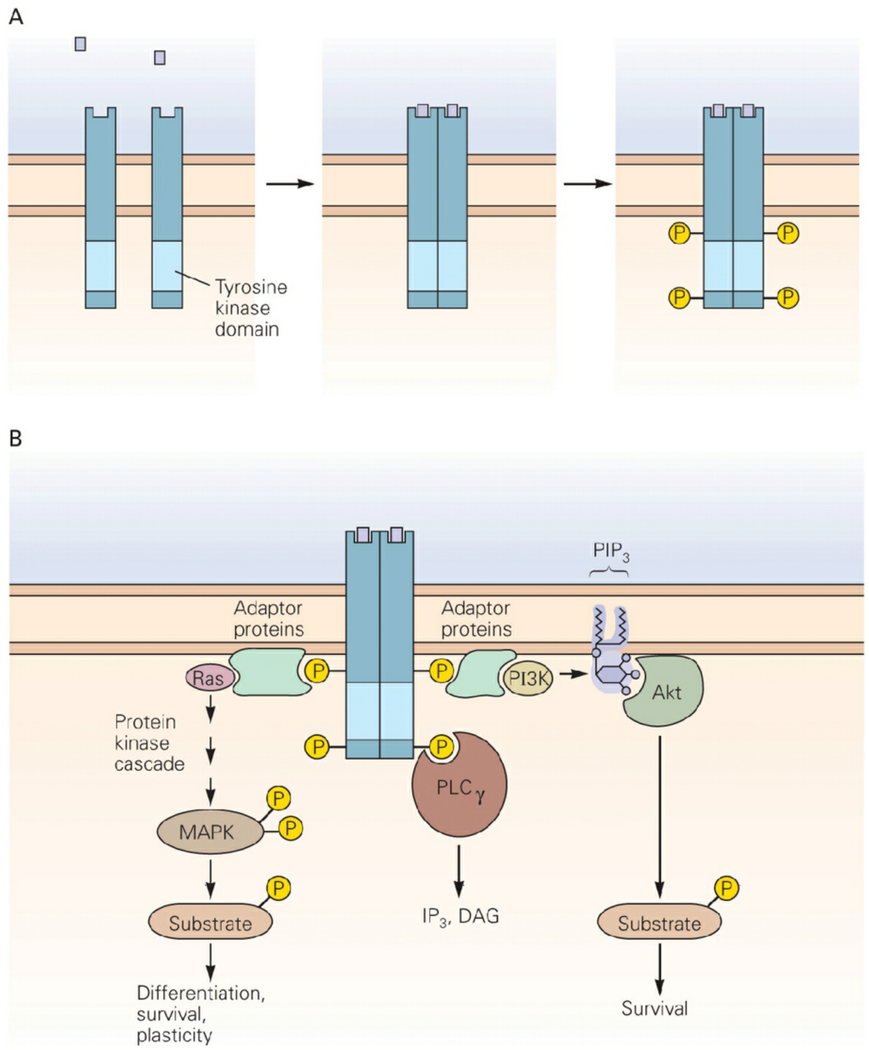


Figure 11-9 Receptor tyrosine kinases are a major class of metabotropic receptors.

A. Receptor tyrosine kinases are monomers in the absence of a ligand. The receptor contains a large extracellular binding domain that is connected by a single transmembrane segment to a large intracellular region that contains a catalytic tyrosine kinase domain. Ligand binding to the receptor often causes two receptor subunits to form dimers, enabling the enzyme to phosphorylate itself on various tyrosine residues on the cytoplasmic side of the membrane.

B. After the receptor is autophosphorylated, several downstream signaling cascades become activated through the binding of specific adaptor proteins to the receptor phosphotyrosine residues (P). The signaling cascade on the left illustrates the activation of mitogen-activated protein kinase (MAPK). A series of adaptor proteins recruits the small guanosine triphosphate (GTP)-binding protein Ras, which activates a protein kinase cascade, leading to the dual phosphorylation of MAP kinase on nearby threonine and tyrosine residues. The activated MAP kinase then phosphorylates substrate proteins on serine and threonine residues, including ion channels and transcription factors. Signaling pathways on the right illustrate the activation of the Akt protein kinase (also called PKB). Adaptor proteins first activate phosphoinositide 3-kinase (PI3K), which adds a phosphate group to PIP_2, yielding PIP_3, which then enables Akt activation. In yet another pathway, phospholipase Cγ becomes activated on binding to a different phosphotyrosine residue, providing a mechanism for producing inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) that does not rely on G proteins. (PLCγ, phospholipase Cγ.)

The ligands for the receptor tyrosine kinases are peptide hormones, including epidermal growth factor (EGF), fibroblast growth factor (FGF), nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), and insulin. Cells also contain important nonreceptor cytoplasmic tyrosine kinases, such as the protooncogene *src*. These nonreceptor tyrosine kinases are often activated by interactions with receptor tyro-sine kinases and are important in regulating growth and development.

Many (but not all) of the receptor tyrosine kinases exist as monomers in the plasma membrane in the absence of ligand. Ligand binding causes one receptor subunit to associate with another to form a homodimer thereby activating the intracellular kinase. Each monomer phosphorylates its counterpart at a tyrosine residue, an action that enables the kinase to phosphorylate other proteins. Like the serine and threonine protein kinases, tyrosine kinases regulate the activity of neuronal proteins they phosphorylate, including the activity of certain ion channels. Tyrosine kinases also activate an isoform of phospholipase C, phospholipase Cγ, which like PLC_β, cleaves PIP_2 into IP_3 and diacylglycerol.

Receptor tyrosine kinases initiate cascades of reactions involving several adaptor proteins and other protein kinases that often lead to changes in gene transcription. The mitogen-activated protein kinases (MAPKs) are an important group of serine-threonine kinases that can be activated by a signaling cascade initiated by receptor tyrosine kinase stimulation. MAP kinases are activated by cascades of protein-kinase reactions (kinase kinases), each cascade specific to one of three types of MAP kinase: extracellular signal regulated kinase (ERK), p38 MAP kinase, and *c-Jun* N-terminal kinase (JNK).

MAP kinase signaling cascades are initiated when a specific adaptor protein binds to a phosphotyrosine residue on the cytoplasmic tail of an activated receptor tyrosine kinase. The binding is mediated by the SH2 domain of the adaptor protein, named for the domain's homology to a region of src. A second adaptor protein domain, SH3 (named for its homology to another region of src), binds to proline-rich regions of effector proteins, thereby coupling the activated receptor to the effector. The signaling complex ultimately binds to a small monomeric GTP-binding protein (MW 20,000-40,000), such as Ras or one of its relatives. Ras was first identified because it acts as a protooncogene. These small GTP-binding proteins are distantly related to the α-subunit of the heterotrimeric G proteins discussed earlier in this chapter.

Ras becomes active following the exchange of a bound molecule of GDP for GTP, similar to the activation of trimeric G proteins. The activated Ras protein then initiates a cascade of reactions involving two upstream kinase kinases that lead to the phosphorylation and activation of MAP kinase. Activated MAP kinases have several important actions. They translocate to the nucleus where they turn on gene transcription by phosphorylating certain transcription factors. This action is thought

to be important in stabilizing long-term memory formation. MAP kinases also phosphor-ylate cytoplasmic and membrane proteins to produce short-term modulatory actions (Figure 11–9).

The Physiological Actions of Ionotropic and Metabotropic Receptors Differ

Second-Messenger Cascades Can Increase or Decrease the Opening of Many Types of Ion Channels

The structural differences between metabotropic and ionotropic receptors are reflected in their functional effects (Table 11-1). Metabotropic receptor actions are much slower than ionotropic ones. The physiological action of the two classes of receptors also differs.

Table 11-1 Comparison of Synaptic Excitation Produced by the Opening and Closing of Ion Channels

	Ion channels involved	Effect on total membrane conductance	Contribution to action potential	Time course	Second messenger	Nature of synaptic action
EPSP caused by opening of channels	Non selective cation channel	Increase	Triggers action potential	Usually fast (milliseconds)	None	Mediating
EPSP caused by closing of channels	K^+ channel	Decrease	Modulates action potential	Slow (seconds or minutes)	Cyclic AMP (or other second messengers)	Modulating

Ionotropic receptors regulate channels that function as simple on-off switches, those whose main job is either to excite a neuron to fire an action potential or to inhibit the neuron from firing. Because these channels are normally confined to the postsynaptic region of the membrane, the action of ionotropic receptors is local. Conversely, metabotropic receptors, because they activate diffusible second messengers, can act on channels some distance from the receptor. As a result, metabo-tropic receptors regulate a variety of channel types, including resting channels, voltage-gated channels that generate the action potential or provide Ca^{2+} influx for neurotransmitter release, and ligand-gated channels.

Finally, whereas transmitter binding always leads to an increase in the opening of ionotropic receptor-channels, the activation of metabotropic receptors can lead to either an increase or a decrease in channel opening. For example, MAP kinase phosphorylation of an inactivating (A-type) K + channel in the dendrites of hippocampal pyramidal neurons decreases the K+ current magnitude, thereby enhancing dendritic action potential firing.

The slow synaptic actions of metabotropic receptors normally are insufficient to cause a cell to fire an action potential. But they can greatly influence elec-trophysiological properties of a neuron. By acting on resting and voltage-gated channels in the neuron's cell body and dendrites, metabotropic receptor actions can alter resting potential, input resistance, length and time constants, threshold potential, action potential duration, and repetitive firing characteristics. By acting on channels in axon terminals and the postsynaptic membrane, metabotropic receptors can also modulate, respectively, neurotransmitter release and the opening of ionotropic receptor-channels, thereby regulating synaptic transmission. These various actions of metabotropic receptors are referred to as *modulatory synaptic actions* (Figure 11–10).

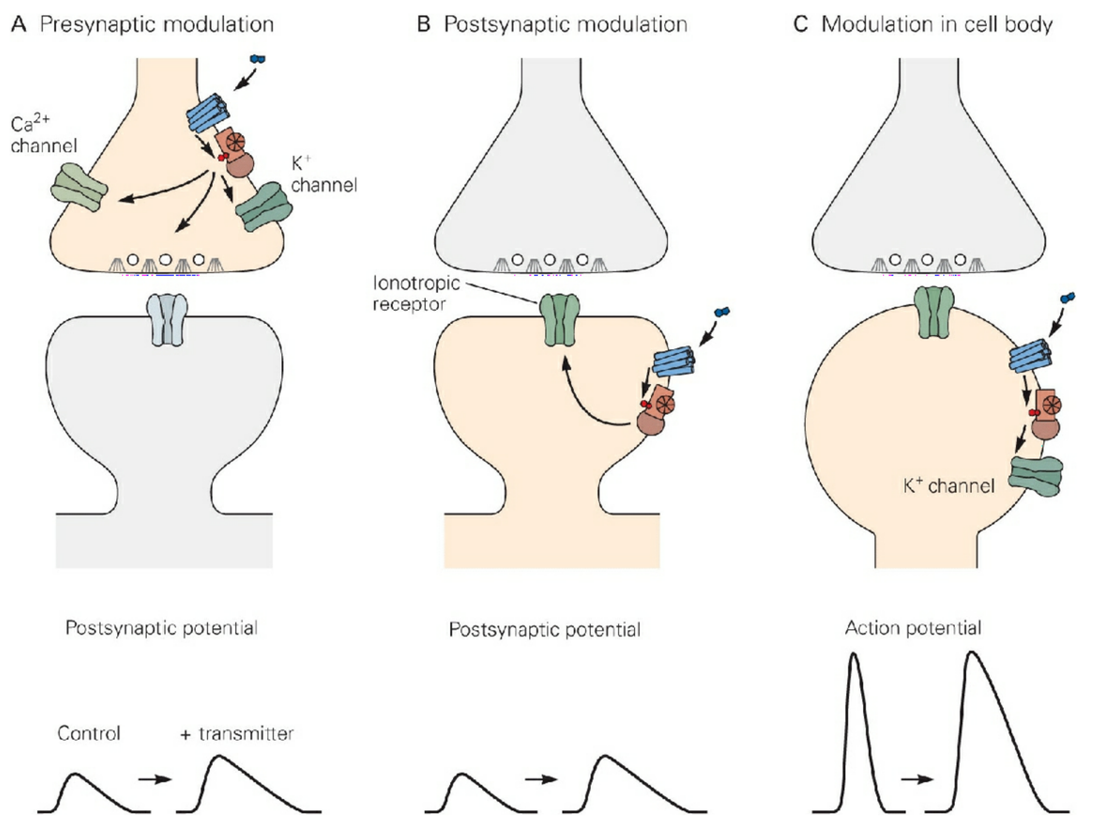


Figure 11-10 The modulatory actions of second messengers can occur at three cellular sites.

A. In the presynaptic neuron second messengers can modulate the activity of K^+, and Ca^{2+} channels, as well as the transmitter release machinery, to regulate the efficacy of transmitter release and thus the size of the fast postsynaptic potential mediated by ionotropic receptors.

B. In the postsynaptic neuron second messengers can alter directly the amplitude of postsynaptic potentials by modulating ionotropic receptors.

C. Second messengers can also affect the function of resting and voltage-gated channels in the soma and dendrites, thus altering a variety of electrical properties of the cell, including resting potential, input resistance, length and time constants, threshold, and action potential duration (as illustrated here).

The distinction between direct and indirect regulation of ion channels is nicely illustrated by cholinergic synaptic transmission in autonomic ganglia. Stimulation of the presynaptic nerve releases ACh from the nerve terminals. This directly opens nicotinic ACh receptor-channels, producing a fast EPSP in the postsynaptic neuron. The fast EPSP is followed by a slow EPSP that takes approximately 100 ms to develop but which then lasts for several seconds. The slow EPSP is produced by the activation of metabotropic muscarinic ACh receptors that close a delayed-rectifier K + channel called the muscarine-sensitive (or M-type) K^+ channel. These voltage-gated channels are partially activated when the cell is at rest, and the current through them helps determine the cell resting potential and input resistance (Figure 11–11).

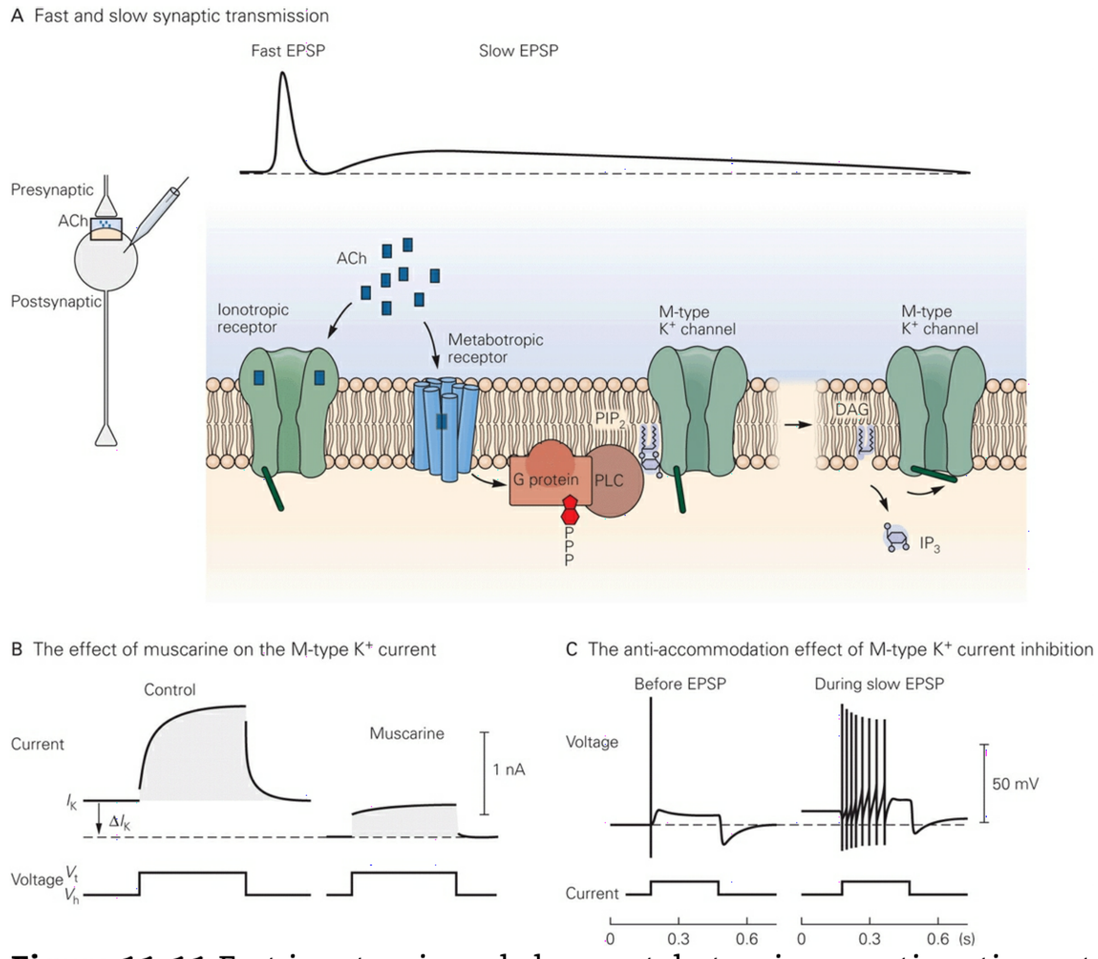


Figure 11-11 Fast ionotropic and slow metabotropic synaptic actions at autonomic ganglia.

A. The release of ACh onto a postsynaptic neuron in autonomic ganglia produces a fast excitatory postsynaptic potential (EPSP) followed by a slow EPSP[1] The fast EPSP is produced by activation of ionotropic nicotinic ACh receptors. The slow EPSP is produced by activation of metabotropic muscarinic ACh receptors. This receptor stimulates PLC to hydrolyze PIP_2, yielding IP_3 and DAG. The decrease in PIP_2 causes the closure of the M-type delayed-rectifier K+ channel.

B. Voltage clamp recordings indicate that ACh decreases the magnitude of the current carried by the M-type K+ channel. The depolarization of the membrane from a negative holding potential (V_h, typically -60 mV) to a more positive test potential (V_v *t* typically -30 mV) normally causes a slow increase in outward K+ current ($/_K$) as the M-type K+

channels activate (control). Application of muscarine, a plant alkaloid that selectively stimulates the muscarinic ACh receptor, decreases the outward K^+ current at the holding potential (note the shift in base line current, $\Delta/_K$) by closing the M-type K+ channels that are open at rest. The functional loss of M-type channels also decreases the magnitude of the slowly activated K^+ current in response to the step depolarization. (Adapted, with permission, from Adams et al. 1986.)

C. In the absence of muscarinic ACh receptor stimulation, the neuron fires only a single action potential in response to a prolonged depolarizing current stimulus, a process termed accommodation (left). This is because the slow activation of the M-type K^+ channel repolarizes the membrane below threshold. When the same current stimulus is applied during a slow EPSP, the neuron fires a more sustained train of action potentials (right) because the decrease in M-type current decreases the extent to which the membrane repolarizes during the stimulus. (Adapted, with permission, from Adams et al. 1986.)

The M-type K^+ channel is distinguished from other delayed-rectifier K + channels by its slow activation. It requires several hundred milliseconds to fully activate on depolarization. Closure of the M-type channels in response to muscarinic stimulation causes a decrease in K+ efflux from the cell at the resting potential. As a result, K+ efflux no longer balances the Na+ influx through resting channels, and the net influx of Na+ leads to membrane depolarization (Figure 11–11).

How far will the membrane depolarize? Membrane depolarization decreases the inward driving force on Na^+, and increases the outward driving force on K^+, such that the net inward current decreases. Thus the membrane will depolarize until the decrease in K^+ conductance (resulting from the closure of the M-type channels) is offset by the increase in the outward driving force on K^+, and the decrease in the inward driving force on Na^+ (as a result of the depolarization). At this new steady-state membrane potential the outward K^+ current and inward Na^+ current are again in balance.

Although the magnitude of the slow EPSP caused by closure of the M-type K^+ channels is relatively modest, the decrease in K^+ conductance profoundly increases action potential firing in response to a fast excitatory input. What are the special properties of the slow EPSP that produce this effect? First, the depolarization resulting from the reduction in resting K^+ conductance drives the membrane closer to threshold. Second, the increase in input resistance decreases the amount of excitatory current necessary to depolarize the cell by a given voltage. Third, the reduction in the delayed K+ current enables the cell to produce a more sustained firing of action potentials in response to a prolonged depolarizing stimulus.

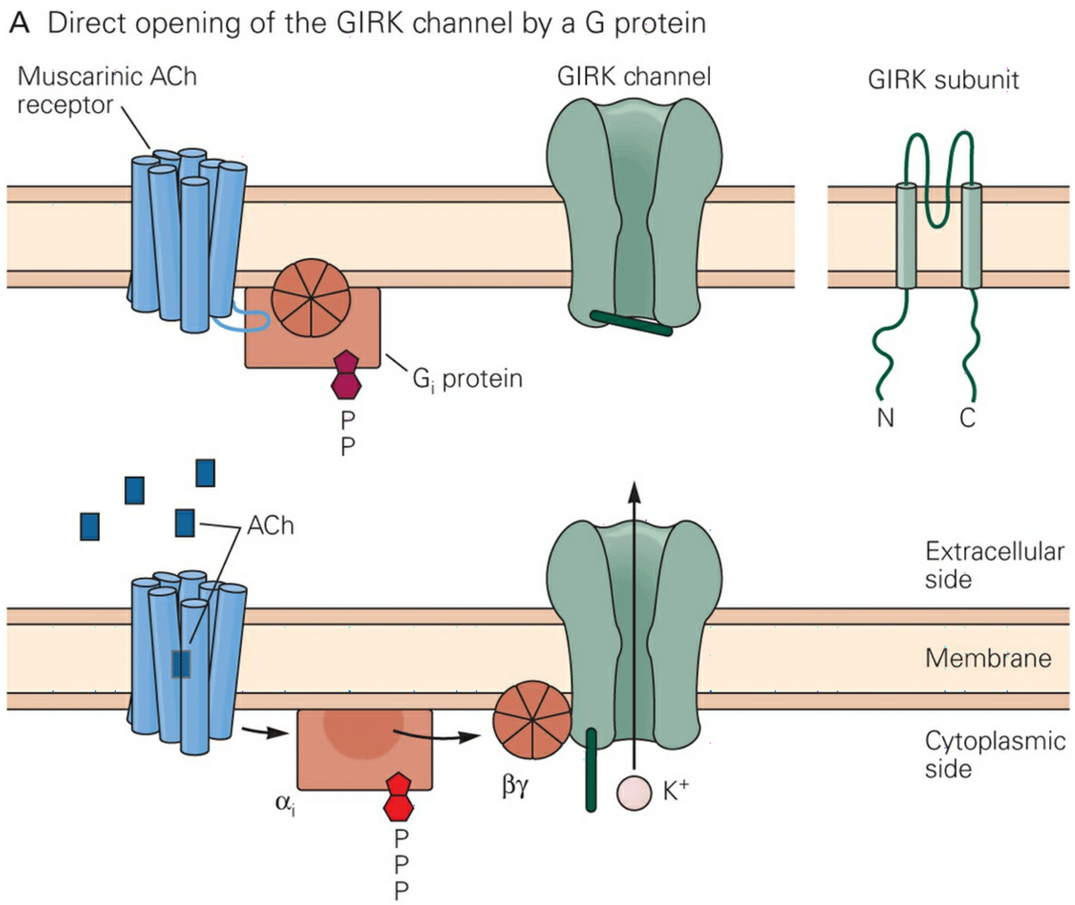
In the absence of ACh, a ganglionic neuron normally fires only one or two action potentials and then stops firing in response to prolonged excitatory stimulation that is just above threshold. This process, termed *accommodation*, results in part from the increase in M-type K+ current in response to the prolonged depolarization, which helps repolarize the membrane below threshold. As a result, if the same prolonged stimulus is applied during a slow EPSP (when the M-type K+ channels are closed), the neuron remains depolarized above threshold during the entire stimulus and thus fires a prolonged burst of impulses, a process termed *anti-accommodation* (Figure 11-11C). As this modulation by ACh illustrates, the M-type K^+ channels do more than help set the resting potential—they also control excitability.

A G protein mediates the modulation of the M-type channel by muscarinic receptor activation. However, until recently the target effector of the G protein remained a mystery. Although a number of lines of evidence implicated the PIP_2 hydrolysis pathway, neither PKC nor IP_3 seemed to play a role in regulating M-type channel opening. It is now known that PIP_2 is a necessary cofactor required for the proper function of many types of channels, including the M-type channels. As a result, stimulation of muscarinic ACh receptors that activate phospholipase C leads to closure of the M-type channels because of the decrease in PIP_2 levels in the membrane. Thus, M-type channels are regulated by the degradation of a substance rather than the synthesis of a chemical messenger. How PIP_2 binding enables proper functioning of the M-type channels remains unknown.

Although we do not know precisely the molecular mechanisms regulating M-type channels, we do have a more detailed understanding of how effectors in other types of modulatory actions regulate channel function. We shall first describe the simplest mechanism, the direct gating of ion channels by G proteins, and then consider a more complex mechanism dependent on protein phosphorylation by PKA.

G Proteins Can Modulate Ion Channels Directly

The simplest mechanism for activating an ion channel is direct gating of an ionotropic receptor-channel, as when ACh binds and opens the nicotinic ACh receptor-channel. The simplest mechanism for the indirect gating of a channel is when a metabotropic receptor releases a G protein subunit that directly interacts with the channel to modify its opening. This mechanism is used to gate two kinds of ion channels: the G protein-gated inward-rectifier K+ channel (GIRK) and a voltage-dependent Ca^{2+} channel. With both kinds of channels, the G protein's βγ complex is the mediator (Figure 11–12A).



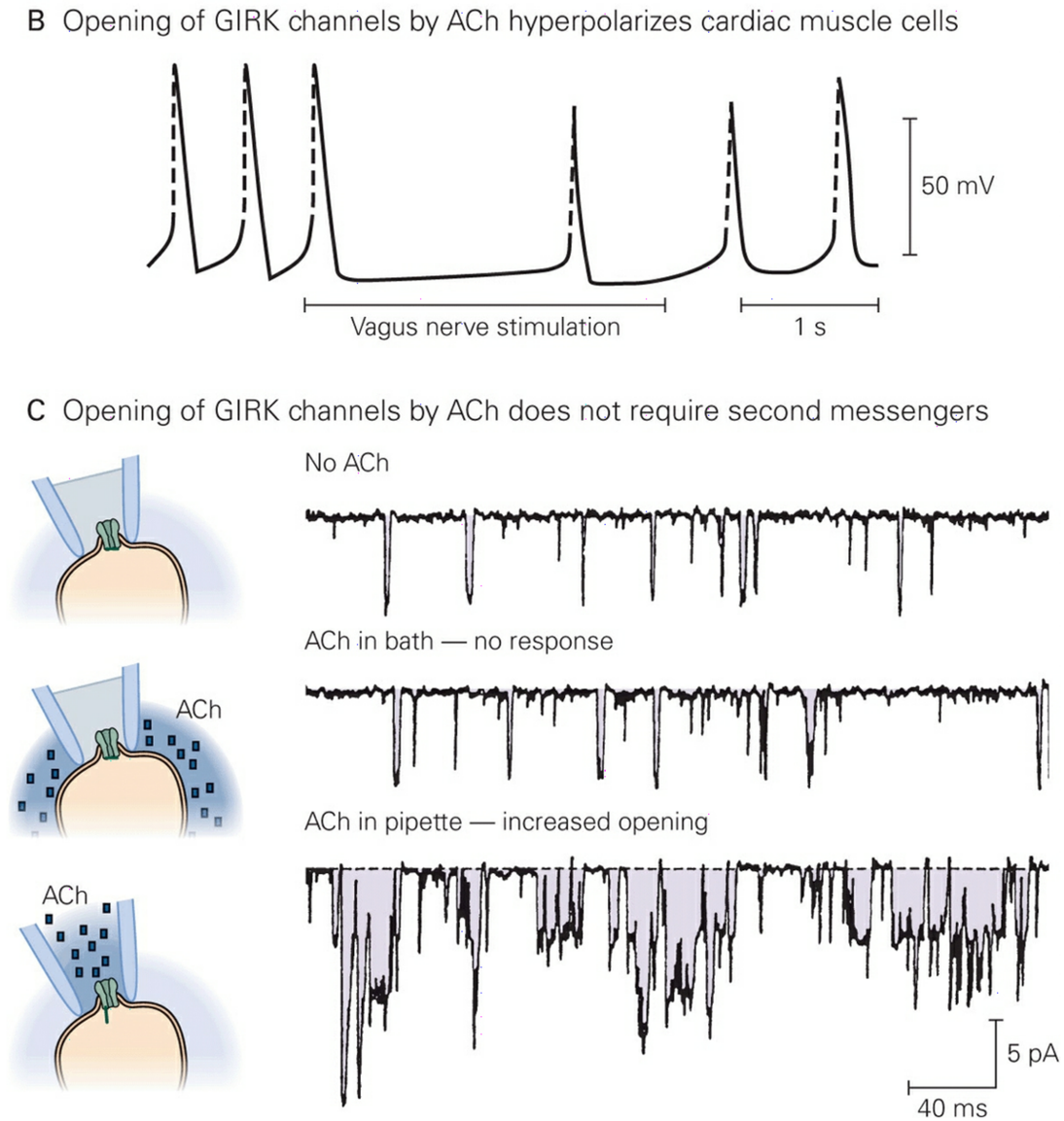


Figure 11-12 Some G proteins open ion channels directly without employing second messengers.

A. An inward-rectifying K+ channel (GIRK) is opened directly by a G protein. Binding of ACh to a muscarinic receptor causes the dissociation of the G_i protein $\alpha_i\beta\gamma$-complex; the free βγ-subunits bind to a cytoplasmic domain of the channel, causing the channel to open.

B. Stimulation of the parasympathetic vagus nerve releases ACh, which acts at muscarinic receptors to open GIRK channels in cardiac muscle

cell membranes. The current through the GIRK channel hyperpolarizes the cells, thus helping to slow the heart rate. (Adapted, with permission, from Toda and West 1967.)

C. Three single-channel records show that opening of GIRK channels does not involve a freely diffusible second messenger. In this experiment the pipette contained a high concentration of K+, which makes E_K less negative. As a result, when GIRK channels open, they generate brief pulses of inward (downward) current. In the absence of ACh, channels open briefly and infrequently (top record). Application of ACh in the bath (outside the pipette) does not increase channel opening in the patch of membrane under the pipette (middle record). The ACh must be in the pipette to activate the channel (bottom record). This is because the free βγ-subunits, released by the binding of ACh to its receptor, remain tethered to the membrane near the receptor and can only activate nearby channels. The subunits are not free to diffuse to the channels under the patch pipette. (Reproduced, with permission, from Soejima and Noma 1984.)

The GIRK channel, like other inward-rectifier channels, passes current more readily in the inward than the outward direction, although in physiological situations K+ current is always outward. As we learned in Chapter 5, inward-rectifier channels resemble a truncated voltage-gated K^+ channel in having two transmembrane regions connected by a P-region loop that forms the selectivity filter in the channel (see Figure 5-12).

In the 1920s Otto Loewi described how the release of ACh in response to stimulation of the vagus nerve slows the heart rate (Figure 11-12B). We now know that the ACh activates muscarinic receptors to stimulate G protein activity, which directly opens the GIRK channel. For many years this transmitter action was puzzling because it has properties of both ionotropic and metabotropic receptor actions. The time course of activation of the K^+ current following release of ACh is slower (50 to 100 ms rise time) than that of ionotropic receptors (submillisecond rise time). However, the rate of K^+ channel activation is much faster than that of second-messenger-mediated actions that depend on protein phosphorylation (which can take many seconds to turn on). Although biochemical and electrophysi-ological studies clearly demonstrated that a G protein was required for this action, patch-clamp experiments showed that the G protein did not trigger production of a diffusible second messenger (Figure 11-12C). These findings were reconciled when it was found that activation of the muscarinic receptors releases the G protein's βγ-subunits, which directly open the K+ channel.

Activation of GIRK channels hyperpolarizes the membrane in the direction of E_K (-80 mV). In certain classes of spontaneously active neurons the outward K^+ current through these channels acts predominantly to decrease the neuron's intrinsic firing rate, opposing the slow depolarization caused by excitatory pacemaker currents carried by the hyperpolarization-acti-vated, cyclic nucleotide-regulated (HCN) channels (see Chapter 7). Because GIRK channels are activated by neurotransmitters, they provide a means for regulating the firing rate of excitable cells. These channels are regulated in a wide variety of neurons by a large number of transmitters and neuropeptides that act on different G protein-coupled receptors to activate either G_i or G_o, thereby releasing β_γ subunits.

Several G protein-coupled receptors also act to inhibit the opening of certain voltage-gated Ca^{2+} channels, again as a result of the direct binding to the channel of the βγ complex of G_i or G_o. Because Ca^{2+} influx through voltage-gated Ca^{2+} channels normally has a depolarizing effect, the dual action of G protein βγ-subunits—Ca^{2+} channel inhibition and K+ channel activation—strongly inhibits neuronal firing. As we will see in Chapter 12, inhibition of voltage-gated Ca^{2+} channels in presynaptic terminals through G protein βγ-subunits can suppress the release of neurotransmitter, a process termed *presynaptic inhibition*.

Cyclic AMP-Dependent Protein Phosphorylation Can Close Potassium Channels

In the marine mollusk *Aplysia*, stimulation of certain interneurons results in the release of the transmitter serotonin. This produces a slow EPSP in a group of mechanoreceptor sensory neurons. These sensory neurons initiate defensive withdrawal reflexes in response to tactile stimuli through fast, excitatory synapses with motor neurons. Serotonin sensitizes this reflex, enhancing the animal's response to a stimulus, resulting in a simple form of learning (see Chapter 66).

The modulatory action of serotonin depends on its binding to a G

protein-coupled receptor that activates a G_s protein, which elevates cAMP and thus activates PKA. This leads to the direct phosphorylation and subsequent closure of the serotonin-sensitive (or S-type) K^+ channel that acts as a resting channel (Figure 11–13). Like the closing of the M-type K + channel with ACh, closure of the S-type K^+ channel decreases K^+efflux from the cell, thereby depolarizing the cell and decreasing its resting membrane conductance.

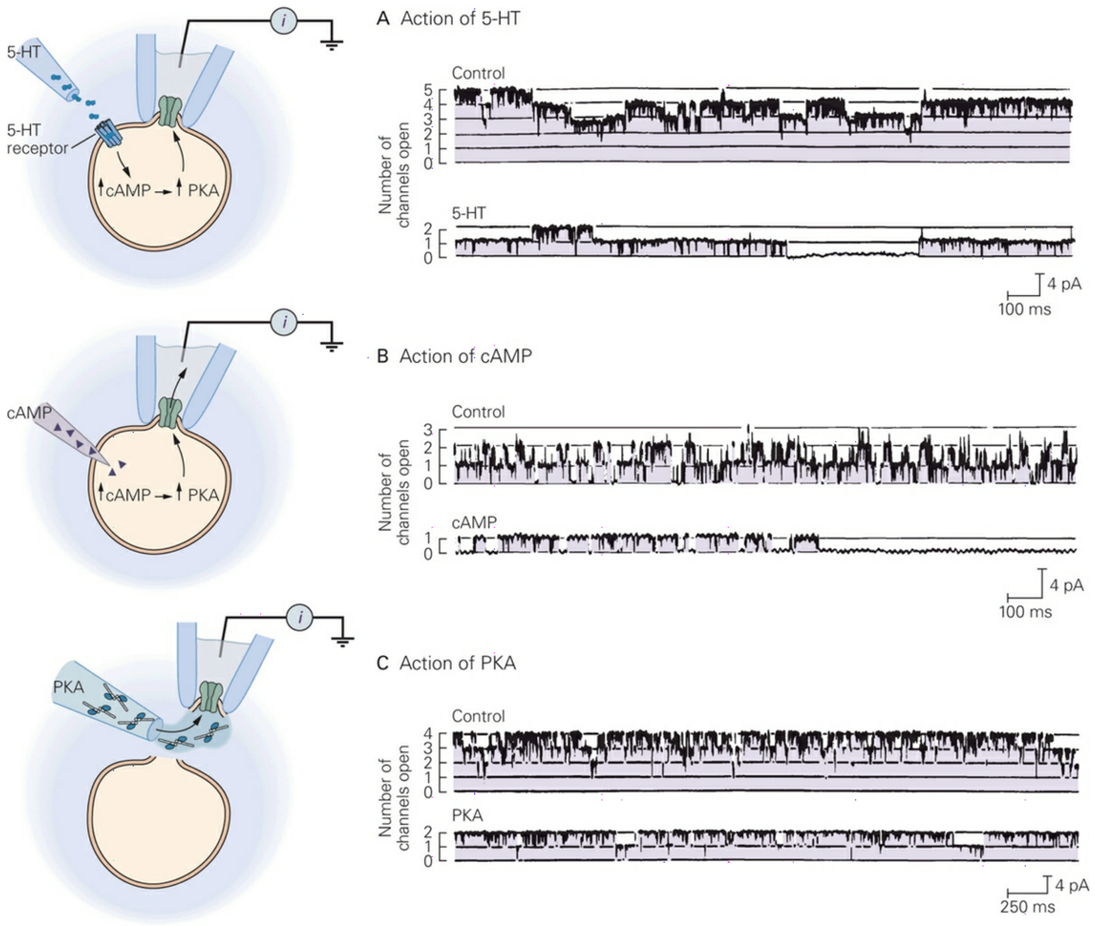


Figure 11-13 Serotonin closes a K+ channel through the diffusible second-messenger cAMP. Serotonin (5-HT) produces a slow excitatory postsynaptic potential (EPSP) in *Aplysia* sensory neurons by closing the serotonin-sensitive or S-type K+ channels. The 5-HT receptor is coupled to G_s, which stimulates adenylyl cyclase. The increase in cAMP activates cAMP-dependent protein kinase A (PKA), which phosphorylates the S-type channel, leading to its closure. Single-channel recordings illustrate the actions of 5-HT, cAMP and PKA on the S-type channels.

A. Addition of 5-HT to the bath closes three of five S-type K+ channels active in this cell-attached patch of membrane. The experiment implicates a diffusible messenger, as the 5-HT applied in the bath has no direct access to the S-type channels in the membrane under the pipette. Each channel opening contributes an outward (positive) current pulse. (Adapted, with permission, from Siegelbaum, Camardo, and Kandel 1982.)

B. Injection of cAMP into a sensory neuron through a micro-electrode closes all three active S-type channels in this patch. The bottom trace shows the closure of the final active channel in the presence of cAMP. (Adapted, with permission, from Siegelbaum, Camardo, and Kandel 1982.)

C. Application of the purified catalytic subunit of PKA to the cytoplasmic surface of the membrane closes two out of four active S-type K^+ channels in this cell-free patch. ATP was added to the solution bathing the inside surface of the membrane to provide the source of phosphate for protein phosphorylation. (Adapted, with permission, from Shuster et al. 1985.)

The opening of the same S-type channels can be enhanced by the neuropeptide FMRFamide, acting through 12-lipoxygenase metabolites of arachidonic acid. This enhanced K^+ channel opening leads to a slow hyperpolarizing IPSP associated with an increase in resting membrane conductance. Thus a single channel can be regulated in opposing ways by distinct second-messenger pathways that produce opposing effects on neuronal excitability. A resting K^+ channel with two pore-forming domains in each subunit (TREK) in mammalian neurons is also dually regulated by PKA and arachidonic acid in a manner very similar to the dual regulation of the S-type channel in *Aplysia*.

Synaptic Actions Mediated by Phosphorylation Are Terminated by Phosphoprotein Phosphatases

Synaptic actions mediated by phosphorylation are terminated by phosphoprotein phosphatases, enzymes that remove phosphoryl groups

from proteins, thereby generating inorganic phosphate. One class of phosphatases dephosphorylates proteins at serine or threonine residues and hence can reverse the actions of PKA, PKC, and Ca^{2+}/calmodulin kinase (Figure 11–14). A second class dephos-phorylates proteins at tyrosine residues. Finally, a third group is specific for the pair of adjacent phosphorylated residues that mediate activation of MAP kinases.

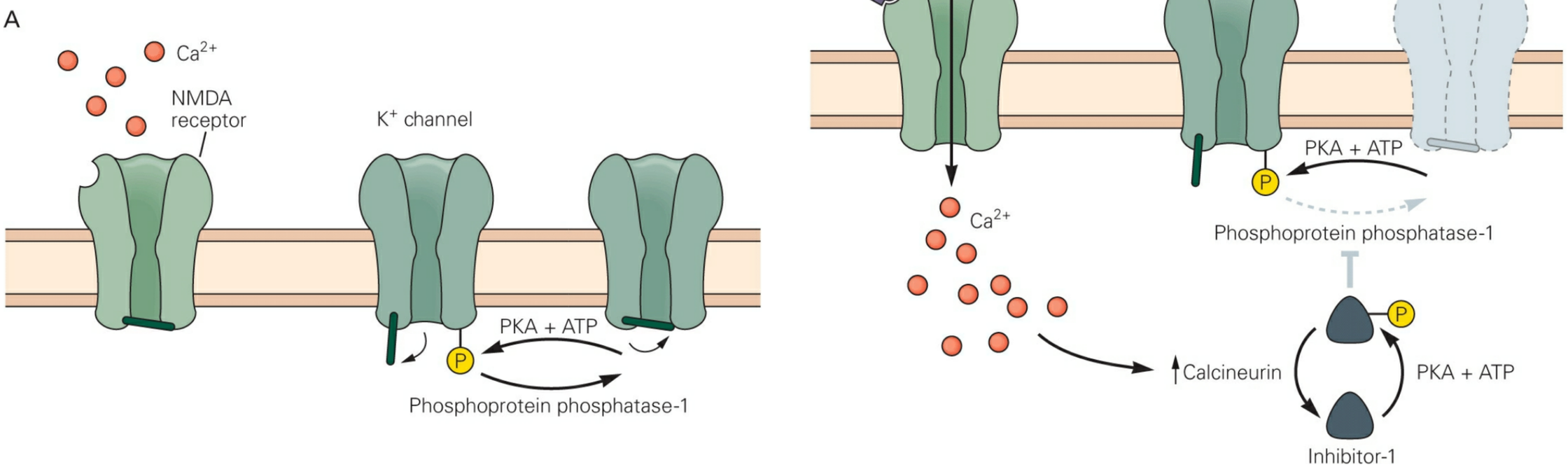


Figure 11-14 Phosphoprotein phosphatases end the actions of protein kinases.

A. The forward rate of phosphorylation of substrate proteins (here a K+ channel) is controlled by protein kinases, the reverse rate by phosphoprotein phosphatases.

B. The extent and duration of phosphorylation can be controlled by regulation of phosphatase activity through a protein called inhibitor-1. When inhibitor-1 is phosphorylated by cAMP-dependent protein kinase (PKA), it binds to and blocks the activity of phosphoprotein phosphatase-1. The extent of phosphorylation of inhibitor-1 is controlled by another phosphatase, calcineurin, which is activated by the Ca^{2+}/calmodulin complex. In this manner Ca^{2+} entering the cell through the *N*-methyl-D-aspartate (NMDA)-type glutamate receptors activates calcineurin and triggers dephosphorylation of inhibitor-1. This in turn leads to the disinhibition of phosphoprotein phosphatase-1, which

then dephosphorylates many substrates, including the K+ channel. (ATP, adenosine triphosphate; P, phosphate; Glu, glutamate.) (Adapted, with permission, from Halpain, Girault, and Greengard 1990.)

Phosphatase activity can be regulated by different mechanisms. One of the major serine-threonine phos-phatases in neurons, phosphatase-1, is under the control of a regulatory protein called inhibitor-1. Inhibitor-1 binds to and inhibits phosphatase-1 only after the inhibitor has itself been phosphorylated by PKA (Figure 11–14). An increase in cAMP therefore has two effects that enhance levels of protein phosphorylation: It increases the rate of phosphorylation by activating PKA and decreases the rate of dephosphorylation by inhibiting phosphatase-1.

Another serine-threonine phosphatase, calcineurin, is activated in response to an increase in the concentration of Ca^{2+} inside a cell. The Ca^{2+} binds to calmodulin and the Ca^{2+}/calmodulin complex then activates the phosphatase. One of the important functions of cal-cineurin is to dephosphorylate inhibitor-1. In neurons of the basal ganglia, Paul Greengard and colleagues showed that dopamine (acting through metabotropic D_1 receptors and cAMP production) activates PKA, which in turn phosphorylates inhibitor-1 (called DARPP-32 in these cells). The resulting inhibition of phosphatase-1 leads to an overall enhancement of phosphorylation in the neuron. However, if NMDA receptors are activated by release of glutamate, the resultant Ca^{2+} influx can stimulate calcineurin. This leads to the dephosphor-ylation of inhibitor-1, which relieves the inhibition of phosphatase-1, resulting in a decrease in overall levels of phosphorylation in the basal ganglion neurons. As we shall learn later in Chapter 67, a similar calcineurin cascade is thought to underlie a long-lasting depression of synaptic transmission in the hippocampus.

Second Messengers Can Endow Synaptic Transmission with Long-Lasting Consequences

So far we have described how synaptic second messengers alter the biochemistry of neurons for periods of time lasting seconds to minutes. Second messengers can also effect long-term changes lasting days to weeks as a result of alterations in a cell's expression of specific genes (Figure 11–15). Such changes in gene expression result from the ability of second-messenger cascades to control the activity of transcription factors, regulatory proteins that control mRNA synthesis.

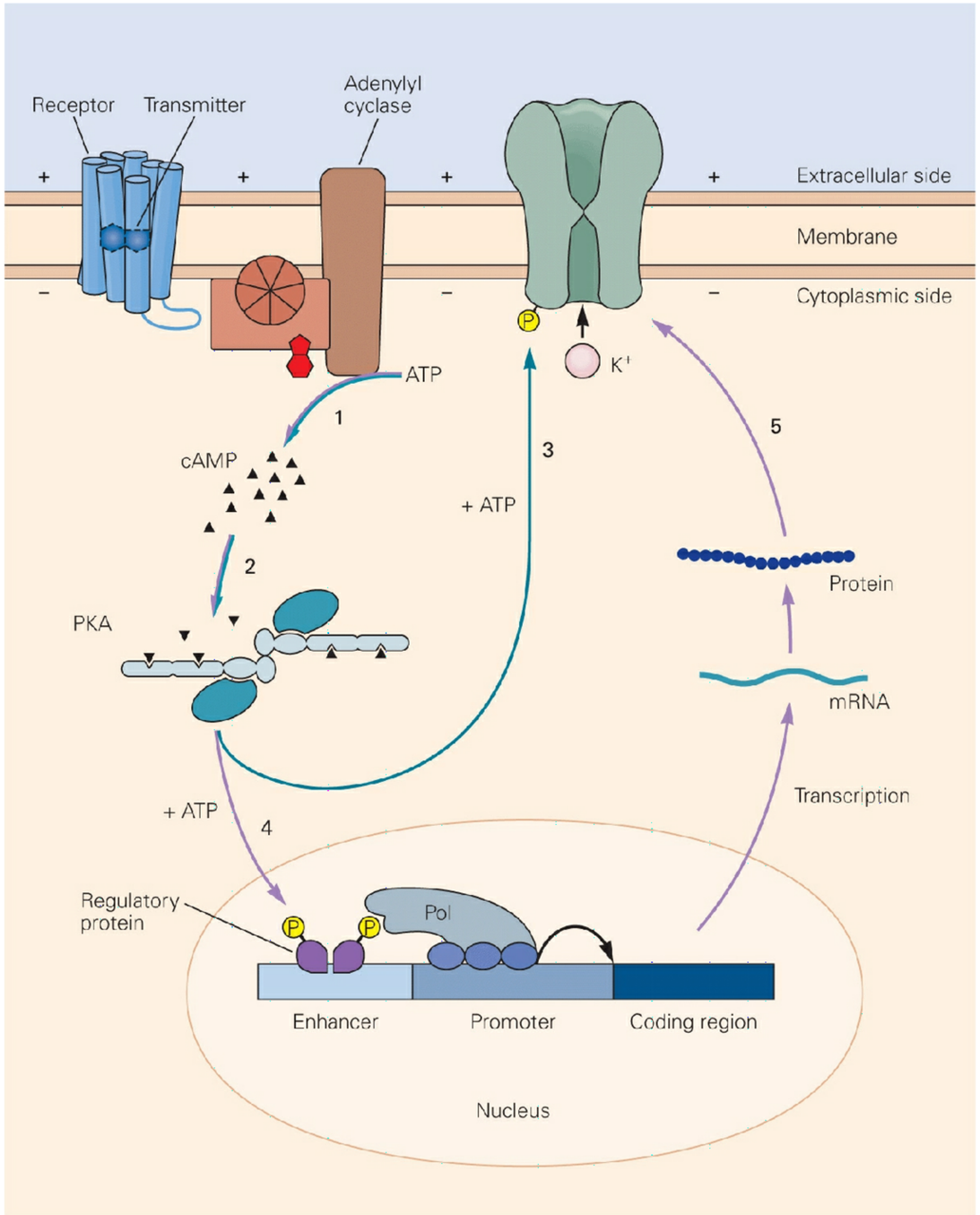


Figure 11-15 A single neurotransmitter can have either short-term or long-term effects on an ion channel. In this example a short exposure to

transmitter activates the cAMP second-messenger system (1), which in turn activates PKA (2). The kinase phosphorylates a K+ channel; this produces a synaptic potential that lasts for several minutes and modifies the excitability of the neuron (3). With sustained activation of the receptor, the kinase translocates to the nucleus, where it phosphorylates one or more transcription factors that turn on gene expression (4). As a result of the new protein synthesis, the synaptic actions are prolonged—closure of the channel and changes in neuronal excitability last days or longer (5). (Pol, polymerase.)

The activity of some transcription factors can be directly regulated by phosphorylation. For example, a transcription factor termed the cAMP response element-binding protein (CREB) is activated when phosphorylated by PKA, Ca^{2+}/calmodulin-dependent protein kinases, PKC, or MAP kinases. Once activated, CREB enhances transcription by binding a component of the transcription machinery, the CREB-binding protein (CBP). CBP activates transcription by recruiting RNA polymerase II and by functioning as a his-tone acetylase, adding acetyl groups to certain histone lysine residues. The acetylation weakens the binding between histones and DNA, which opens up the chromatin structure and enables specific genes to be transcribed. The changes in transcription and chromatin structure are important for regulating neuronal development, as well as for long-term learning and memory (see Chapters 66 and 67).

An Overall View

Signaling between neurons occurs when neurotransmitters bind to their postsynaptic receptors. Two distinct classes of receptors, ionotropic and metabotropic, differ widely in biochemical mechanism, duration of action, and physiological function.

Binding of transmitter to an ionotropic receptor directly opens an ion channel that is part of the receptor macromolecule. These ligand-gated receptor-channels produce the fastest and briefest type of syn-aptic action, lasting only a few milliseconds. This fast synaptic transmission mediates most motor actions and sensory processing.

Longer-lasting effects of transmitters are mediated by two major types of metabotropic receptors: G protein-coupled receptors and receptor tyrosine kinases. G protein-coupled receptors are proteins with seven transmembrane segments. They are members of a large gene superfamily and all act through G proteins, either to activate second-messenger cascades or directly alter ion channel activity. Prominent second messengers are cAMP and the products of hydrolysis of phospholipids: IP_3, diacylglycerol, and arachidonic acid.

Many second-messenger actions involve phosphorylation of a variety of target proteins, including ion channels, thereby changing the functional state of the channels. These second-messenger actions generally last from seconds to minutes and thus do not mediate rapid behaviors. Rather they modulate the strength and efficacy of fast synaptic transmission—by modulating transmitter release or the responsiveness of ionotropic receptors to their ligand—or the electrical excitability of postsynaptic cells. Second-messenger actions not only open ion channels, as do the fast syn-aptic actions, but also close channels that are normally open in the absence of transmitter, thereby decreasing membrane conductance.

These modulatory actions are important in producing emotional states, mood, arousal, and simple forms of learning and memory. Many neurological and psychiatric disorders, including Parkinson disease, depression, anxiety, and schizophrenia, are thought to involve alterations in metabotropic receptor-dependent forms of synaptic transmission. Drugs that act to enhance or depress metabotropic receptor activation are important for treating these diseases.

The longest-lasting effects of neurotransmitters involve changes in gene expression, changes that can persist for days or longer. These more permanent actions are mediated by many of the same types of receptors and second-messenger pathways that operate in the shorter-term modulatory actions of transmitters. The long-term processes, however, may require repeated stimulation of the receptors and more prolonged action of the second messengers. As we shall see in Chapters 66 and 67, synaptically induced changes in gene expression are critical for long-term memory storage.

Steven A. Siegelbaum
David E. Clapham
James H. Schwartz

Selected Readings

Berridge MJ. 2009. Inositol trisphosphate and calcium signalling mechanisms. Biochim Biophys Acta 1793:933-940.

Brown DA, Passmore GM. 2009. Neural KCNQ (Kv7) channels. Br J Pharmacol 156:1185-1195.

Greengard P. 2001. The neurobiology of slow synaptic transmission. Science 294:1024-1030.

Hernandez CC, Zaika O, Tolstykh GP, Shapiro MS. 2008. Regulation of neural KCNQ channels: signalling pathways, structural motifs and functional implications. J Physiol 586:1811-1821.

Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M. 2009. Endocannabinoid-mediated control of synaptic transmission. Physiol Rev 89:309-380.

Levitan IB. 1999. Modulation of ion channels by protein phosphorylation. How the brain works. Adv Second Messenger Phosphoprotein Res 33:3-22.

Milligan G, Kostenis E. 2006. Heterotrimeric G-proteins: a short history. Br J Pharmacol 147 (Suppl 1):S46-55.

Nishizuka Y. 2003. Discovery and prospect of protein kinase C research: epilogue. J Biochem 133:155-158.

Schwartz JH. 2001. The many dimensions of cAMP signaling. Proc Natl Acad Sci USA 98:13482-13484.

Suh BC, Hille B. 2008. PIP2 is a necessary cofactor for ion channel function: how and why? Annu Rev Biophys 37:175-195.

References

Adams PR, Jones SW, Pennefather P, Brown DA, Koch C, Lancaster B. 1986. Slow synaptic transmission in frog sympathetic ganglia. J Exp Biol 124:259-285.

Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD. 1994. *Molecular Biology of the Cell*, 3rd ed. New York: Garland.

Carnegie GK, Means CK, Scott JD. 2009. A-kinase anchoring proteins: from protein complexes to physiology and disease. IUBMB Life 61:394-406.

Chen Z, Gibson TB, Robinson F, Silvestro L, Pearson G, Xu B, Wright A, Vanderbilt C, Cobb MH. 2001 MAP kinases. Chem Rev 101:2449-2476.

Fantl WJ, Johnson DE, Williams LT. 1993. Signalling by receptor tyrosine kinases. Annu Rev Biochem 62:453-481.

Francis SH, Corbin JD. 1994. Structure and function of cyclic nucleotide-dependent protein kinases. Annu Rev Physiol 56:237-272.

Frielle T, Kobilka B, Dohlman H, Caron MG, Lefkowitz RJ. 1989. The β-adrenergic receptor and other receptors coupled to guanine nucleotide regulatory proteins. In: S Chien (ed). *Molecular Biology in Physiology*, pp. 79-91. New York: Raven.

Greenberg SM, Castellucci VF, Bayley H, Schwartz JH. 1987. A molecular mechanism for long-term sensitization in *Aplysia*. Nature 329:62-65.

Halpain S, Girault JA, Greengard P. 1990. Activation of NMDA receptors induces dephosphorylation of DARPP-32 in rat striatal slices. Nature 343:369-372.

Logothetis DE, Kurachi Y, Galper J, Neer EJ, Clapham DE. 1987. The βγ subunits of GTP-binding proteins activate the muscarinic K^+ channel in heart. Nature 325:321-326.

Murphy RC, Fitzpatrick FA (eds). 1990. Arachidonate related lipid mediators. Methods Enzymol 187:1-683.

Needleman P, Turk J, Jakschik BA, Morrison AR, Lefkowith JB. 1986. Arachidonic acid metabolism. Annu Rev Biochem 55:69-102.

Osten P, Valsamis L, Harris A, Sacktor TC. 1996. Protein synthesis-dependent formation of protein kinase Mzeta in long-term potentiation. J Neurosci 16:2444-2451.

Pfaffinger PJ, Martin JM, Hunter DD, Nathanson NM, Hille B. 1985. GTP-binding proteins couple cardiac muscarinic receptors to a K channel. Nature 317:536-538.

Piomelli D, Volterra A, Dale N, Siegelbaum SA, Kandel ER, Schwartz JH, Belardetti F. 1987. Lipoxygenase metabolites of arachidonic acid as second messengers for presynaptic inhibition of *Aplysia* sensory cells. Nature 328:38-43.

Shuster MJ, Camardo JS, Siegelbaum SA, Kandel ER. 1985. Cyclic AMP-dependent protein kinase closes the serotonin-sensitive K+ channels of Aplysia sensory neurones in cell-free membrane patches. Nature 313:392-395.

Siegel GJ, Agranoff BW, Albers RW, Molinoff PB (eds). 1994. *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*, 7th ed. Amsterdam: Elsevier.

Siegelbaum SA, Camardo JS, Kandel ER. 1982. Serotonin and cyclic AMP close single K^+ channels in *Aplysia* sensory neurones. Nature 299:413-417.

Soejima M, Noma A. 1984. Mode of regulation of the ACh-sensitive K-channel by the muscarinic receptor in rabbit atrial cells. Pflugers Arch 400:424-431.

Takai Y, Sasaki T, Matozaki T. 2001. Small GTP-binding proteins. Physiol Rev 81:153-208.

Tedford HW, Zamponi GW. 2006. Direct G protein modulation of Cav2 calcium channels. Pharmacol Rev 58:837-862.

Toda N, West TC. 1967. Interactions of K, Na, and vagal stimulation in the S-A node of the rabbit. Am J Physiol 212:416-423.

Wayman GA, Lee YS, Tokumitsu H, Silva AJ, Soderling TR. 2008. Calmodulin-kinases: modulators of neuronal development and plasticity. Neuron 59:914-931.