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 reticulum, 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+} channel that spans the reticulum membrane; however, cytoplasmic Ca^{2+}, not IP_3, opens the ryanodine receptor-channel.

Calcium often acts by binding to the small cytoplasmic protein calmodulin. An important function of the calcium/calmodulin complex is to activate *calcium/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 calcium/calmodulin complex is absent, the C-terminal regulatory domain of the kinase binds and inactivates the catalytic portion. Binding to the calcium/calmodulin complex causes conformational changes of the kinase molecule that unfetter the catalytic domain for action (Figure 14–4B). Once activated, CaM kinase can phosphorylate itself through intramolecular reactions at many sites in the molecule. Autophosphorylation has an important functional effect: It converts the enzyme into a form that is independent of calcium/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 that underlie long-term changes in synaptic function associated with certain forms of memory. In addition to the persistent activation of calcium/calmodulin-dependent protein kinase, PKA can also become persistently active following a prolonged increase in cAMP because of a slow enzymatic degradation of free 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 through the expression of a PKC isoform that lacks a regulatory domain. Finally, the duration of phosphorylation can be enhanced by certain proteins that act to inhibit the activity of phosphoprotein phosphatases. One such protein, inhibitor-1, inhibits phosphatase activity only when the inhibitor is itself phosphorylated by PKA.

Receptor Tyrosine Kinases Compose the Second Major Family of Metabotropic Receptors

The *receptor tyrosine kinases* represent a distinct family of receptors from the G protein–coupled receptors. The receptor tyrosine kinases 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 cytoplasmic region contains a protein kinase domain that phosphorylates both itself (autophosphorylation) and other proteins on tyrosine residues (Figure 14–5A). This phosphorylation results in the activation of a large number of proteins, including other kinases that are capable of acting on ion channels.

Receptor tyrosine kinases are activated when bound by 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 tyrosine 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 two monomeric receptor subunits to form a dimer, 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 PLCβ cleaves PIP_2 into IP_3 and DAG.

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 (MAP kinases) are an important group of serine-threonine kinases that can be activated by a signaling cascade initiated by receptor tyrosine kinase. 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). Activated MAP kinases have several important actions. They translocate to the nucleus where they

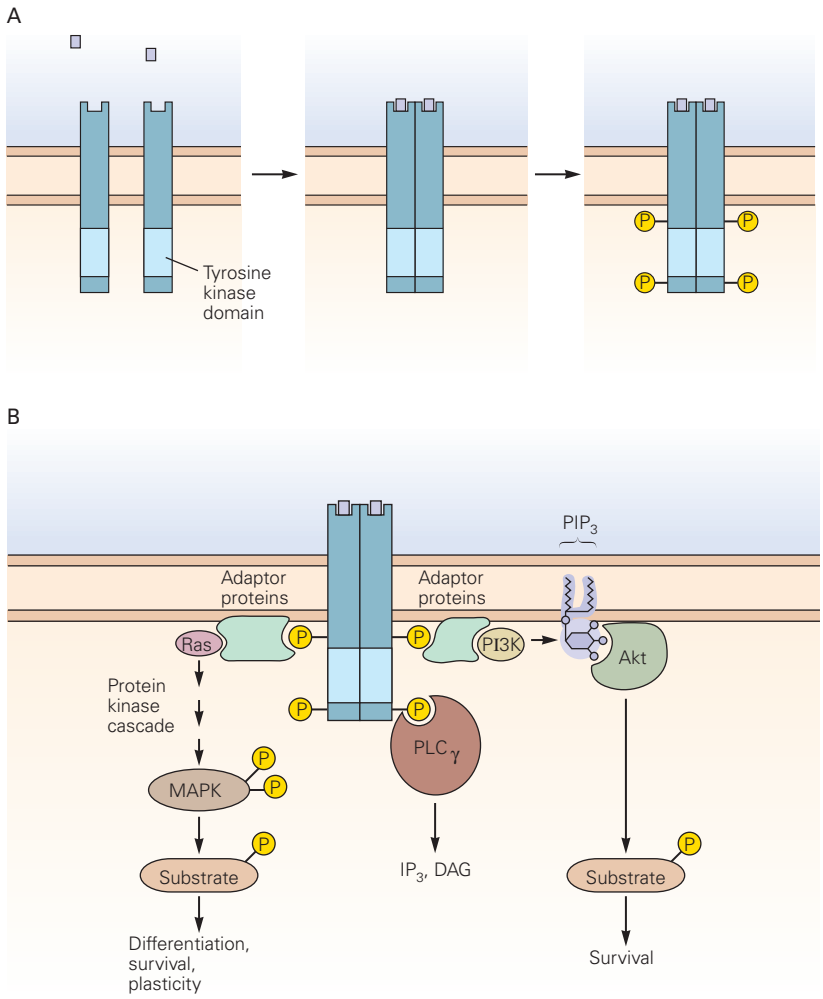

Figure 14–5 Receptor tyrosine kinases.

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**). *Left:* 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. *Center:* Phospholipase C_γ (**PLC_γ**) 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. *Right:* 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.

turn on gene transcription by phosphorylating certain transcription factors. This action is thought to be important in stabilizing long-term memory formation (Chapters 53 and 54). MAP kinases also phosphorylate cytoplasmic and membrane proteins to produce short-term modulatory actions (Figure 14–5B).

Several Classes of Metabolites Can Serve as Transcellular Messengers

The metabolic products we have considered so far in response to metabotropic receptor actions do not readily cross the cell membrane. As a result, they act as true intracellular second messengers: They only affect the cell that produces them. However, cells can also synthesize metabolites that are lipid soluble and so can both act on the cell that produces them and diffuse across the plasma membrane to affect neighboring cells. We refer to such molecules as transcellular messengers.

Although these molecules have some functional resemblance to neurotransmitters, they differ in a number of important ways. They are not contained within vesicles and are not released at specialized synaptic contacts. They often do not act on membrane receptors but cross the plasma membrane of neighboring cells to reach intracellular targets. And their release and actions are much slower than those at fast synapses. We will consider three broad classes of transcellular messengers: the cyclooxygenase and lipoxygenase metabolites of the lipid molecule arachidonic acid, the endocannabinoids, and the gas nitric oxide.

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

Phospholipase A_2 hydrolyzes phospholipids that are distinct from PIP_2, 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 other metabolites; and (3) the cytochrome P450 complex, which oxidizes arachidonic acid itself as well as cyclooxygenase and lipoxygenase metabolites (Figure 14–6). 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 flow). These metabolites can all be released by the cell that synthesizes them and thus act as transcellular signals. Many of the actions of prostaglandins are mediated by acting in the plasma membrane on a family of G protein–coupled receptors. The members of this receptor family can, in turn, activate or inhibit adenylyl cyclase or activate phospholipase C.

Endocannabinoids Are Transcellular Messengers That Inhibit Presynaptic Transmitter Release

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, Δ^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 both the central and peripheral nervous systems. Activation of these receptors inhibits release of several types of neurotransmitters, including both GABA and 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 *endocannabinoids*. Two major endocannabinoids have been identified; both contain an arachidonic acid moiety and bind to both CB1 and CB2 receptors. *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 phospholipids 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 14–6). However, whereas 2-AG is synthesized in nearly all neurons, the sources of anandamide are less well characterized.

Because the endocannabinoids are lipid metabolites that can diffuse through the membrane, they function as transcellular signals that act on neighboring cells, including presynaptic terminals. 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.

The Gaseous Second Messenger Nitric Oxide Is a Transcellular Signal That Stimulates Cyclic GMP Synthesis

Nitric oxide (NO) acts as a transcellular messenger in neurons as well as in other cells of the body. The modulatory function of NO was discovered through its action as 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 readily passes through cell membranes and can affect nearby cells without acting on a surface receptor. Nitric oxide is a free radical and so is highly reactive and short-lived.

Nitric oxide produces many of its actions by stimulating 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 activates 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 activated by NO. In some instances, NO is thought

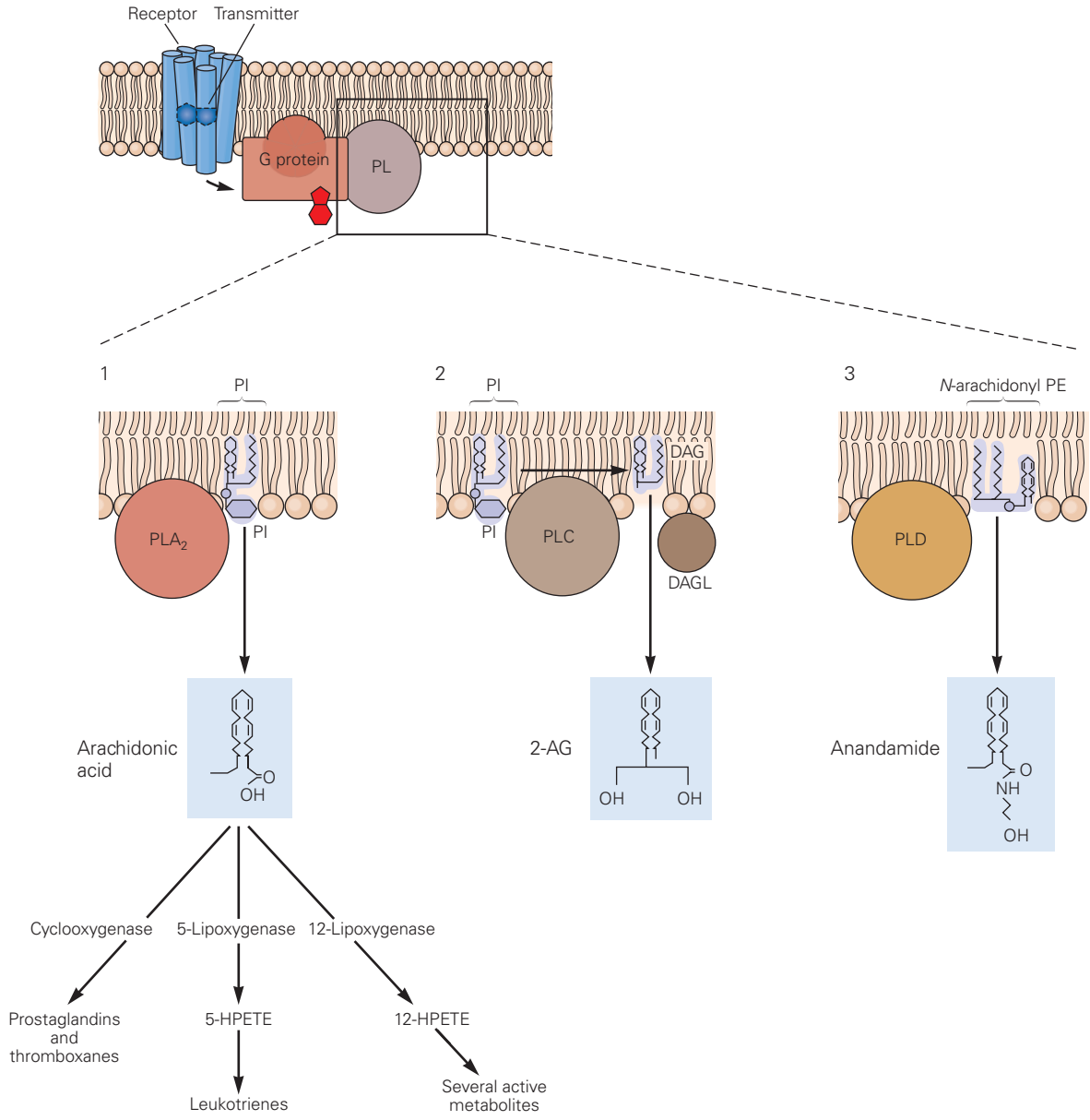

Figure 14–6 Three phospholipases generate distinct second messengers by hydrolysis of phospholipids containing arachidonic acid.

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 with four double bonds 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 thromboxanes. 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. (**HPETE**, hydroperoxyeicosatetraenoic acid.)

Pathway 2. Other G proteins activate phospholipase C (**PLC**), which hydrolyzes PI in the membrane to generate DAG (see Figure 14–4). Hydrolysis of DAG by a second enzyme, diacylglycerol lipase (**DAGL**), leads to production of 2-arachidonylglycerol (**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 anandamide (arachidonylethanolamide).

to act directly by modifying sulfhydryl groups on cysteine residues of various proteins, a process termed nitrosylation.

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 22 and 29, respectively), and it activates the *cGMP-dependent protein kinase* (PKG), which like PKA phosphorylates substrate proteins on certain serine or threonine residues. PKG differs from the PKA 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. It also phosphorylates a distinct set of substrates from PKA.

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.

The Physiological Actions of Metabotropic Receptors Differ From Those of Ionotropic Receptors

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

The functional differences between metabotropic and ionotropic receptors reflect the differences in their properties. For example, metabotropic receptor actions are much slower than ionotropic ones (Table 14–1). The physiological actions of the two classes of receptors also differ.

Ionotropic receptors are channels that function as simple on-off switches; their main job is either to excite a neuron to bring it closer to the threshold for firing or inhibit the neuron to decrease its likelihood to fire. Because these channels are normally confined to the postsynaptic region of the membrane, the action of ionotropic receptors is local. Metabotropic receptors, on the other hand, because they activate diffusible second messengers, can act on channels some distance from the receptor. Moreover, metabotropic receptors regulate a variety of channel types, including resting channels, ligand-gated channels, and voltage-gated channels that generate action potentials, underlie pacemaker potentials, and provide Ca^{2+} influx for neurotransmitter release.

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

The binding of transmitter to metabotropic receptors can greatly influence the electrophysiological properties of a neuron (Figure 14–7). Metabotropic receptors in a presynaptic terminal can alter transmitter release by regulating either Ca^{2+} influx or the efficacy of the synaptic release process itself (Figure 14–7A). Metabotropic receptors in the postsynaptic cell can influence the strength of a synapse by modulating the ionotropic receptors that mediate the postsynaptic potential (Figure 14–7B). By acting on resting and voltage-gated channels in the postsynaptic neuron's cell body, dendrites, and axon, metabotropic receptor actions can also alter the resting potential, membrane resistance, length and time constants, threshold potential, action potential

Table 14–1 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	Nonselective 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

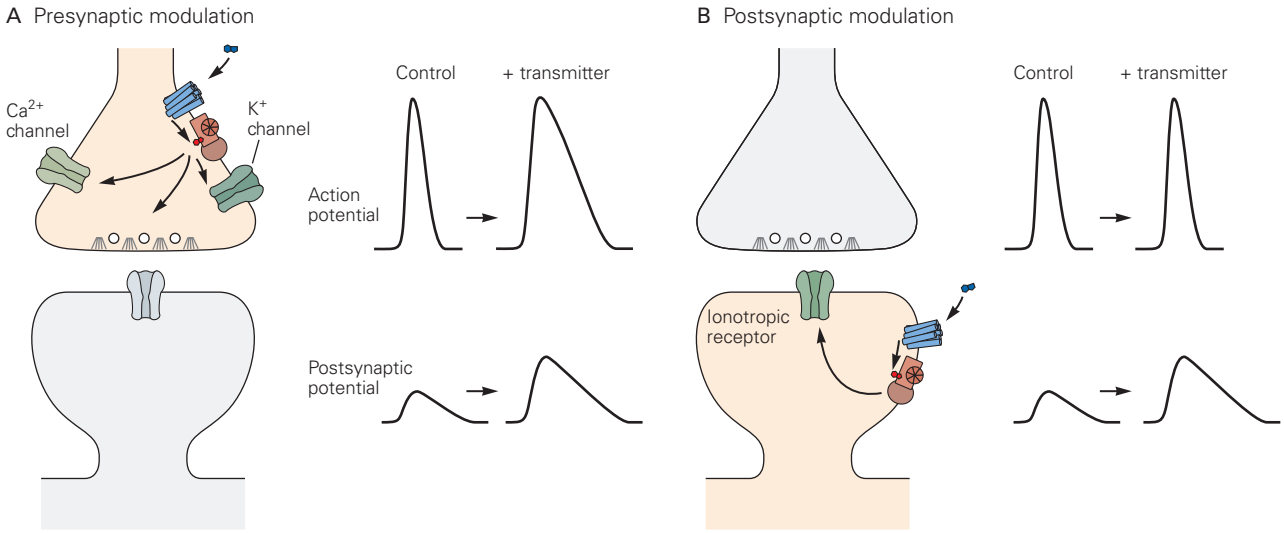

Figure 14–7 The modulatory actions of second messengers can regulate fast synaptic transmission by acting at two synaptic sites.

A. In the presynaptic terminal, second messengers can regulate the efficacy of transmitter release and thus the size of the fast postsynaptic potential mediated by ionotropic receptors. This can occur by altering presynaptic Ca^{2+} influx, either directly by modulating presynaptic voltage-gated Ca^{2+} channels or indirectly by modulating presynaptic K^+ channels, which alters Ca^{2+} influx by controlling action potential duration as illustrated (and thereby the length of time Ca^{2+} channels remain open). Some modulatory transmitters act to directly modulate the efficacy of the release machinery.

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

duration, and repetitive firing characteristics. Such modulation of the intrinsic excitability of neurons can play an important role in regulating information flow through neuronal circuits to alter behavior.

The distinction between direct and indirect regulation of ion channels is nicely illustrated by cholinergic synaptic transmission in autonomic ganglia of the peripheral nervous system. Stimulation of the presynaptic nerve releases ACh from the nerve terminals, directly opening nicotinic ACh receptor-channels in the postsynaptic neuron, thereby producing a fast excitatory postsynaptic potential (EPSP). The fast EPSP is followed by a slow EPSP that takes approximately 100 ms to develop but then lasts for several seconds. The slow EPSP is produced by an action of ACh on metabotropic muscarinic receptors that leads to the closing of a delayed-rectifier K^+ channel called the muscarine-sensitive (or M-type) K^+ channel (Figure 14–8A). These voltage-gated channels, which are formed by members of the KCNQ gene family, are partially activated when the cell is at rest; as a result, the current they carry helps determine the cell resting potential and membrane resistance.

The M-type K^+ channel differs from other delayed-rectifier K^+ channels by its much slower activation. It requires several hundred milliseconds to fully activate on depolarization. Because M-type channels are partially open at the resting potential, their closure in response to muscarinic stimulation causes a decrease in resting K^+ conductance, thus depolarizing the cell (Figure 14–8B). How far will the membrane depolarize? This can be calculated using the equivalent circuit form of the Goldman equation (Chapter 9) by decreasing the g_K term from its initial value. As the change in g_K due to closure of M-type K^+ channels is relatively modest, the depolarization at the peak of the slow EPSP is small, only a few millivolts. Nonetheless, M-type K^+ channel closure by ACh can lead to a striking increase in action potential firing in response to a depolarizing input.

What are the special properties of M-type K^+ channel closure that dramatically enhance excitability? First, the depolarization resulting from the reduction in resting g_K drives the membrane closer to threshold. Second, the increase in membrane resistance decreases the amount of excitatory current necessary to depolarize the cell to 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 *spike-frequency adaptation*, results in part from

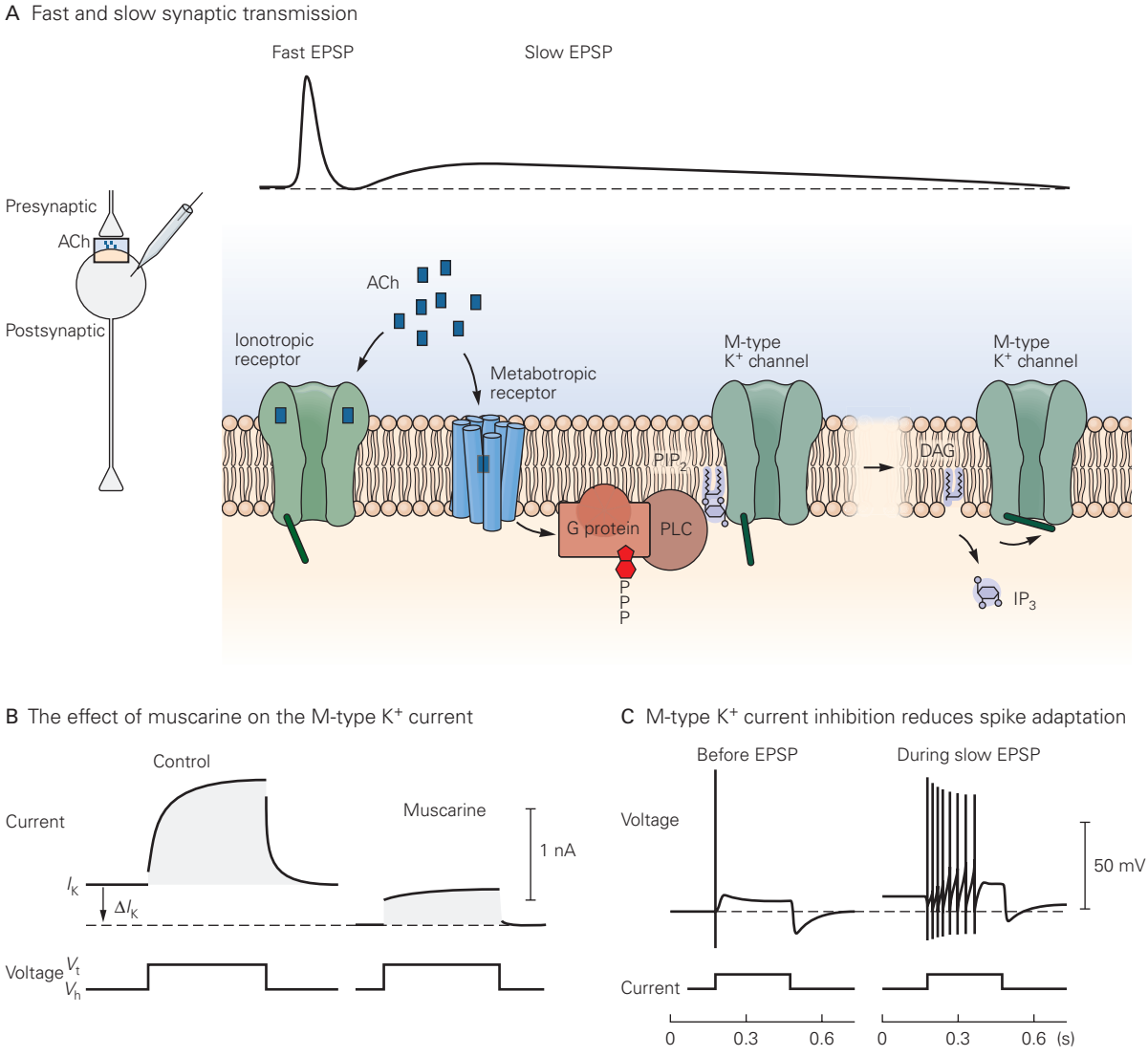

Figure 14–8 Fast ionotropic and slow metabotropic synaptic actions at autonomic ganglia.

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

B. Voltage-clamp recordings from an autonomic ganglion neuron indicate that ACh decreases the magnitude of the current carried by the voltage-gated M-type K^+ channels. In this experiment, the cell is initially clamped at a holding potential (V_h) near the resting potential in the absence of ACh (typically –60 mV). At this potential, the M-type K^+ channels are partially open, leading to a steady outward K^+ current. The voltage is then stepped for 1 second to a more positive test potential (V_t, typically –40 mV), which normally causes a slow increase in outward K^+ current (I_K) as the M-type K^+ channels respond to the more positive voltage by increasing their opening (control). Application of muscarine, a plant alkaloid that selectively stimulates the muscarinic ACh receptor, causes a fraction of the M-type K^+ channels to close. This decreases the outward K^+ current at the holding potential (note the shift in baseline current, ΔI_K), by closing the M-type K^+ channels that are open at rest, and decreases the magnitude of the slowly activating K^+ current in response to the step depolarization. (Adapted 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 spike-frequency adaptation (*left*). This is because the slow activation of the M-type K^+ channel during the depolarization generates an outward current that repolarizes the membrane below threshold. When the same current stimulus is applied during a slow EPSP, when a large fraction of M-type channels are now unable to open, the neuron fires a more sustained train of action potentials (*right*). (Adapted from Adams et al. 1986.)

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 (Figure 14–8C). As this modulation by ACh illustrates, the M-type K^+ channels do more than help set the resting potential—they also control excitability.

Although it has been known for some time that muscarinic receptor actions in autonomic ganglia result in the activation of PLC and the production of DAG and IP_3, the precise mechanism by which this signaling cascade produces M-type channel closure remained mysterious. However, it is now clear that M-channel closure upon muscarinic receptor activation is not due to the production of a second messenger. Rather, the M-channels, as well as a number of other types of channels (eg, see Figure 14–10), bind membranous PIP_2 as a cofactor for their proper functioning. Thus, muscarinic receptor activation closes M-type channels by activating PLC, and thereby decreasing the levels of PIP_2 in the membrane due to hydrolysis by PLC. We shall next discuss the mechanisms by which other signaling cascades are capable of modulating other types of ion channels. We start by describing 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 the indirect gating of a channel occurs when transmitter binding to 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^+* channels (GIRK1–4; encoded by the *KCNJ1–4* genes) and a voltage-dependent Ca^{2+} channel. With both kinds of channels, it is the G protein's βγ complex that binds to and regulates channel opening (Figure 14–9A).

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. 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 8–11).

In the 1920s, Otto Loewi described how the release of ACh in response to stimulation of the vagus nerve slows the heart rate (Figure 14–9B). We now know that 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 (rise time <1 ms). However, the rate of GIRK 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 electrophysiological 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 14–9C). These findings were reconciled when it was found that the GIRK channel was activated directly by the G protein's βγ-subunit complex, which becomes available to interact with the GIRK channel when it dissociates from the G protein α-subunit upon activation of the muscarinic receptors.

The mechanism by which the βγ-subunits activate the GIRK channel was recently elucidated at the atomic resolution through the solving of the X-ray crystal structure of the GIRK channel in a complex with the βγ-subunits. Each of the four GIRK channel subunits binds a single βγ-subunit complex, which interacts with the cytoplasmic surface of the channel, leading to a conformational change that promotes channel opening (Figure 14–10).

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-activated, cyclic nucleotide-regulated channels, which are encoded by the *HCN* gene family (Chapter 10). Because GIRK channels are activated by neurotransmitters, they provide a means for synaptic modulation of 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 the βγ-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 of the βγ complex of G_i or G_o to the channel. 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 15, inhibition

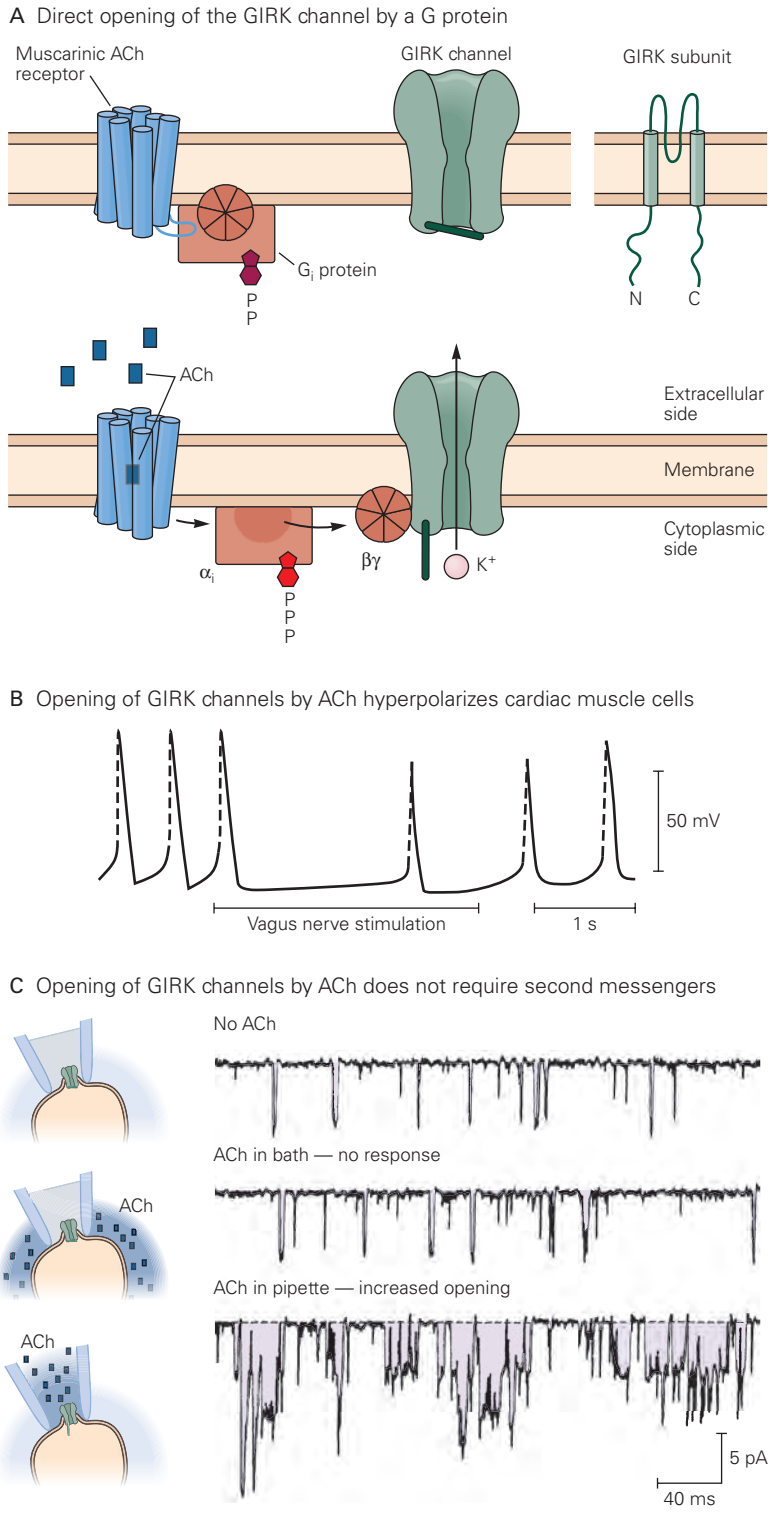

Figure 14–9 Some G proteins can open ion channels directly without employing second messengers.

A. An inward-rectifying K^+ channel (**GIRK**) is opened directly by a G protein. Binding of ACh to a muscarinic receptor causes the G_i protein and $\alpha_i\beta\gamma$ complex to dissociate; the free $\beta\gamma$-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 slowing the heart rate. (Adapted 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**). This is because the free $\beta\gamma$-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. The ACh must be in the pipette to activate the channel (**bottom record**). (Reproduced, with permission, from Soejima and Noma 1984. Copyright © 1984 Springer Nature.)