

Box 6.4**OF SPECIAL INTEREST****The Brain's Exciting Poisons**

Neurons of the brain do not regenerate, so each dead neuron is one less we have for thinking. One of the fascinating ironies of neuronal life and death is that glutamate, the most essential neurotransmitter in the brain, is also one of the biggest killers of neurons. A large percentage of the brain's synapses releases glutamate, which is stored in large quantities. Even the cytosol of nonglutamatergic neurons has a very high glutamate concentration, greater than 3 mM. An ominous observation is that when you apply this same amount of glutamate to the outside of isolated neurons, they die within minutes.

The voracious metabolic rate of the brain demands a continuous supply of oxygen and glucose. If blood flow ceases, as in cardiac arrest, neural activity will stop within seconds, and permanent damage will result within a few minutes. Disease states such as cardiac arrest, stroke, brain trauma, seizures, and oxygen deficiency can initiate a vicious cycle of excess glutamate release. Whenever neurons cannot generate enough ATP to keep their ion pumps working hard, membranes depolarize, and Ca^{2+} leaks into cells. The entry of Ca^{2+} triggers the synaptic release of glutamate. Glutamate further depolarizes neurons, which further raises intracellular Ca^{2+} and causes still more glutamate to be released. At this point, there may even be a reversal of the glutamate transporter, further contributing to the cellular leakage of glutamate.

When glutamate reaches high concentrations, it kills neurons by overexciting them, a process called excitotoxicity. Glutamate simply activates its several types of receptors, which allow excessive amounts of Na^+ , K^+ , and

Ca^{2+} to flow across the membrane. The NMDA subtype of the glutamate-gated channel is a critical player in excitotoxicity, because it is the main route for Ca^{2+} entry. Neuron damage or death occurs because of swelling resulting from water uptake and stimulation by Ca^{2+} of intracellular enzymes that degrade proteins, lipids, and nucleic acids. Neurons literally digest themselves.

Excitotoxicity has been implicated in several progressive neurodegenerative human diseases such as *amyotrophic lateral sclerosis* (ALS, also known as Lou Gehrig's disease), in which spinal motor neurons slowly die, and *Alzheimer's disease*, in which brain neurons slowly die. The effects of various environmental toxins mimic aspects of these diseases. Eating large quantities of a certain type of chickpea can cause lathyrism, a degeneration of motor neurons. The pea contains an excitotoxin called β -oxalylaminoalanine, which activates glutamate receptors. A toxin called domoic acid, found in contaminated mussels, is also a glutamate receptor agonist. Ingesting small amounts of domoic acid causes seizures and brain damage. And another plant excitotoxin, β -methylaminoalanine, may cause a hideous condition that combines signs of ALS, Alzheimer's disease, and Parkinson's disease in individual patients on the island of Guam.

As researchers sort out the tangled web of excitotoxins, receptors, enzymes, and neurological disease, new strategies for treatment emerge. Already, glutamate receptor antagonists that can obstruct these excitotoxic cascades and minimize neuronal suicide show clinical promise. Genetic manipulations may eventually thwart neurodegenerative conditions in susceptible people.

property has a significant impact on synaptic integration at many locations in the CNS.

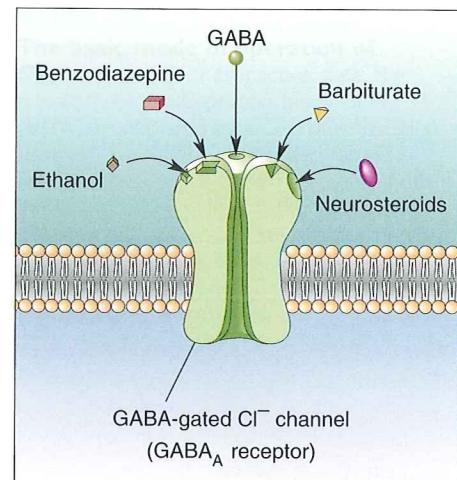
GABA-Gated and Glycine-Gated Channels. GABA mediates most synaptic inhibition in the CNS, and glycine mediates most of the rest. Both the GABA_A receptor and the glycine receptor gate a chloride channel. Surprisingly, inhibitory GABA_A and glycine receptors have a structure very similar to that of excitatory nicotinic ACh receptors, despite the fact that the first two are selective for anions while the last is selective for cations. Each receptor has α subunits that bind the transmitter and β subunits that do not.

Synaptic inhibition must be tightly regulated in the brain. Too much causes a loss of consciousness and coma; too little leads to a seizure. The need to control inhibition may explain why the GABA_A receptor has, in addition to its GABA binding site, several other sites where chemicals can dramatically modulate its function. For example, two classes of drugs, **benzodiazepines** (such as the tranquilizer diazepam, or Valium) and **barbiturates**

(including phenobarbital and other sedatives and anticonvulsants), each bind to their own distinct site on the outside face of the GABA_A channel (Figure 6.22). By themselves, these drugs do very little to the channel. But when GABA is present, benzodiazepines increase the frequency of channel openings, while barbiturates increase the duration of channel openings. The result in each case is more inhibitory Cl^- current, stronger IPSPs, and the behavioral consequences of enhanced inhibition. The actions of benzodiazepines and barbiturates are selective for the GABA_A receptor, and the drugs have no effect on glycine receptor function. Some of this selectivity can be understood in molecular terms; only receptors with the γ type of GABA_A subunit, in addition to α and β subunits, respond to benzodiazepines.

Another popular drug that strongly enhances the function of the GABA_A receptor is ethanol, the form of alcohol imbibed in beverages. Ethanol has complex actions that include effects on NMDA, glycine, nicotinic ACh, and serotonin receptors. Its effects on GABA_A channels depend on their specific structure. Evidence indicates that particular α , β , and γ subunits are necessary for constructing an ethanol-sensitive GABA_A receptor, similar to the structure that is benzodiazepine sensitive. This explains why ethanol enhances inhibition in some brain areas but not others. By understanding this molecular and anatomical specificity, we can begin to appreciate how drugs like ethanol lead to such powerful, and addictive, effects on behavior.

These myriad drug effects present an interesting paradox. Surely the GABA_A receptor did not evolve modulatory binding sites just for the benefit of our modern drugs. The paradox has motivated researchers to search for endogenous ligands, natural chemicals that might bind to benzodiazepine and barbiturate sites and serve as regulators of inhibition. Substantial evidence indicates that natural benzodiazepine-like ligands exist, although identifying them and understanding their functions are proving difficult. Other good candidates as natural modulators of GABA_A receptors are the *neurosteroids*, natural metabolites of steroid hormones that are synthesized from cholesterol primarily in the gonads and adrenal glands, but also in glial cells of the brain. Some neurosteroids enhance inhibitory function while others suppress it, and they seem to do so by binding to their own site on the GABA_A receptor (see Figure 6.22), distinct from those of the other drugs we've mentioned. The functions of natural neurosteroids are also obscure, but they suggest a means by which brain and body physiology could be regulated in parallel by the same chemicals.

**FIGURE 6.22**

The binding of drugs to the GABA_A receptor. The drugs by themselves do not open the channel, but they change the effect that GABA has when it binds to the channel at the same time as the drug.

▼ G-PROTEIN-COUPLED RECEPTORS AND EFFECTORS

There are multiple subtypes of G-protein-coupled receptors in every known neurotransmitter system. In Chapter 5, we learned that transmission at these receptors involves three steps: (1) binding of the neurotransmitter to the receptor protein, (2) activation of G-proteins, and (3) activation of effector systems. Let's focus on each of these steps.

The Basic Structure of G-Protein-Coupled Receptors

Most G-protein-coupled receptors are simple variations on a common plan, consisting of a single polypeptide containing seven membrane-spanning alpha helices (Figure 6.23). Two of the extracellular loops of the polypeptide form the transmitter binding sites. Structural variations in this region determine which neurotransmitters, agonists, and antagonists bind to the receptor. Two of the intracellular loops can bind to and activate G-proteins.

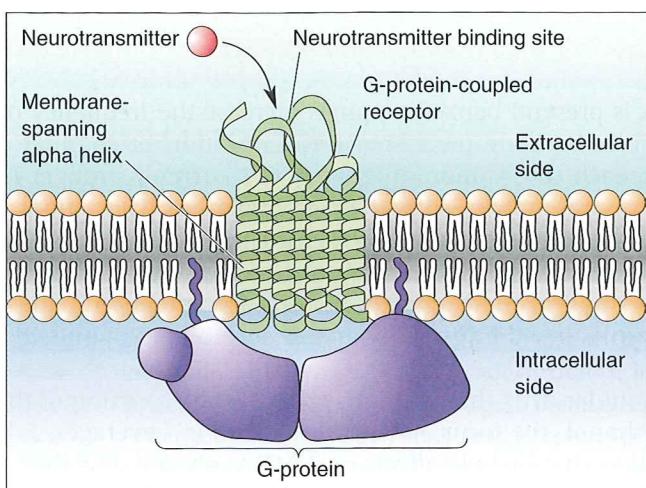


FIGURE 6.23

The basic structure of a G-protein-coupled receptor. Most metabotropic receptors have seven membrane-spanning alpha helices, a transmitter binding site on the extracellular side, and a G-protein binding site on the intracellular side.

Structural variations here determine which G-proteins and, consequently, which effector systems are activated in response to transmitter binding.

A partial list of G-protein-coupled receptors appears in Table 6.2. About 100 such receptors have been described. Most of these were unknown about 15 years ago, before the powerful methods of molecular neurobiology were applied to the problem.

The Ubiquitous G-Proteins

G-proteins are the common link in most signaling pathways that start with a neurotransmitter receptor and end with effector proteins. G-protein is short for guanosine triphosphate (GTP) binding protein, which is actually a diverse family of about 20 types. There are many more transmitter receptors than G-proteins, so some types of G-proteins can be activated by many receptors.

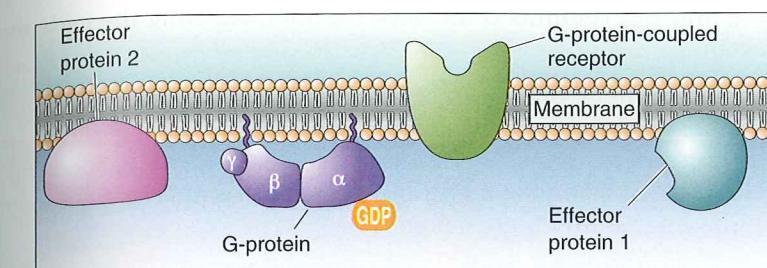
G-proteins all have the same basic mode of operation (Figure 6.24):

1. Each G-protein has three subunits, termed α , β , and γ . In the resting state, a guanosine diphosphate (GDP) molecule is bound to the G_{α} .

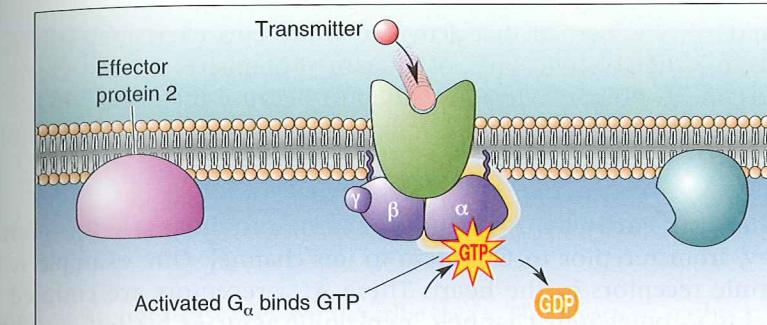
Table 6.2 Some G-Protein-Coupled Neurotransmitter Receptors

NEUROTRANSMITTER RECEPTOR(S)

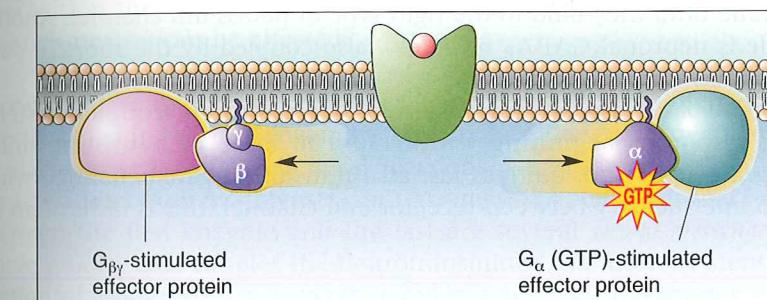
Acetylcholine (ACh)	Muscarinic receptors (M_1, M_2, M_3, M_4, M_5)
Glutamate (Glu)	Metabotropic glutamate receptors (mGluR1–8)
GABA	$GABA_A R_1, GABA_A R_2$
Serotonin (5-HT)	$5-HT_1(A, B, C, D_\alpha, D_\beta, E, F)$
Dopamine (DA)	$5-HT_2, 5-HT_4, 5-HT_{5\alpha, 5\beta}$
Norepinephrine (NE)	D_1, D_2, D_3, D_4
Enkephalin	$\alpha_1, \alpha_2, \beta_1, \beta_2, \beta_3$
Cannabinoid	μ, δ, κ
ATP	CBD, CB2
	A1, A2a, A2b, A3, P2y, P2z, P2t, P2u



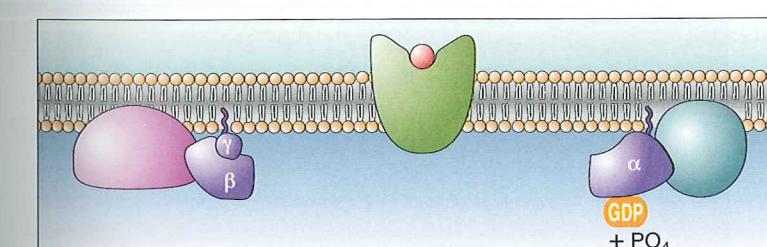
(a)



(b)



(c)



(d)

FIGURE 6.24
The basic mode of operation of G-proteins. (a) In its inactive state, the α subunit of the G-protein binds GDP. (b) When activated by a G-protein-coupled receptor, the GDP is exchanged for GTP. (c) The activated G-protein splits, and both the G_{α} (GTP) subunit and the $G_{\beta\gamma}$ subunit become available to activate effector proteins. (d) The G_{α} subunit slowly removes phosphate (PO_4) from GTP, converting GTP to GDP and terminating its own activity.

subunit, and the whole complex floats around on the inner surface of the membrane.

2. If this GDP-bound G-protein bumps into the proper type of receptor *and* if that receptor has a transmitter molecule bound to it, then the G-protein releases its GDP and exchanges it for a GTP that it picks up from the cytosol.
3. The activated GTP-bound G-protein splits into two parts: the G_{α} subunit plus GTP, and the $G_{\beta\gamma}$ complex. Both can then move on to influence various effector proteins.
4. The G_{α} subunit is itself an enzyme that eventually breaks down GTP into GDP. Therefore, G_{α} eventually terminates its own activity by converting the bound GTP to GDP.

5. The G_{α} and $G_{\beta\gamma}$ subunits come back together, allowing the cycle to begin again.

The first G-proteins that were discovered had the effect of stimulating effector proteins. Subsequently, it was found that other G-proteins could inhibit these same effectors. Thus, the simplest scheme for subdividing the G-proteins is G_s , designating that the G-protein is stimulatory, and G_i , designating that the G-protein is inhibitory.

G-Protein-Coupled Effector Systems

In Chapter 5, we learned that activated G-proteins exert their effects by binding to either of two types of effector proteins: G-protein-gated ion channels and G-protein-activated enzymes. Because the effects do not involve any other chemical intermediaries, the first route is sometimes called the shortcut pathway.

The Shortcut Pathway. A variety of neurotransmitters use the shortcut pathway, from receptor to G-protein to ion channel. One example is the muscarinic receptors in the heart. These ACh receptors are coupled via G-proteins to potassium channels, explaining why ACh slows the heart rate (Figure 6.25). In this case, the $\beta\gamma$ subunits migrate laterally along the membrane until they bind to the right type of potassium channel. Another example is neuronal GABA_B receptors, also coupled by the shortcut pathway to potassium channels.

Shortcut pathways are the fastest of the G-protein-coupled systems, having responses beginning within 30–100 msec of neurotransmitter binding. Although not quite as fast as a transmitter-gated channel, which uses no intermediary between receptor and channel, this is faster than the

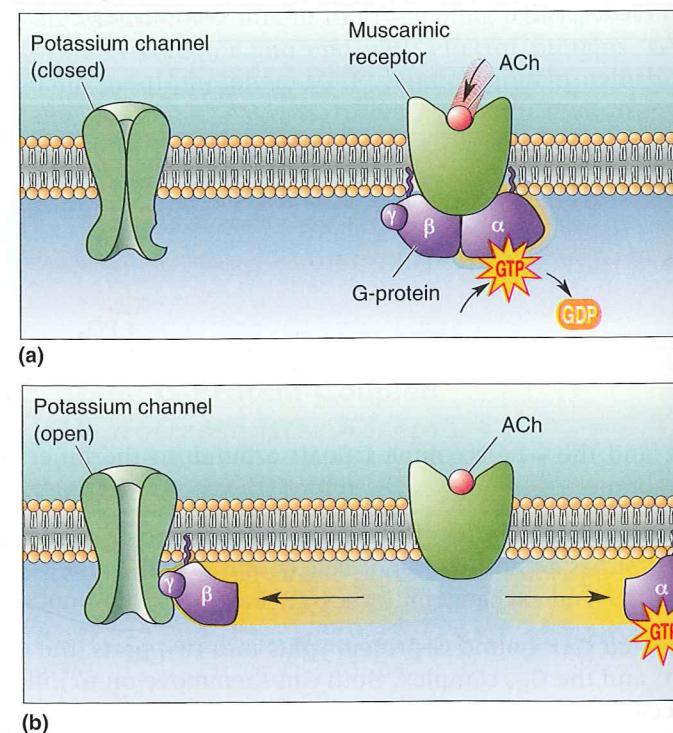


FIGURE 6.25
The shortcut pathway. (a) G-proteins in heart muscle are activated by ACh binding to muscarinic receptors. (b) The activated G $\beta\gamma$ subunit directly gates a potassium channel.

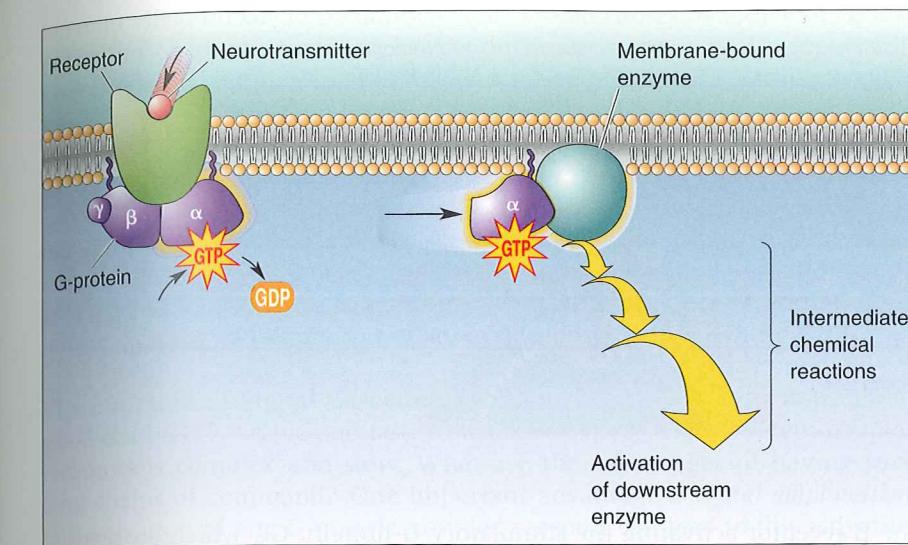


FIGURE 6.26
The components of a second messenger cascade.

second messenger cascades we describe next. The shortcut pathway is also very localized compared with other effector systems. As the G-protein diffuses within the membrane, it apparently cannot move very far, so only channels nearby can be affected.

Second Messenger Cascades. G-proteins can also exert their effects by directly activating certain enzymes. Activation of these enzymes can trigger an elaborate series of biochemical reactions, a cascade that often ends in the activation of other “downstream” enzymes that alter neuronal function. Between the first enzyme and the last are several *second messengers*. The whole process that couples the neurotransmitter, via multiple steps, to activation of a downstream enzyme is called a **second messenger cascade** (Figure 6.26).

In Chapter 5, we introduced the cAMP second messenger cascade initiated by the activation of the NE β receptor (Figure 6.27a). It begins with

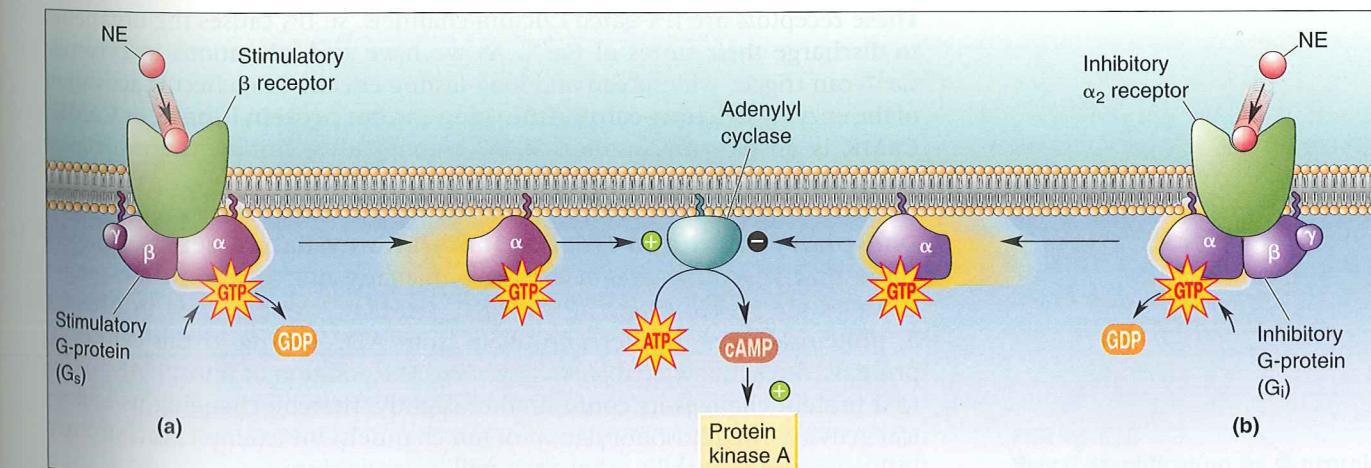


FIGURE 6.27
The stimulation and inhibition of adenyl cyclase by different G-proteins.
(a) Binding of NE to the β receptor activates G_s , which in turn activates adenyl cyclase. Adenyl cyclase generates cAMP, which activates the downstream enzyme protein kinase A.
(b) Binding of NE to the α_2 receptor activates G_i , which inhibits adenyl cyclase.

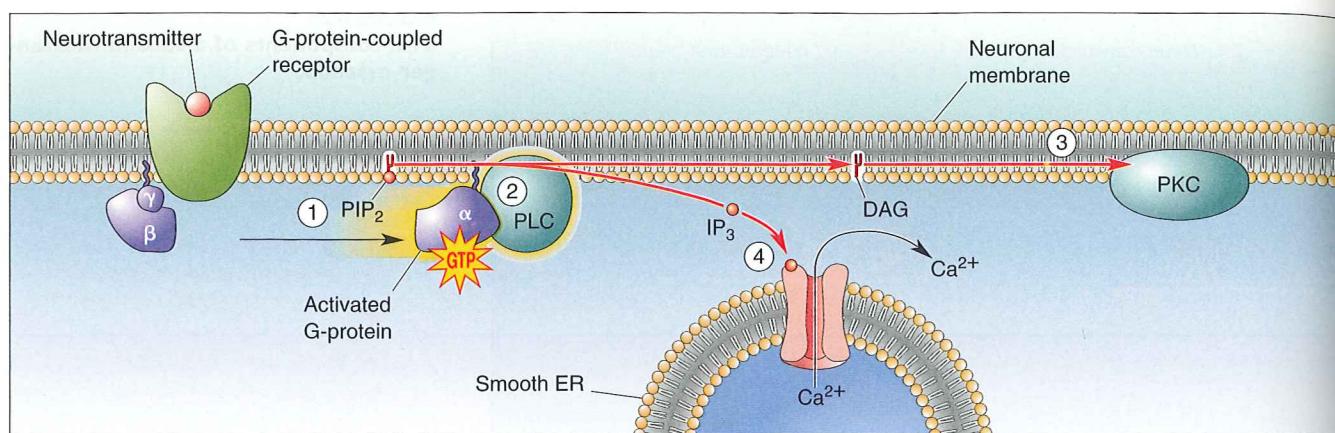


FIGURE 6.28

Second messengers generated by the breakdown of PIP₂, a membrane phospholipid. ① Activated G-proteins stimulate the β receptor activating the stimulatory G-protein, G_s, which proceeds to stimulate the membrane-bound enzyme adenylyl cyclase. Adenylyl cyclase converts ATP to cAMP. The subsequent rise of cAMP in the cytosol activates a specific downstream enzyme called **protein kinase A (PKA)**.

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Many biochemical processes are regulated with a push-pull method, one to stimulate them and one to inhibit them, and cAMP production is no exception. The activation of a second type of NE receptor, called the α₂ receptor, leads to the activation of G_i (the inhibitory G-protein). G_i suppresses the activity of adenylyl cyclase, and this effect can take precedence over the stimulatory system (Figure 6.27b).

Some messenger cascades can branch. Figure 6.28 shows how the activation of various G-proteins can stimulate **phospholipase C (PLC)**, an enzyme that floats in the membrane-like adenylyl cyclase. PLC acts on a membrane phospholipid (PIP₂, or phosphatidylinositol-4,5-bisphosphate), splitting it to form two molecules that serve as second messengers: **diacylglycerol (DAG)** and **inositol-1,4,5-triphosphate (IP₃)**. DAG, which is lipid-soluble, stays within the plane of the membrane where it activates a downstream enzyme, **protein kinase C (PKC)**. At the same time, the water-soluble IP₃ diffuses away in the cytosol and binds to specific receptors on the smooth ER and other membrane-enclosed organelles in the cell. These receptors are IP₃-gated calcium channels, so IP₃ causes the organelles to discharge their stores of Ca²⁺. As we have said, elevations in cytosolic Ca²⁺ can trigger widespread and long-lasting effects. One effect is activation of the enzyme **calcium-calmodulin-dependent protein kinase**, or **CaMK**. CaMK is an enzyme implicated in, among other things, the molecular mechanisms of memory (see Chapter 25).

Phosphorylation and Dephosphorylation. The preceding examples show that key downstream enzymes in many of the second messenger cascades are **protein kinases** (PKA, PKC, CaMK). As mentioned in Chapter 5, protein kinases transfer phosphate from ATP floating in the cytosol to proteins, a reaction called **phosphorylation**. The addition of phosphate groups to a protein changes its conformation slightly, thereby changing its biological activity. The phosphorylation of ion channels, for example, can strongly influence the probability that they will open or close.

Consider the consequence of activating the β type of NE receptors on cardiac muscle cells. The subsequent rise in cAMP activates PKA, which phosphorylates the cell's voltage-gated calcium channels, and this *enhances* their activity. More Ca²⁺ flows, and the heart beats more strongly. By contrast, the stimulation of β-adrenergic receptors in many neurons seems to

have no effect on calcium channels, but instead causes *inhibition* of certain potassium channels. Reduced K⁺ conductance causes a slight depolarization, increases the length constant, and makes the neuron more excitable (see Chapter 5).

If transmitter-stimulated kinases were allowed to phosphorylate without some method of reversing the process, all proteins would quickly become saturated with phosphates, and further regulation would become impossible. Enzymes called **protein phosphatases** save the day, because they act rapidly to remove phosphate groups. The degree of channel phosphorylation at any moment therefore depends on the dynamic balance of phosphorylation by kinases and dephosphorylation by phosphatases (Figure 6.29).

The Function of Signal Cascades. Synaptic transmission using transmitter-gated channels is simple and fast. Transmission involving G-protein-coupled receptors is complex and slow. What are the advantages of having such long chains of command? One important advantage is *signal amplification*: The activation of one G-protein-coupled receptor can lead to the activation of not one, but many, ion channels (Figure 6.30).

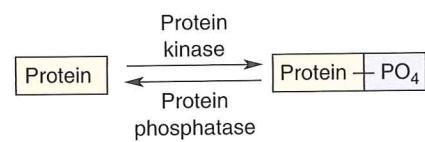


FIGURE 6.29
Protein phosphorylation and dephosphorylation.

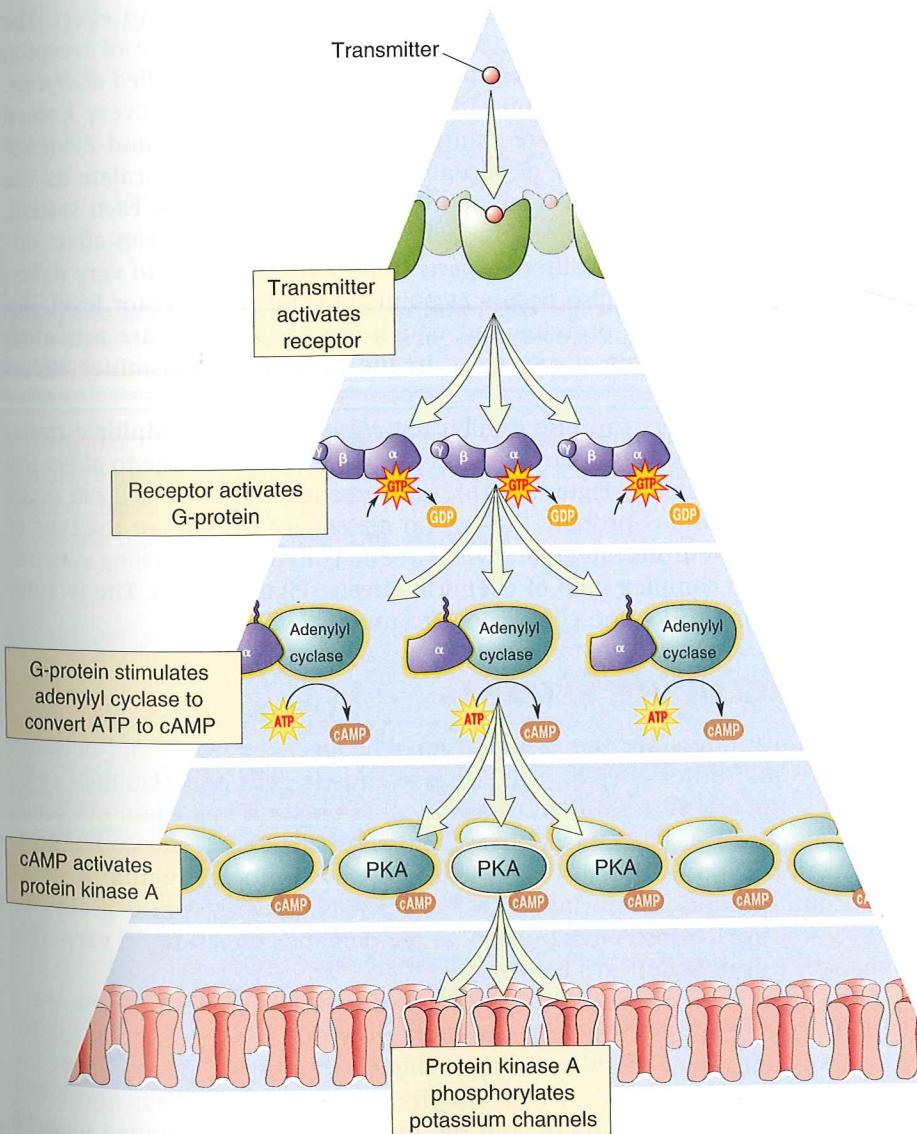


FIGURE 6.30
Signal amplification by G-protein-coupled second messenger cascades. When a transmitter activates a G-protein-coupled receptor, there can be amplification of the messengers at several stages of the cascade, so that ultimately many channels are affected.

Signal amplification can occur at several places in the cascade. A single neurotransmitter molecule, bound to one receptor, can activate perhaps 10–20 G-proteins; each G-protein can activate an adenylyl cyclase, which can make many cAMP molecules that can spread to activate many kinases; each kinase can then phosphorylate many channels. If all cascade components were tied together in a clump, signaling would be severely limited. The use of small messengers that can diffuse quickly (such as cAMP) also allows signaling at a distance, over a wide stretch of cell membrane. Signal cascades also provide many sites for further regulation, as well as interaction between cascades. Finally, signal cascades can generate very long-lasting chemical changes in cells, which may form the basis for, among other things, a lifetime of memories.

DIVERGENCE AND CONVERGENCE IN NEUROTRANSMITTER SYSTEMS

Glutamate is the most common excitatory neurotransmitter in the brain, while GABA is the pervasive inhibitory neurotransmitter. But this is only part of the story, because any single neurotransmitter can have many different effects. A molecule of glutamate can bind to any of several kinds of glutamate receptors, and each of these can mediate a different effect. The ability of one transmitter to activate more than one subtype of receptor, and cause more than one type of postsynaptic response, is called *divergence*.

Divergence is the rule among neurotransmitter systems. Every known neurotransmitter can activate multiple receptor subtypes, and evidence indicates that the number of receptors will continue to escalate as the powerful methods of molecular neurobiology are applied to each system. Because of the multiple receptor subtypes, one transmitter can affect different neurons (or even different parts of the same neuron) in very different ways. Divergence also occurs at points beyond the receptor level, depending on which G-proteins and which effector systems are activated. Divergence may occur at any stage in the cascade of transmitter effects (Figure 6.31a).

Neurotransmitters can also exhibit *convergence* of effects. Multiple transmitters, each activating their own receptor type, can converge to affect the same effector systems (Figure 6.31b). Convergence in a single cell can occur at the level of the G-protein, the second messenger cascade, or the type of ion channel. Neurons integrate divergent and convergent signaling systems, resulting in a complex map of chemical effects (Figure 6.31c). The wonder is that it ever works; the challenge is to understand how.

CONCLUDING REMARKS

Neurotransmitters are the essential links between neurons, and between neurons and other effector cells, such as muscle cells and glandular cells. But it is important to think of transmitters as one link in a chain of events, inciting chemical changes both fast and slow, divergent and convergent. You can envision the many signaling pathways onto and within a single neuron as a kind of information network. This network is in delicate balance, shifting its effects dynamically as the demands on a neuron vary with changes in the organism's behavior.

The signaling network within a single neuron resembles in some ways the neural networks of the brain itself. It receives a variety of inputs, in the form of transmitters bombarding it at different times and places. These inputs cause an increased drive through some signal pathways and a decreased drive through others, and the information is recombined to yield

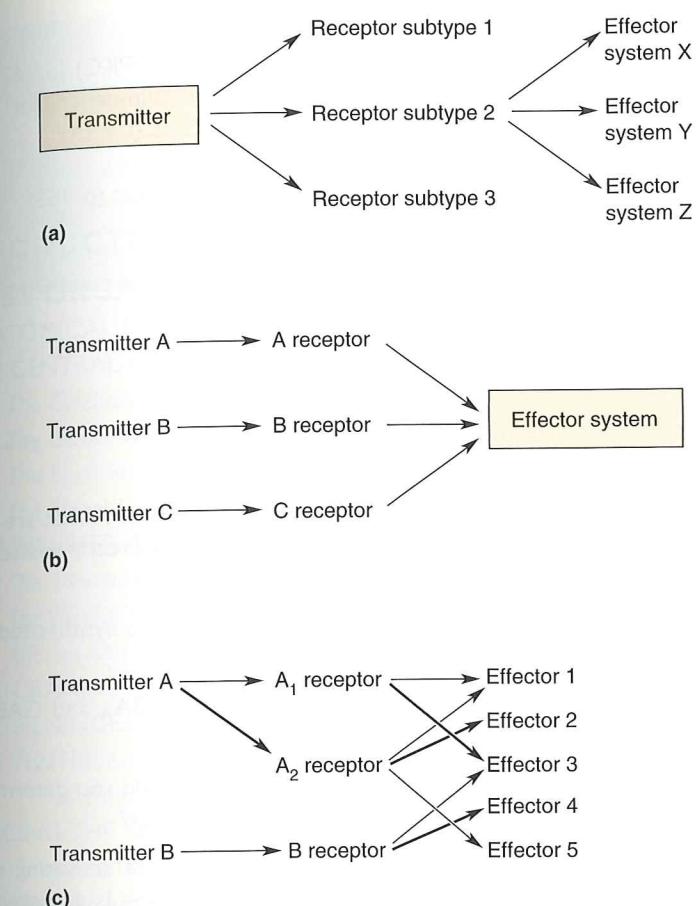


FIGURE 6.31
Divergence and convergence in neurotransmitter signaling systems.
(a) Divergence. **(b)** Convergence. **(c)** Integrated divergence and convergence.

a particular output that is more than a simple summation of the inputs. Signals regulate signals, chemical changes can leave lasting traces of their history, drugs can shift the balance of signaling power—and, in a literal sense, the brain and its chemicals are one.

KEY TERMS

Introduction

- cholinergic (p. 134)
- noradrenergic (p. 134)
- glutamatergic (p. 134)
- GABAergic (p. 134)
- peptidergic (p. 134)

Studying Neurotransmitter Systems

- immunocytochemistry (p. 135)
- in situ* hybridization (p. 137)
- autoradiography (p. 137)
- microionophoresis (p. 138)
- receptor subtype (p. 138)

nicotinic ACh receptor (p. 139)
muscarinic ACh receptor (p. 139)

AMPA receptor (p. 139)
NMDA receptor (p. 139)

kainate receptor (p. 139)
ligand-binding method (p. 141)

Neurotransmitter Chemistry

- Dale's principle (p. 142)
- co-transmitter (p. 142)
- acetylcholine (ACh) (p. 142)
- transporter (p. 142)
- rate-limiting step (p. 143)

catecholamines (p. 143)
dopamine (DA) (p. 143)

norepinephrine (NE) (p. 143)
epinephrine (adrenaline) (p. 143)

dopa (p. 143)
serotonin (5-HT) (p. 146)

serotonergic (p. 146)
glutamate (Glu) (p. 146)

glycine (Gly) (p. 146)
gamma-aminobutyric acid (GABA) (p. 146)

endocannabinoid (p. 148)
retrograde messenger (p. 148)
nitric oxide (NO) (p. 151)