acid (jasmonate) and salicylic acid act as plant defense signals.[377,378] Salicylic acid activates a large number of transcription factors, which induce resistance to a variety of pathogenic organisms, a response referred to as **systemic acquired resistance**. See also Chapter 31, Section G. Among other compounds synthesized as part of the systemic acquired resistance of plants are proteins known as **systemins**.[382] Initially discovered in tomatoes, systemins have been discovered in more than 100 other species of plants. Jasmonic acid emitted from tomato plants acts as a pheromone that attracts wasps to attack caterpillars that feed on the tomato plants.[379] In fact, wounding by herbivores may stimulate emission of a variety of volatile compounds that may attract predators to the attacking herbivores.[380,381]

Many other compounds influence plant growth. Among them are the vitamins, thiamine, pyridoxine, and nicotinic acid, which are synthesized in the leaves and transported downward to the roots. Since they promote growth of roots, they are sometimes referred to as **root growth hormones**. However, they are nutrients universally needed by cells. Various compounds secreted by other organisms can either stimulate or inhibit growth of a given plant. Some are powerful toxins. Others, such as the previously mentioned NOD factors, and evidently also the riboflavin degradation product **lumichrome** (Box 15-B), are beneficial.[383] These **plant growth regulators** may be produced by other plants, by microorganisms, or by fungi.[384,385] Much use is made in agriculture of synthetic growth regulators.

10. Secretion of Hormones

In Chapter 11 the effects of binding of hormones to cell surface receptors have been emphasized. Equally important are the mechanisms that control the secretion of hormones. The topic of exocytosis has been considered briefly in Chapter 8, Section C,6 and aspects of the Golgi in Fig. 20-8 and associated text. Both hormones and neurotransmitters are secreted by exocytosis of vesicles. Cells have two pathways for secretion.[386,387] The **constitutive pathway** is utilized for continuous secretion of membrane constituents, enzymes, growth factors, viral proteins, and components of the extracellular matrix. This pathway carries small vesicles that originate in the trans-Golgi network (TGN; Fig. 20-8). The **regulated pathway** is utilized for secretion of hormones and neurotransmitters in response to chemical, electrical, or other stimuli.

Many neurotransmitters are packaged into **small synaptic vesicles** ~50 nm in diameter. These may originate from large endosomes rather than from the Golgi. They are usually recycled and refilled repeatedly.[386] Secretion of hormones, and of some neurotransmitters, occurs via **large dense-core vesicles** of ~100 nm diameter. These originate from the TGN and are not recycled. They are prominent in chromaffin cells and other cells that secrete large amounts of a signaling molecule. Secretion of hormones and that of neurotransmitters have several common features. Indeed, hormones of the hypothalamus, neurohypophysis, and the adrenal medulla are secreted by specialized neurons. However, while hormones are often carried in the bloodstream, neurotransmitters are most often secreted into the very small volume of a single synapse. The exocytosis must occur very rapidly from a small number of SSVs.

A common feature, and also a puzzle, of vesicular signaling is the nearly universal response to calcium ions. Exocytosis is usually triggered by a rise in the concentration of Ca^{2+}, and most receptor signaling also leads to an increase in cytosolic Ca^{2+}.[388–391] The puzzle lies in the ability of cells to use a common mechanism for so many specific purposes. This topic is considered further in Section B,8. There are also many other factors that can control exocytosis. Recent evidence suggests that NO may play a role.[392]

B. Neurochemistry

The nervous system, a network of neurons in active communication, reaches its ultimate development in the 1.5 kg human brain.[149,393–396] Many invertebrates, such as leeches,[396a] crayfish, insects, and snails, have brains containing no more than 10^4 to 10^5 neurons,[396b,397,398] but the human brain contains ~10^{11}. Each of these neurons interconnects through **synapses** with hundreds or thousands of other neurons. The number of connections is estimated to be as many as 60,000 with each Purkinje cell of the human cerebellum. There may be many more than 10^{14} synapses in the human brain.[399,400]

In addition to neurons, the brain contains 5–10 times as many **glial** cells of several types. The neuroglia occupy 40% of the volume of brain and spinal cord in the human. Some glial cells seem to bridge the space between neurons and bloodcarrying capillaries. Others synthesize myelin. Some are very irregular in shape.

1. The Anatomy and Functions of Neurons

Although neurons have many shapes and forms, a common pattern is evident.[400a] At one end of the elongated cell (Fig. 30-8) is a series of **dendrites**, thin fibers often less than 1 μm in diameter. The ends of the dendrites form synapses with other neurons and act as receivers of incoming messages. Additional messages come into synapses on the **cell body**, while

the **axon** serves as the output end of the cell. The axon, a long fiber of diameter 1–20 μm, is also branched. As a consequence, the nervous system contains both highly branching and highly converging pathways. Many of the axons are wrapped in a myelin sheath (Fig. 30-9; pp. 390 and 1767).

The ends of the fine nerve fibers are thickened to form the **synaptic knobs**, which make synaptic contacts with dendrites on cell bodies of other neurons. In most instances the arrival of a nerve signal at the **presynaptic** end of a neuron causes the release of a transmitter substance (neurohormone). The transmitter passes across the 10–50 nm (typically 20 nm) **synaptic cleft** between the two cells and induces a change in the electrical potential of the **postsynaptic** membrane of the next neuron (Fig. 30-10).[149,401] Excitatory transmitters usually cause **depolarization** of the membrane. By this we mean that the membrane potential, which in a resting neuron is –50 to –70 mv (Chapter 8), falls to nearly zero often as a consequence of an increased permeability to Na^+ and a resultant inflow of sodium ions. The resulting **postsynaptic potential** (really a drop in the potential difference) is propagated to the cell body and axon and under appropriate circumstances may initiate an **action potential**. This is a narrow spikelike region of depolarization that travels down the axon at a constant velocity and with undiminished intensity (Fig. 30-11).

A characteristic of many neurons is an *all-or-none response* or firing. An action potential passes down the axon only if there is sufficient depolarization. In general, a stimulus must reach a neuron through *more than one synapse* before the neuron will fire. Furthermore, neurons are often *inhibitory*, releasing transmitters that counter the excitatory synapses and tend to prevent firing. Inhibition is important in damping out small excitations; thus sharpening the response of the nervous system toward strong stimuli. Another characteristic of basic importance to the operation of the brain is that neurons fire at longer or shorter intervals depending upon the strength and duration of the stimulus. The stronger the stimulus to a given neuron, the more rapid the train of spikes that passes down the axon. Thus, the brain functions to a large extent in

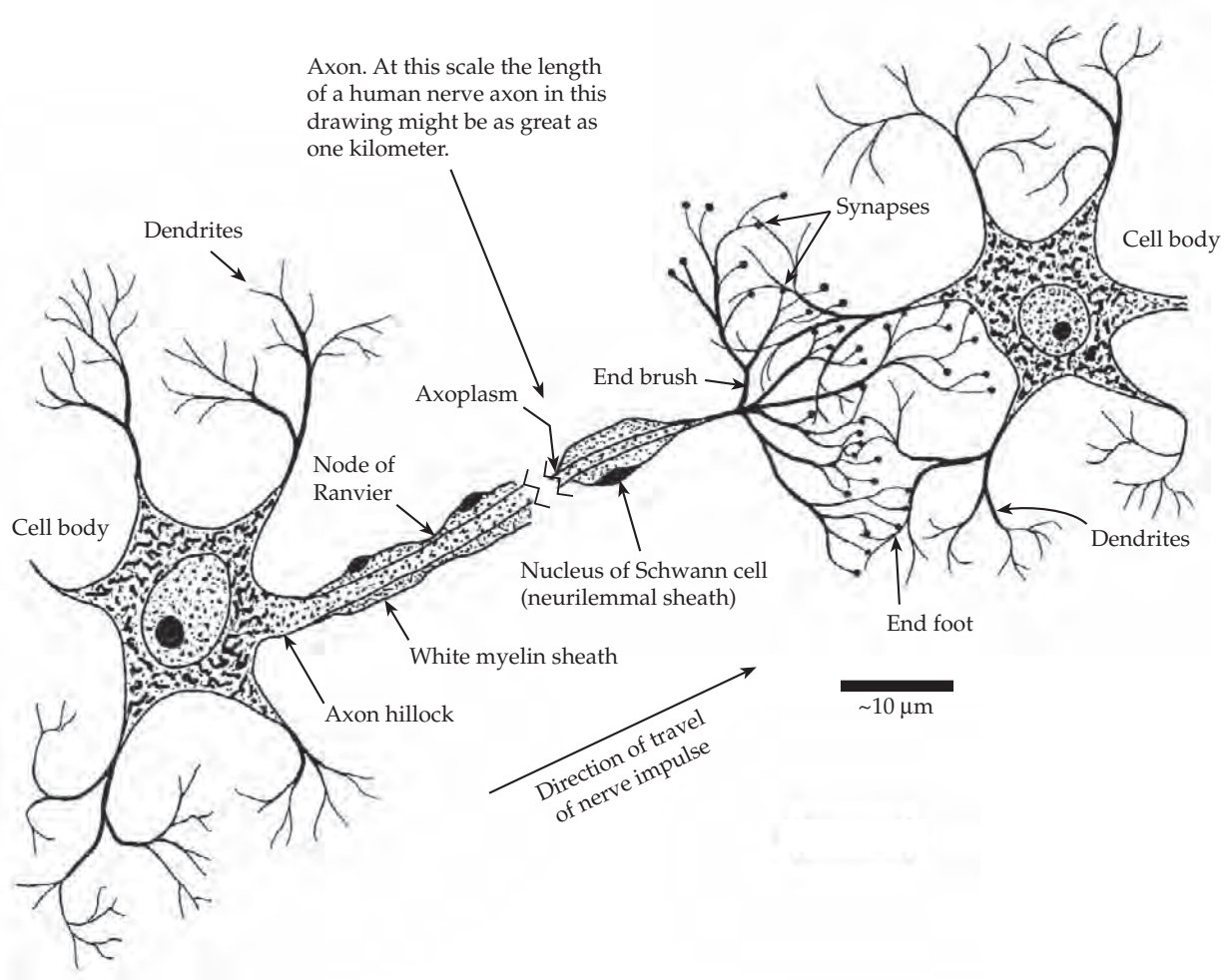


Figure 30-8 Schematic drawing of a neuron (after Brand and Westfall,[401] p. 1192).

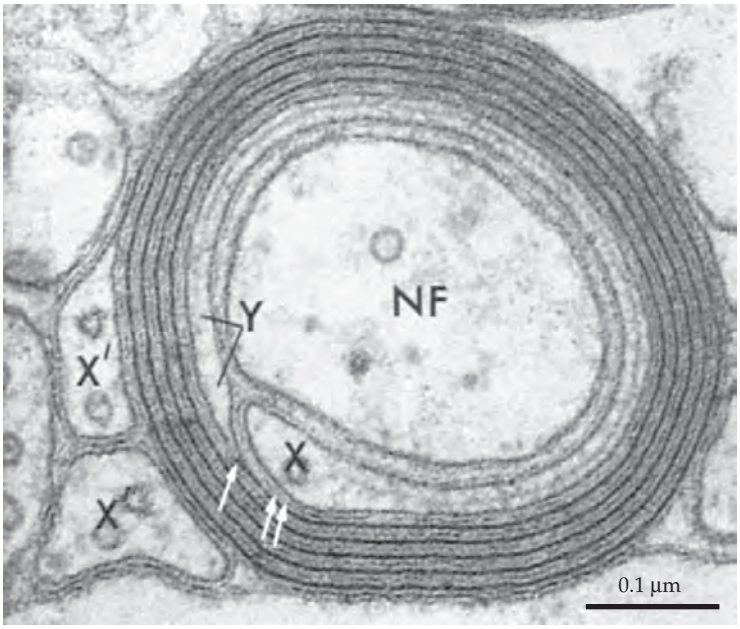
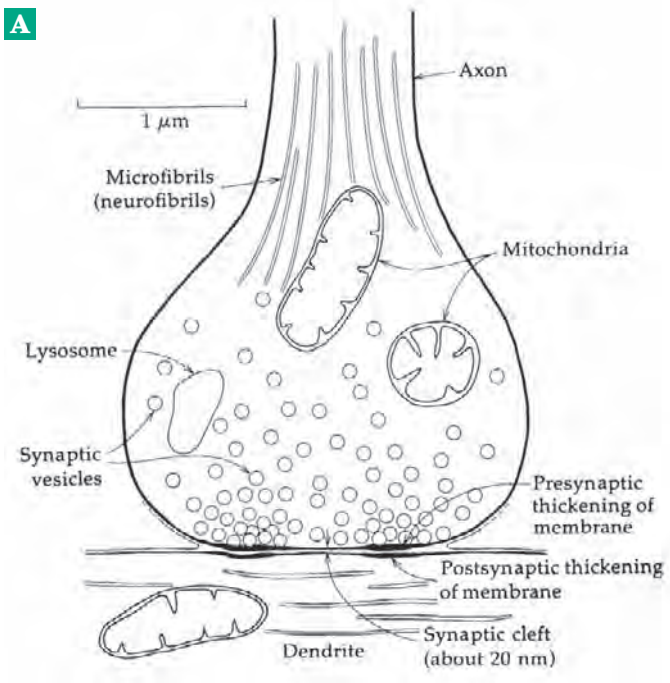


Figure 30-9 Micrograph of a section through an axon of a neuron from rat brain. The structure of the myelin sheath can be seen clearly. The growing lip of cytoplasm (X) from a neuroglial cell is advancing around the axon process (NF) and insinuating itself into the space between the plasma membrane of the axon and the membrane that limits the thin layer of cytoplasm (Y) left behind by the growing lip during its previous turn. This cytoplasmic layer disappears as the inner leaflets of its plasma membrane fuse to form the major dense line of the myelin sheath. This process is occurring at the point indicated by the single arrow. The outer leaflet of the plasma membrane surrounding the lip fuses with its own outer leaflet laid down on the previous turn. The two outer leaflets thus give rise to the less dense intermediate line of the sheath (double arrow). The cell body from which the investing cytoplasmic sheet originated cannot be seen in this micrograph, but cytoplasm within the lateral margins of the sheet does appear (X′). The micrograph, by A. Hirano and H. M. Dembritzer, originally appeared in *J. Cell Biol.* **34**, 555 (1967), where a more complete explanation of myelin sheath formation is provided. Figure copied from Porter and Bonneville.[402] Courtesy of Mary Bonneville.



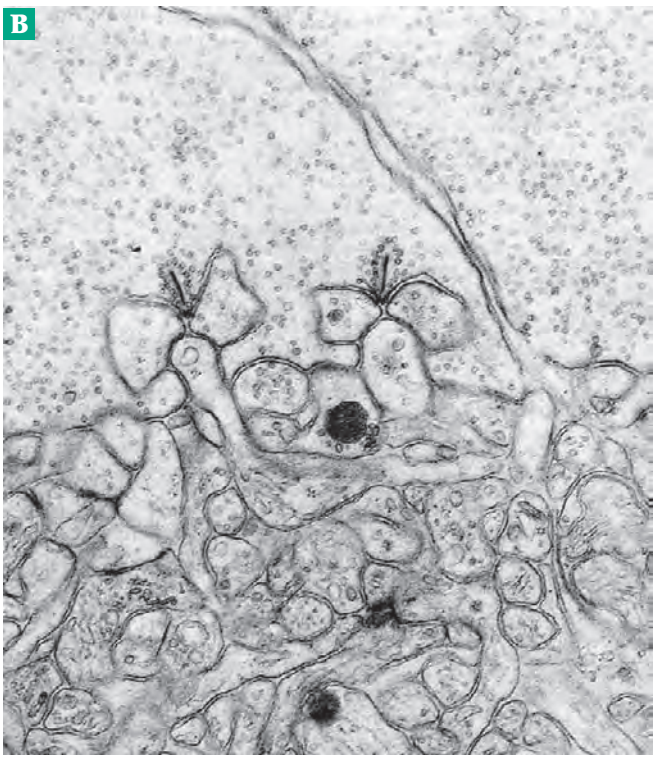


Figure 30-10 (A) Schematic drawing of a synapse. (B) Electron micrograph showing the synaptic junctions in the basal part (pedicle) of a retinal cone cell of a monkey.[403] Each pedicle contains synaptic contacts with 12 triads, each made up of processes from a bipolar cell center that carries the principal output signal and processes from two horizontal cells that also synapse with other cones. A ribbon structure within the pedicle is characteristic of these synapses. Note the numerous synaptic vesicles in the pedicle, some arranged around the ribbon, the synaptic clefts, and the characteristic thickening of the membranes surrounding the cleft (below the ribbons). Micrograph courtesy of John Dowling.

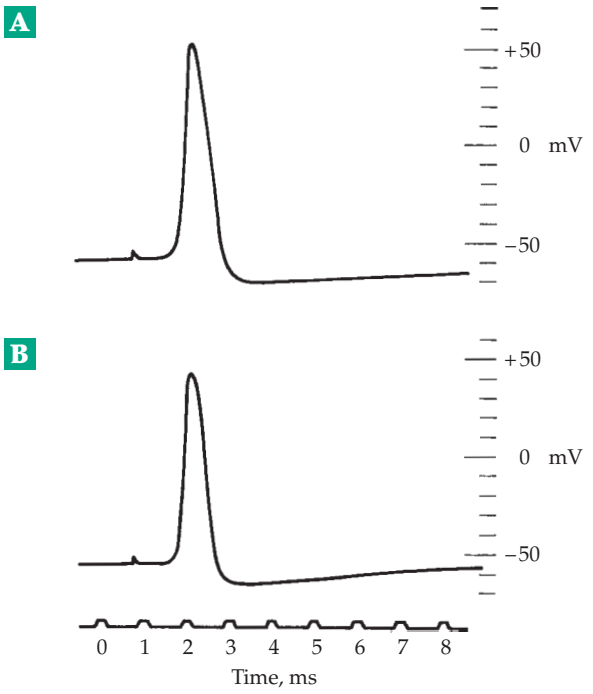


Figure 30-11 (A) Action potential recorded with internal electrode from extruded axon filled with potassium sulfate (16°C). (B) Action potential of an intact axon, with same amplification and time scale (18°C). The voltage scale gives the potential of the internal electrode relative to its potential in the external solution with no correction for junction potential. From A. Hodgkin, *Conduction of Nervous Impulses*, 1964. Courtesy of Charles C. Thomas, Publisher, Springfield, Illinois.

decoding trains of impulses. The frequency of the impulses from neurons varies from a few per second to a maximum of about 200 s^{-1} in most nerves (up to 1600 s^{-1} in the Renshaw cells of the spinal cord). The maximum frequency is dictated by the refractory period of ~1 ms (Section B,3).

Although the concepts of neuronal function outlined in the preceding paragraphs have been accepted for many years, more recent discoveries require that they be modified somewhat. Dendrites seem to be able to transmit information as well as to receive it. Furthermore, while information is certainly transmitted long distances by spike action potentials, shorter neurons and dendrites may communicate extensively by exchange of chemicals through low resistance gap junctions, also called **electric synapses** (Chapter 1). Small changes in membrane potential transmitted through these junctions may alter the behavior of adjacent neurons. Chemical transmitters do not always have an electrical effect on postsynaptic neurons but may influence metabolism or gene transcription.

2. Organization of the Brain

The anatomy of the brain is quite complex, and only a few terms will be defined here. The **cerebrum**, which is made up of two hemispheres, accounts for the largest part of the brain. The deeply folded outermost layer, the **cerebral cortex**, consists of **gray matter**, a mass of cell bodies, and fine unmyelinated nerve fibers. Beneath this lies a layer of **white matter** made up of myelin-covered axons connecting the cerebral cortex with other parts of the brain. The two cerebral hemispheres are connected by the **corpus callosum**, a band of ~2 x 10^8 nerve fibers. Remarkably, these fibers can be completely severed with a relatively minimal disruption of the nervous system. In the past the corpus callosum was sometimes cut to control almost incessant epileptic seizures that could not be prevented by drugs. The "split-brain" patients suffered relatively little disability as long as both eyes functioned normally. Studies of these patients provided some insights into the differing functions of the two hemispheres of the cerebrum.[395]

Deeper in the cerebrum lie the **basal ganglia,** which include the caudate, lenticular, and amygdaloid nuclei. The lenticular nuclei are further divided into putamen (an outer portion) and the globus pallidus. The putamen and caudate nuclei together are known as the **striatum** (Fig. 30-12). The lower lying subthalamic nuclei and substantia nigra are sometimes also included in the basal ganglia.

The outer parts of the cerebrum, including the basal ganglia, make up the telencephalon. Deep in the center of the brain is the diencephalon consisting of the **thalamus** (actually two thalami), **hypothalamus**, **hypophysis** (Figs. 30-1, 30-13), and other attached regions. A major structure at the back of the brain is the **cerebellum**. Like the cerebrum, its cortex is highly folded. The 30 billion neurons of the cerebellum are organized in a highly regular fashion.[393,404] The interconnections of the seven types of neurons present in this part of the brain have been worked out in fine detail.

The basal part of the brain or **brain stem** consists of the medulla oblongata and the pons. While the bulk of the tissue consists of myelinated nerve tracts passing into the spinal cord, synaptic regions such as the olivary nucleus are also present.

The brain, which must function in a chemically stable environment, is protected by a tough outer covering, the **arachnoid membrane**, and by the **blood–brain barrier**[406,407] and the **blood–cerebrospinal barrier**. Both of these barriers consist of tight junctions similar to those seen in Fig. 1-15A. They are formed between the endothelial cells of the cerebral capillaries and between the epithelial cells that surround the capillaries of the **choroid plexus**. The choroid plexus consists of capillary beds around portions

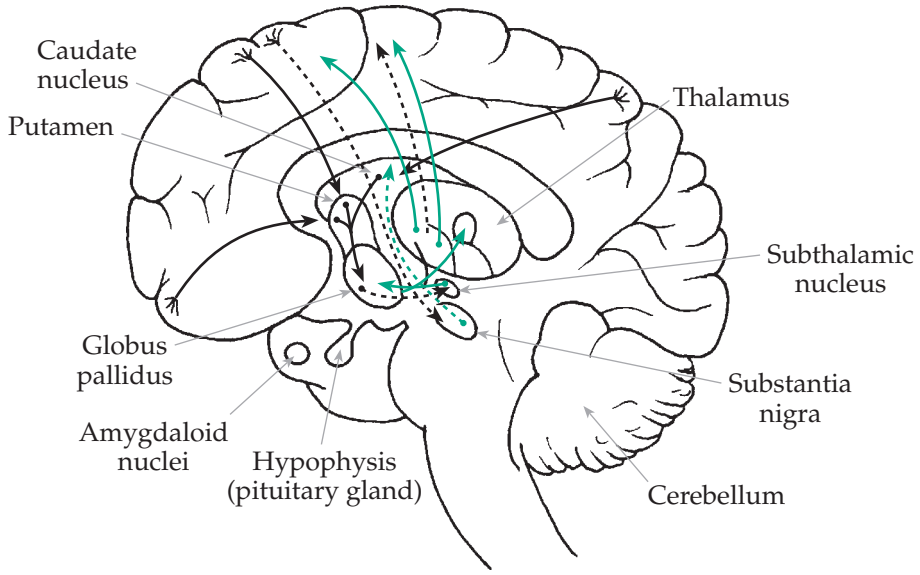


Figure 30-12 Diagram illustrating some of the major interconnections of the "extrapyramidal system" of the brain. Arrows indicate major direction of projections. The nigrostriatal (substantia nigra to striatum) and related neuronal pathways are indicated with dashed lines. After Noback and Demarest,[405] pp. 182 and 183.

of the fluid-filled **ventricles** deep in the interior of the brain. They serve as a kind of "kidney" for the brain assisting in bringing nutrients in from the blood and helping to keep dangerous compounds out.[406]

3. Neuronal Pathways and Systems

Consider a message originating with a nerve receptor in the skin or in another sense organ. A nerve signal passes via a **sensory neuron (afferent fiber)** upward toward the brain. It may pass through two or more synapses (often through one in the spinal cord and one in the thalamus) finally reaching a spot in the sensory region of the cerebral cortex. From there the signal in modified form spreads through the **interneurons** of virtually the entire cortex. In each synapse, as well as in the cortex, the impulse excites inhibitory fibers that dampen impulses flowing through adjacent fibers. Likewise, if a given impulse is not strong enough, it will itself be inhibited before reaching the cortex. Among the important sensory neurons are those from the seven million cone cells and 100 million rod cells of the eye. The nerve signals pass out of the retina by way of a million axons from retinal ganglion cells reaching, among other parts of the brain, the **visual cortex** (Fig. 30-14).[408]

The neuronal events that occur within the cerebral cortex are extraordinarily complex and little understood.[409] In what way the brain is able to initiate voluntary movement of muscles is obscure. However, it is established that the signals that travel out of the brain down the **efferent fibers** to the muscles arise from large **motor neurons** of the **motor cortex**,[410] a region that extends in a band across the brain and adjacent to the sensory cortex (Fig. 30-14). The axons of these cells form the **pyramidal tract** that carries impulses downward to synapses in the spinal cord and from there to the **neuromuscular junctions**. These are specialized synapses at which acetycholine is released, carrying the signal to the muscle fibers themselves. Passing over the cell surface and into the

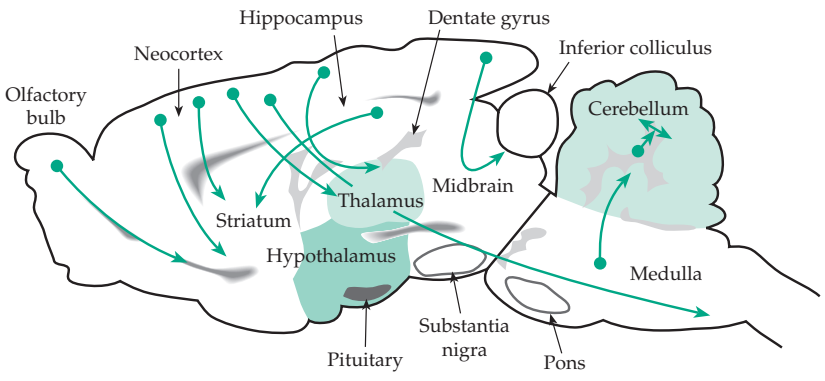


Figure 30-13 Section through a rat brain. This brain, which has been very widely used in neurochemical studies, appears superficially to be quite different from the human brain (Fig. 30-1), which is characterized by its large cerebral cortex. However, basic pathways are the same. Some major pathways for glutamate-secreting (glutamatergic) neurons are marked by arrows. Most of these originate in the neocortex (outer layers of the cerebral cortex) and the hippocampus. From Nicholls.[149] Courtesy of David G. Nicholls.

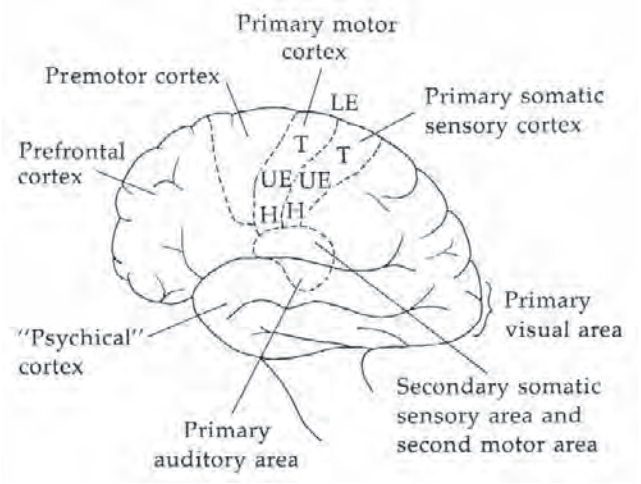


Figure 30-14 The location of several functional areas of the cerebral cortex. The representation of body parts on the primary motor and somatic sensory cortices include the head (H), upper extremity (UE), trunk (T), and lower extremity (LE). After Noback and Demarest,[405] p. 193.

T tubules (Chapter 19, Section B,4; Fig. 19-21), a wave of depolarization initiates the release of calcium and muscular contraction.

At the same time that the motor neurons send signals to the muscles, branches travel into other parts of the brain including the olivary nuclei, which send neurons into the cerebellum. The cerebellum acts as a kind of computer needed for fine tuning of the impulses to the muscles. Injury to the cerebellum leads to difficulty in finely coordinated motions. Input to the Purkinje cells arises from the climbing fibers, which originate in the inferior olive of the brain stem. Each climbing fiber activates a single Purkinje cell, but the dendrites of each Purkinje cell also form as many as 200,000 different synapses with parallel fibers that run across the cortex of the cerebellum (Fig. 30-15). The parallel fibers receive input from many sources via a complex series of mossy fibers and granule cells and influence the firing of the Punkinje cells. The output from the Purkinje cells is entirely inhibitory. It is transmitted via synapses in the cerebellar nuclei to neurons that lead back to the cerebral cortex, into the thalamus, and down the spinal cord.[411] The pathway to the cortex completes an inhibitory feedback loop, of which there are many in the nervous system. For details see Llinás[404] and Nicholls.[149]

In addition to the **somatic motor system** that operates the voluntary (striated) muscles via the pyramidal tract, there is the **autonomic system,** which controls the involuntary (smooth) muscles, glands, heartbeat, blood pressure, and body temperature. This system has its origins in both the cerebral cortex and hypothalamus. It is subdivided into two systems, the **sympathetic** and **parasympathetic** systems, which are anatomically distinct. The sympathetic system is geared to the fight and fright reactions. Its **postganglionic fibers** (those below the ganglia in the spinal cord) liberate norephinephrine (noradrenaline) and include the adrenal medulla, which consists of specialized neurons, the **chromaffin** cells. The parasympathetic system has to do more with homeostasis and maintenance of body systems. Biochemically it is characterized by the release of acetylcholine as a transmitter substance.

The hypothalamus, a four gram portion of the brain, receives a great deal of biochemical attention because of its function in the autonomic nervous system, in homeostasis, and in endocrine secretion. Its liberation of neurohormones that stimulate the hypophysis has already been considered in Section A,3. The hypothalamus is also involved in the regulation of the body temperature, of water balance, and possibly of glucose concentration.

Two other systems of importance in the brain are the **reticular system** and the **limbic system**. The former is the mediator of the sleep–wake cycle and is responsible for characteristic waves in the electroencephalogram. The limbic system is the mediator of **affect** or mood and of **instincts**. It is anatomically complex with centers in the amygdala, other subcortical nuclei, and the limbic lobe of the cortex. The limbic cortex forms a ring lying largely within the longitudinal fissure between the two hemispheres. It includes the olfactory cortex, the **hippocampus**, a region associated with formation of conscious memories, and other evolutionarily older regions of the cerebral cortex. Within the limbic lobe are the **pleasure centers**. When electrodes are implanted in these regions, animals will repeatedly push levers that are designed to electrically stimulate these centers. There are also **punishing centers,** whose stimulation causes animals to avoid further stimulation.

4. The Propagation of Nerve Impulses

Although the chemical basis of the conduction of nerve impulses via an action potential is not entirely clear, the electrical events have been described with precision. If the permeability of a membrane toward sodium ions is increased in a local region, sodium ions flow through the membrane into the cell neutralizing the negative charge inside and depolarizing the membrane. Such depolarization leads to propagation of an electrical signal of diminishing intensity over the surface of the membrane in a manner analogous to the flow of electrical current along a coaxial cable. It is thought that local increases in Na^+ permeability of the plasma membrane often trigger nerve impulses. Other

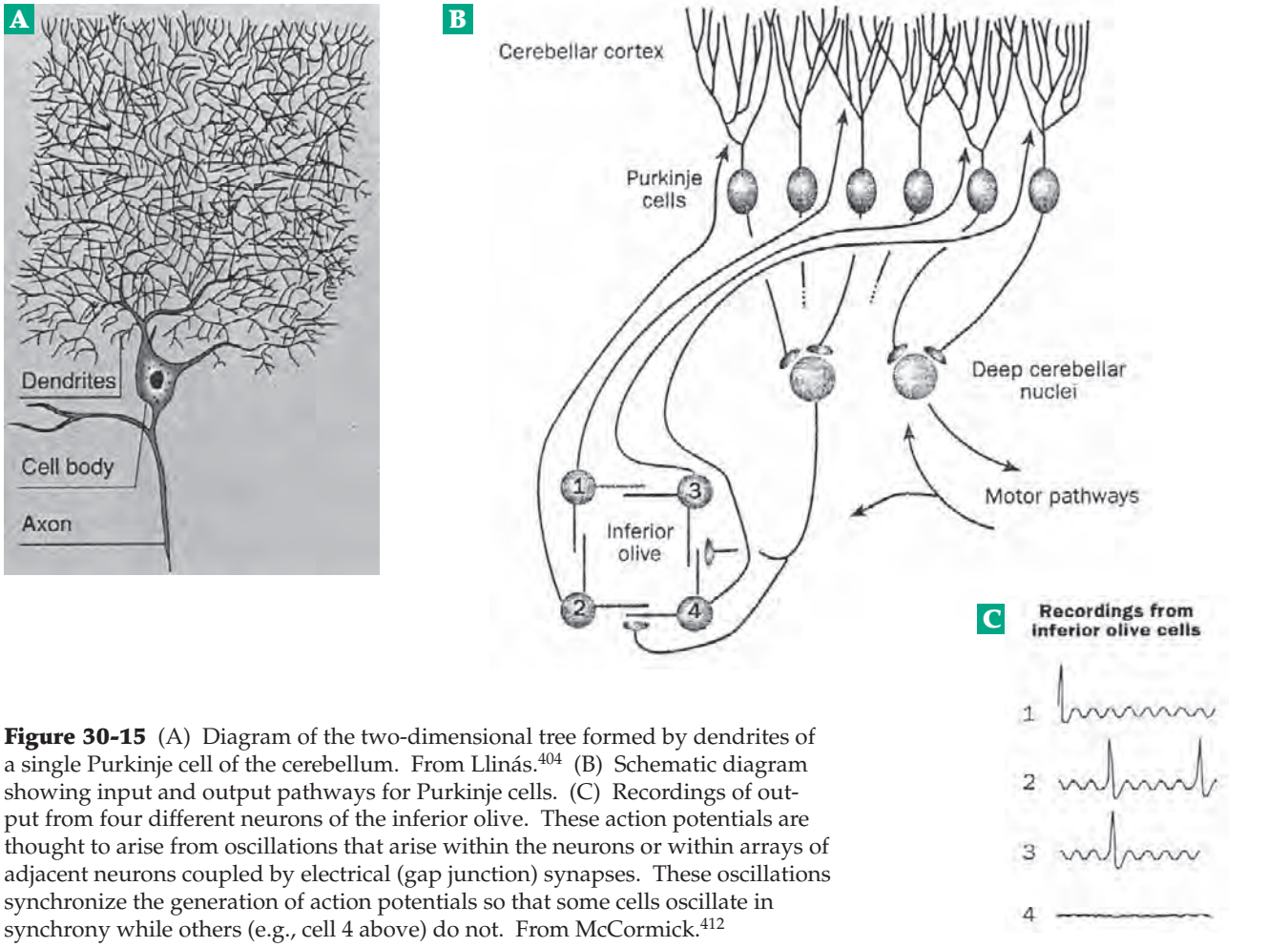


Figure 30-15 (A) Diagram of the two-dimensional tree formed by dendrites of a single Purkinje cell of the cerebellum. From Llinás.[404] (B) Schematic diagram showing input and output pathways for Purkinje cells. (C) Recordings of output from four different neurons of the inferior olive. These action potentials are thought to arise from oscillations that arise within the neurons or within arrays of adjacent neurons coupled by electrical (gap junction) synapses. These oscillations synchronize the generation of action potentials so that some cells oscillate in synchrony while others (e.g., cell 4 above) do not. From McCormick.[412]

ions such as Ca^{2+} may also play a role. While the kind of passive transmission of electrical signals that results from a local depolarization of the membrane is suitable for very short nerve cells, it cannot be used to send signals for long distances. Most nerve axons employ the more efficient action potential. This is an impulse that passes along the axon and for a short fraction of a second (~0.5 ms in mammalian nerves) changes the membrane potential in the characteristic way shown in Fig. 30-11. Initially, the negative potential of 50–70 mV drops rapidly to zero and then becomes positive by as much as 40–50 mV, after which it returns to the resting potential. The remarkable thing about the action potential is that it is propagated down the axons at velocities of 1–100 m / s without loss of intensity.

To establish the chemical basis of the action potential, A. L. Hodgkin and A. F. Huxley in the 1950s devised the **voltage clamp**, a sophisticated device by which the transmembrane current can be measured while using a feedback mechanism to fix the membrane potential at a preselected value.[413–417] Using the voltage clamp the membrane conductance could be measured as a function of the membrane potential and of time. It was found that immediately after a decrease in membrane potential was imposed with the voltage clamp, the permeability of the membrane toward sodium ions rose rapidly. Since an increased sodium ion permeability automatically leads to depolarization in an adjacent region of the membrane, a self-propagating wave is established and moves down the axon. The voltage clamp studies also revealed that after a fraction of a millisecond the permeability to potassium ions also increases. At the same time the sodium ion permeability decreases again, and the normal membrane potential is soon reestablished. However, during an **absolute refractory period** of ~0.5 ms no other nerve impulse can be passed. The sequence of events during passage of the nerve impulse can be described as the opening of sodium channels followed by the opening of potassium channels, and then by a closing of the channels in the same sequence. The results of these investigations led Hodgkin and Huxley to propose equations that quantitatively describe the action potential and that predict the observed conduction velocities and other features of nerve impulses.

A special feature of nerves that are designed to transmit impulses very rapidly is the presence of the wrapping of **myelin** (Fig. 30-9). As can be seen in this figure, the extracellular surfaces of the consecutive wraps bind tightly together, and the cytoplasm of the cell interior is squeezed out to form the compact myelin sheath.[418] Mutations in the integral membrane proteolipid protein (p. 401) are associated with a variety of defects in myelin formation. Some of these are severe, for example, leading to loosely wrapped myelin.[419,420] The proteolipid protein is encoded by an X-linked gene. The most abundant protein in peripheral nerve myelin is the integral membrane **peripheral myelin glycoprotein P_0**. It is encoded by an autosomal gene for which 29 known defects account for a variety of human diseases,[421–422a] including an autoimmune inner ear disease.[423] The extracellular domain of P_0, like many other cell adhesion molecules (p. 407), has a structure related to that of immunoglobulins. Four molecules of P_0, each of which carries a single immunoglobulin domain, associate via these domains in a kind of square donut that protrudes from the outer cell surface. There it can interact with four similar donuts from the apposed cell surface, zipping up the cell–cell interface by a kind of Velcro action.[422,424,425] Protein P_0 accounts for 50% of the total protein of peripheral myelin, but the **myelin basic protein**, which constitutes 20% of the total protein, is also essential.[426] This protein exists as a variety of forms that arise from differential splicing of its mRNA and extensive posttranslational modification. Deimination of argine side chains to form citrulline residues has been associated with development of the autoimmune disease **multiple sclerosis**.[427,428] **Peripheral myosin protein 22** is a 160-residue polypeptide with four membrane-spanning helices. It accounts for 2–5% of the myelin protein and is the site of defects that cause the demyelinating **Charcot–Marie–Tooth disease** and other serious human diseases.[428a,b]

The axon is effectively insulated from the surrounding medium by the myelin sheets except for special regions, the **nodes of Ranvier**, which lie at 1- to 2-mm intervals along the nerve. The nerve impulse in effect jumps from one nerve to the next. This **saltatory conduction** occurs much more rapidly (up to 100 m / s) than conduction in unmyelinated axons. It depends upon Na^+ and K^+ channels that are concentrated in the nodes of Ranvier.

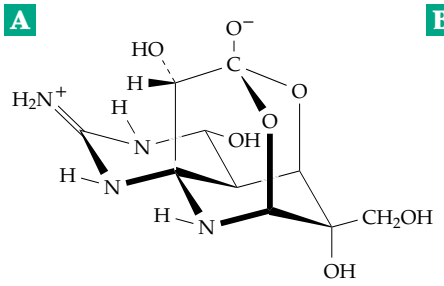
5. Ion Conducting Channels

What is known about the channels through which Na^+ and K^+ flow during nerve excitation? That the channels for the two ions are separate was shown by the fact that **tetrodotoxin** (found in the puffer fish)[429,429a] and **saxitoxin** of dinoflagellates, as well as scorpion toxins (see Fig. 30-16), exert their toxic action by blocking the Na^+ channels while having no effect upon conductance for K^+. At the same time the K^+ channels can be blocked by certain quaternary ammonium salts. Since the binding constants for the toxins are high ($K_f \sim 3 \times 10^8\ M^{-1}$ for tetrodotoxin), it is possible to titrate the sodium channels. The number is usually quite small, about 10–400 Na^+ channels / μm^2 of surface[430] (the same surface area contains 2×10^6 phopholipid molecules). However, membranes in the nodes of Ranvier of mammalian nerve fibers[431] contain ~12,000 channels / μm^2. Note that the ion channels described here are not the same as those in the ion pump, i.e., the Na^+,K^+-ATPase (Fig. 8-25). In some neurons the number of conduction channels for Na^+ appears to be ten times less than the number of pumping channels, i.e., of Na^+,K^+-ATPase.[432,432a]

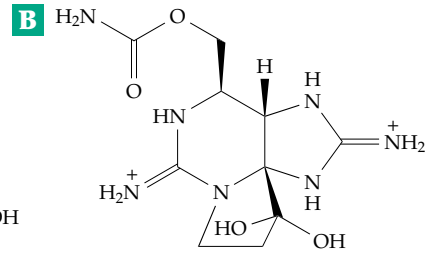
Since the number of ion-conducting channels is small, the rate of sodium passage through the open channels must be extremely rapid and has been estimated as ~10^8 ions / s.[433] This is within an order of magnitude of the diffusion-limited rate (Eq. 9-30). On this basis it is clear that the channels cannot act by means of ionophoric carriers but form pores that can be opened and closed (**gated**) in response to changes in the membrane potential. They are **voltage-sensitive ion channels**.[433,434] The channels are selective for specific ions and the selectivity parallels that of sites in some cation-exchange resins such as those containing carboxylate groups. This suggested that the inside surface of the channel might contain one or more carboxylate groups from protein side chains as well as other polar groups. A Na^+ ion approaching the channel entrance might exchange some of its hydration sphere for ligands from the channel surface. The differing affinity of the "ion exchange" sites for various cations could ensure that it is predominately Na^+ that passes through the channel. Anions could be excluded by electrostatic repulsion. Recent structural studies have allowed these speculations to be replaced with experimental findings as described in the following paragraphs. They have revealed that the selectivity mechanism are similar for Na^+ and Ca^{2+} channels.

The sodium ion channel of the electric eel.

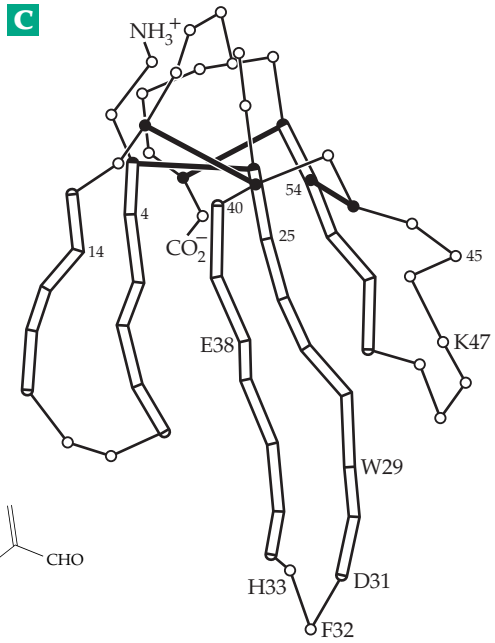
Making use of the binding of radioactively labeled specific toxins to identify them, the subunits of the sodium channel proteins were purified from several sources including the electrical tissue of the electric eel *Electrophorus electricus*,[437–439] heart and skeletal muscle, and brain.[440–441b] In all cases a large ~260-kDa glycoprotein, which may be 30% carbohydrate, is present. The saxitoxin-binding protein from rat brain has two additional 33–36 kDa subunits with a stoichiometry of $\alpha\beta_1\beta_2$. The *Electrophorus* α subunit consists of 1820 residues,[437] while rat brain contains α proteins of 2009



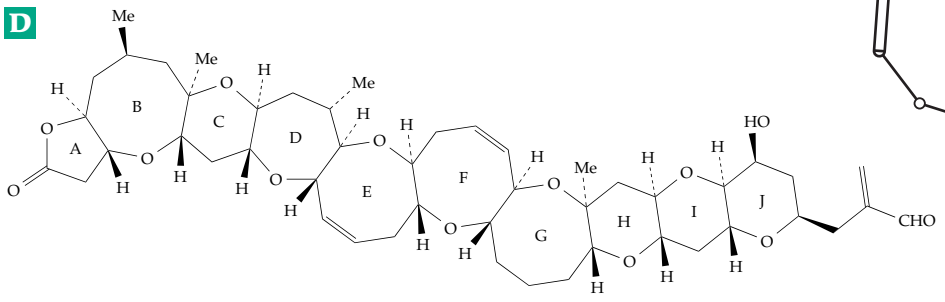
Tetrodotoxin of the puffer fish and certain newts



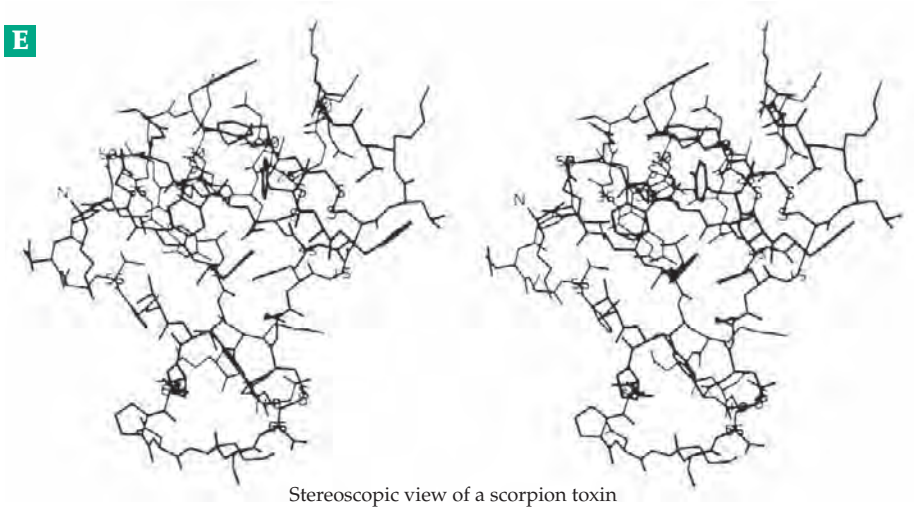
Saxitoxin of *Gonyaulax*, a dinoflagellate whose toxin accumulates in shellfish. As little as 0.3 mg of toxin can be fatal.



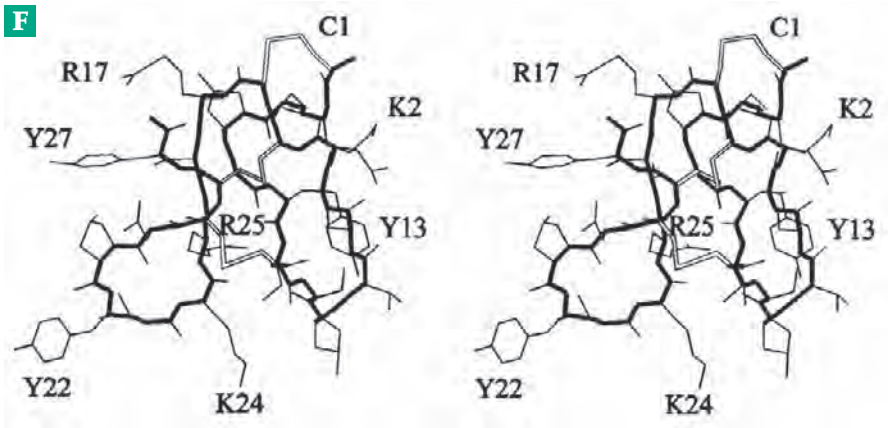
Structure of erabutoxin b. Many snake venom neurotoxins have similar structures with conserved disulfide bridge positions. See Hatanaka, H. *et. al.*[513]



Brevetoxin A



Stereoscopic view of a scorpion toxin



ω Conotoxin, blocks presynaptic voltage-regulated Ca^{2+} channels

Figure 30-16 Structures of some neurotoxins that affect ion channels. Other neurotoxins include the Na^+, K^+-ATPase inhibitor ouabain (Fig. 22-12), batrachotoxin (Fig. 22-12), and picrotoxin (Fig. 22-4). The structure of a scorpion toxin is from Almassy *et al.*,[494a] that of ω conotoxin is from Pallaghy *et al.*,[435] and that of brevetoxin is redrawn after Shimizu *et al.*[436]

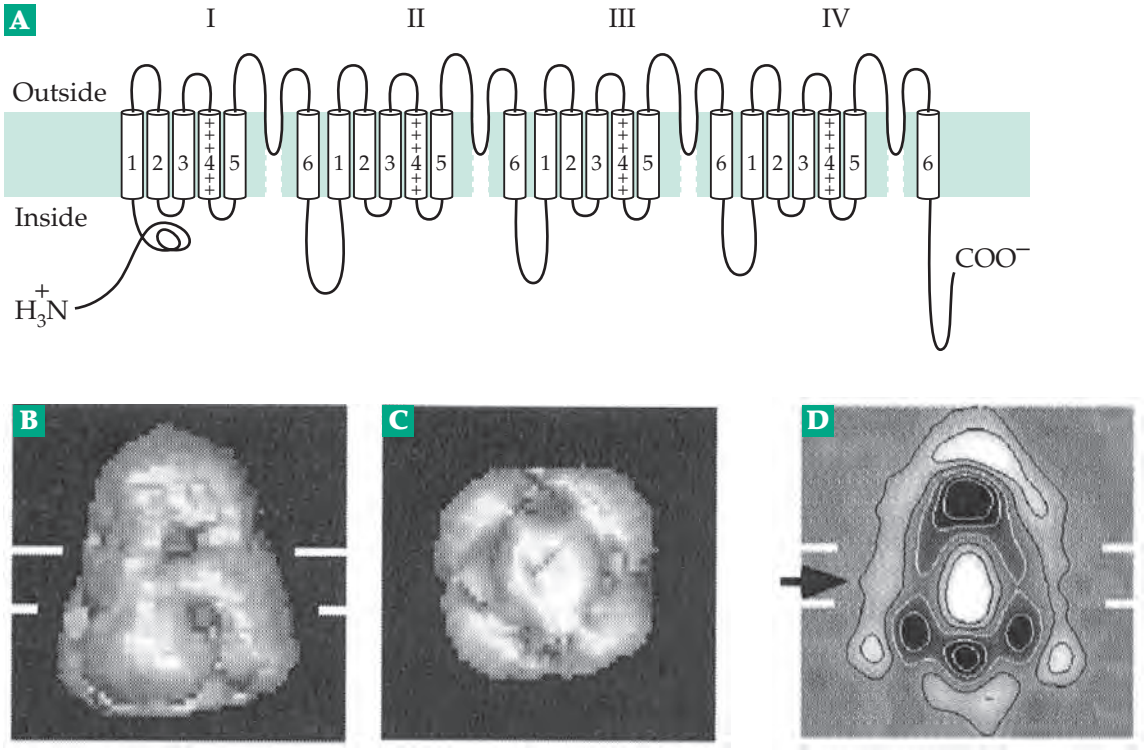


Figure 30-17 (A) Two-dimensional map of the ~260-kDa α subunit of the voltage-gated Na^+ channel from the electric eel *Electrophorus electris*.[438,441] (B) Image of the sodium channel protein obtained by cryo-electron microscopy and image analysis at 1.9 nm resolution. In this side view the protein appears to be bell-shaped with a height of ~13.5 nm, a square bottom (cytoplasmic surface) ~10 nm on a side, and a hemispherical top with a diameter of ~6.5 nm. (C) Bottom view of the protein. (D) Axial section which cuts the bottom, as viewed in (C), approximately along a diagonal. From Sato *et al.*[438] Notice the cavities (dark) and domain structures (light). The black arrow marks a constriction between upper (extracelllar) and lower (cytoplasmic) cavities. White lines indicate approximate position of the lipid bilayer. From Sato *et al.*[438] Courtesy of Chikara Sato.

and 2005 residues, respectively, for Na^+ channels designated I and II.[441] In fact, mammals contain ten distinct Na^+ channel genes.[442] In every case the channel proteins contain four consecutive homologous sequences of about 300 residues apiece. Within these the hydro-pathy plots (see Fig. 2-30) suggest that each homology region forms six membrane-spanning helices as shown in Fig. 30-17A.[441] The four sets may then fold together into a square arrangement that provides a pore somewhat familar to that of the voltage-gated K^+ channel (see Fig. 30-18). The three-dimensional structure of the sodium channel protein, based on cry-electron microscopy, appears to be complex. The central channel may resemble that of Fig. 30-18, but there also seem to be smaller peripheral channels (Fig. 30-17).[438] Bacteria also contain Na^+ channels but they are tetramers of smaller subunits, resembling in this respect bacterial K^+ channels (Figs. 30-18).[442a,b]

How do the "gates" to ion channels open? Presumably some part of the channel protein senses the change in potential and undergoes an appropriate alteration in conformation that opens the gate.[434] The current carried by the ions flowing out through a small number or even a single channel can be measured with tiny **patch electrodes** having openings ~1 μm^2 in area. These are pressed against the nerve membrane, where they form a tight seal. With such a small patch of membrane surface the electrical noise level is low, and it is possible to measure the conductance of the pore.[149,443] From such measurements it was found that a single pore can allow $>10^8$ ions to pass through in one second. Another thing that is apparently measured with patch electrodes is a small **gating current**, which precedes the opening of the channels by ~0.1 ms. This has been interpreted as a flow of ~6 charges across the membrane or the movement of a larger number of dipoles needed to open the gate. One possibility is that a loss of the electrical field from the surface charges on the bilayer induces a rearrangement of charges on protein side chains within the bilayer or induces changes in interactions between two or more dipoles. Such changes could trigger conformation alterations within the proteins, allowing the channel to switch from open to closed.

Recordings with single channels indicate that after a sodium channel is open for a random length of time

it spontaneously closes and passes into a third state, an "inactive" state from which it cannot reopen during the refractory period. After the membrane is repolarized it can function again.[444a]

Calcium ion channels. Immediately after the Na^+ pores open as a result of membrane depolarization, voltage-sensitive Ca^{2+} channels also open. These allow a rapid influx of Ca^{2+}, which can trigger many processes including the secretion of neurotransmitters within the synapses.[434,444] There are several types of voltage-sensitive Ca^{2+} channels.[444a,b] The most abundant type are specifically inhibited by dihydropyridines and are called **dihydropyridine-sensitive** or L-type channels.[434,445–445b] They are most numerous in the transverse tubular membranes of skeletal muscle where they appear to form a complex with the very large calcium release channels, the **ryanodine receptors** (Fig. 19-21 and associated discussion).[446,446a] These channels appear to have a structure similar to that of the Na^+ channels.[434] Calcium channels are also discussed on p. 422 and on pp. 1114-1115. Calcium ions play a central role in cell signaling and there are a large number of different calcium channels in bacteria, plants, and animals. Many of these are coupled to specific receptors.[445b,447] Some are involved in controlling intracellular stores.[447–449] Some release Ca^{2+} in response to mechanical movement and function in feeling, hearing, maintaining balance, and cardiovascular regulation. Plants sense wind and gravity, and microorganisms sense changes in osmotic pressure with the aid of these channel proteins.[450–452]

Potassium ion channels. Several types of K^+-selective cation channels have been recognized on the basis of electrophysiological and pharmacological studies.[149] More recently, the cloning of channel genes has permitted the study of the proteins by X-ray crystallography. The first structure determined[452a] was that of the *Streptococcus lividans* K^+ channel (designated KcsA; Fig. 8-21). There are three large structural families of K^+ channel proteins.[453–455a] One group consists of voltage-regulated (K_v) channels, such as those involved in the action potential of neurons. Like the *S. lividans* channel, they are tetramers whose predicted structure contains six transmembrane helices per subunit with a pore-forming loop (P region) between helices 5 and 6. This is just what is seen in the *S. lividans* channel and in one-fourth of the much larger Na^+ channel protein (Fig. 30-17A). Furthermore, all known potassium channels, from bacteria to human beings, have the conserved sequence GYGD in the C-terminal half of the P region.[453] A great variety of K_v channels are known. There are ~70 genes for these channels in the *Caenorhabditis elegans* genome.[456] One of the first K_v channel genes to be cloned was from a *Drosophilia* mutant known for its neurological defect as *shaker*. Its structure (Fig. 30-18), which is based in part on modeling from the KcsA channel, has the ion selective filter with the conserved sequence **TVGYG** in the expected location. At the cytoplasmic end of the pore is an additional structure not found in the KcsA channel. This is the **inactivation gate**, so called because it accomplished the rapid self-inactivation of the K^+ channels during passage of the action potential (Fig. 30-18A). This is one of the factors necessary for recovery and repolarization of the axon membrane. The inactivation gate is composed of N-terminal ~130 residue "T1" domains of the α subunits together with parts of the β subunits, which are associated as a tetramer beneath the channel in the cytoplasm (Fig. 30-18B). Various experimental data including mutational analysis suggest that small ball-like domains at the N termini of the β subunits block the channel.[456–458] Zhou *et al*. propose that the N termini unfold into an extended conformation, passing through "windows" between the T1 domains and the channel and allowing the $-NH_3^+$ ends to bind into the central cavity in the channel.[459] The same site can be blocked by well-known quaternary amine inhibitors such as tetraethylammonium, tetrabutylammonium ions, or tetrabutylantimony, an analog used for X-ray crystallography.

The T1 domain of the channel not only participates in control of the ion flux but also stabilizes the pore complex.[459a] Among the various K^+, Ca^{2+}, and Na^+ channels the regulatory β subunits are quite variable in their structures and mechanisms of gating.[459b] Some β subunits have bound NADH. A speculative possibility (p. 737) is that the rapid interconversion of the positively charged thiazolium ion and negatively charged thiolate ion forms of thiamin (Eq. 7-19) plays some role in nerve conduction, e.g., voltage sensing.

Some questions about ion channels have been hard to answer. For example, how are small cations allowed to flow rapidly through a very small opening in a 2–3 nm thick nonpolar core of a membrane?[460,461] From basic electrostatic principles ΔG for transfer of an ion to the center of a membrane has been estimated as ~160 kJ/mol, a high thermodynamic barrier to transport. A solution to this problem apparently lies partly in the fact that at the center of the lipid bilayer the ion channel contains a cavity large enough (~0.5 nm diameter) to hold about 50 water molecules. Cations tend to enter this cavity, and X-ray studies have shown that the electron-dense Rb^+ does occupy the cavity. A second stabilizing factor is that four helices have their negative (C-terminal) ends pointing toward the cavity. Although the electrostatic effect of these helix dipoles (Fig. 2-20A) might be regarded as negligible, computations indicate that within the low dielectric bilayer the stabilizing effect of the helices becomes significant.[460,461]

How are the pores in these channels opened and closed? Different channels are gated in different ways.

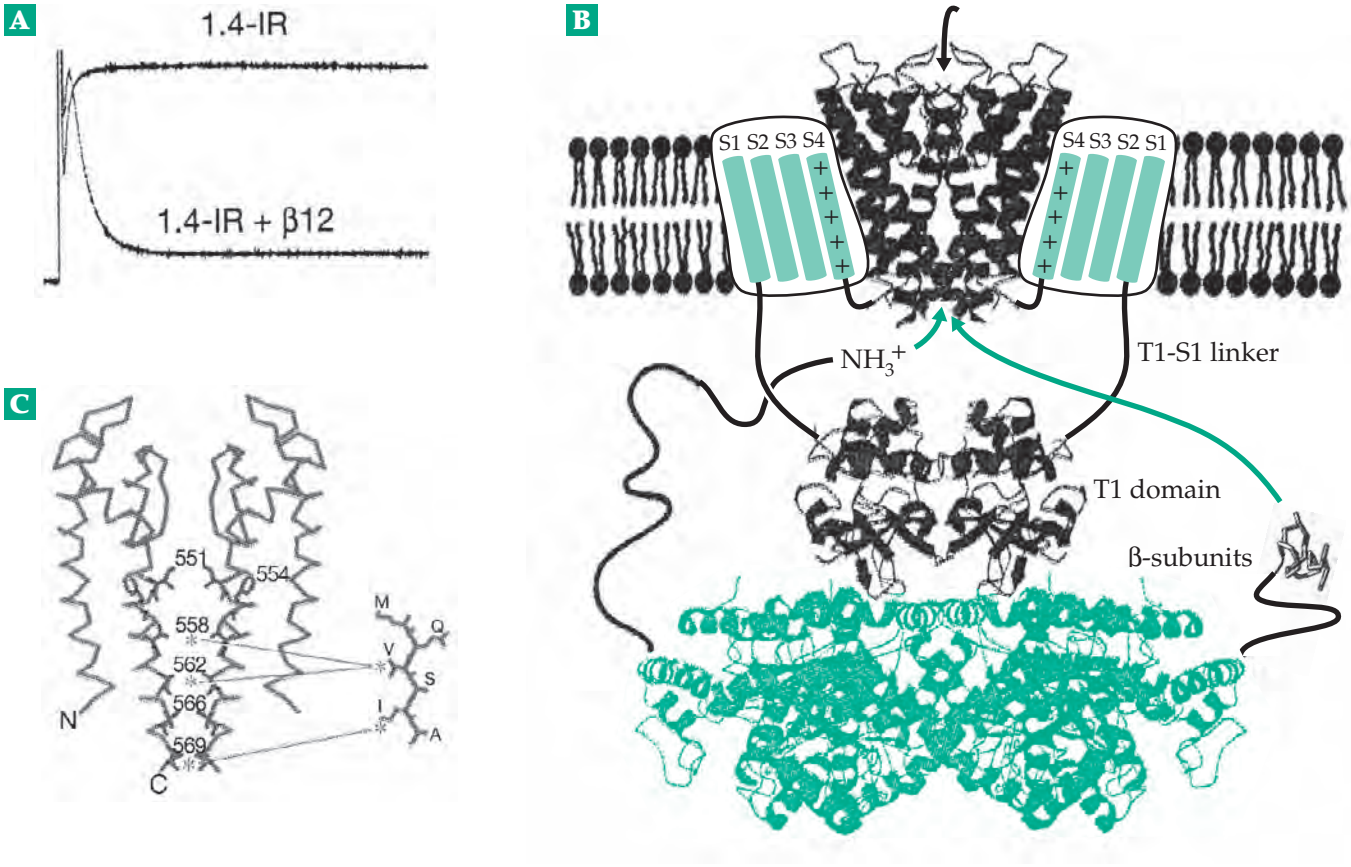


Figure 30-18 (A) K^+ currents recorded from *Xenopus laevus* oocytes carrying cloned genes of *Drosophila* shaker K^+ channels under two-electrode voltage-clamp conditions. Trace 1.4-IR was obtained from a cell expressing channels that lack the inactivation gate. Trace 1.4-IR + β_{12}, obtained from a cell expressing β subunits as well, shows rapid self-inactivation. (From Zhou *et al.*[459]) (B) Composite model of a voltage-dependent K^+ channel. The pore structure in the α subunit is represented by the KcsA channel (Fig. 8-21). The structure of the T1-β complex is from Gulbis *et al.*[458a] The drawing is modified from that of Zhou.[459] (C) Ball-and-stick view of the selectivity filter showing positions of four bound K^+ ions. Two of the four TVGYG peptide strands of the conduction pore are shown. Courtesy of Roderick MacKinnon.

The KcsA channel, which is mostly closed at neutral pH, responds by opening at a low external pH.[462] Using methods of spin labeling and EPR spectroscopy, Perozo *et al.* found small translational and rotational movements of the helices that form the pore (Fig. 30-18). These may alter the diameter of the pore, opening or closing it.[463] How do the electrostatic sensors control the process? The details are uncertain, but the sensor is thought to lie in a conserved sequence of arginine and lysine residues interspersed with hydrophobic amino acids in transmembrane helix 4 of the channel protein (Fig. 30-18; see also Fig. 30-17).[456] How do potassium pores select K^+ over Na^+ or Ca^{2+}? One factor is that Na^+ is more heavily hydrated than K^+ (p. 311). This allows K^+ to pass through the channel more readily than Na^+.[464] Potassium ions travel through the 1.2-nm-long selectivity filter at a rate of ~10^8 s^{-1} in consecutive steps of dehydration, movement, and rehydration occurring in ~10 ns.[464a–d] The process is catalyzed by polypeptides and may depend upon competition between a state in which a ring of four hydrogen-bonded peptide groups is formed and a state in which the four carbonyl groups coordinate a K^+ ion.[464d]

Belonging to the same structural group as the K_v channels are Ca^{2+}-regulated K^+ channels[465,466] Some bacterial channels are controlled by binding of Ca^{2+} ions to a "gating ring" on the intracellular membrane surface.[466a] A mammalian channel is controlled by a complex of calmodulin with the intracellular end of the α subunits of the channel[466b] and others.[453,467] A second large group of K^+ channels, containing seven subfamilies, are the **inward rectifying** (Kir) channels.[455,468] They are tetramers of 360- to 500-residue polypeptide chains, each chain forming two transmembrane helices with a P region between them.[453,469] These channels support a large conductance when K^+ ions flow out from a cell but only a small conductance when they flow in.[470] Kir channels are subject to a variety of controls, which include effects of pH.[471,472]

Some are inhibited by ATP,[473–474b] and others by eicosanoids[475] or inositol hexaphosphate.[476] Some of the ATP-sensitive channels contain an ABC transporter subunit and are binding sites for sulfonylureas and other drugs. See discussion on p. 421. A number of human disorders in Kir channels have been identified.[468] The human Kir channels participate in regulation of resting membrane potentials in K^+ homeostasis, control of heart rate, and hormone secretion.[468] A third group of K^+ channels are dimeric, but each subunit contains two tandem P regions and 4–8 transmembrane helices.[455]

Chloride channels and the ionic environment of neurons. All cells contain voltage-gated chloride channels, which are encoded by the *Clc* genes mentioned on pp. 420, 421.[477,477a] Recently crystal structures[477a–c] have revealed chloride channels formed in single polypeptide chains arranged as dimers. The selectivity filter involves stabilization by the positive ends of α-helix dipoles. The importance of the corresponding proteins to the human body is shown by the existence of several specific diseases arising from mutations in their genes (p. 420).[478,479] A calcium-regulated Cl^- channel is also present[480] as is the ATP-gated CFTR channel (Box 26-A).[480a] In addition, other ligand-gated Cl^- channels, such as γ-aminobutyrate receptor channels (Section B,9), are found in the central nervous system.[481] A glutamate-gated chloride channel in invertebrate organisms is the site of action of the antihelminthic and insecticidal compound **ivermectin**.[481a]

The significance of ion channels can be better appreciated by considering the ionic environment of nerve axons.[149] Mammalian neurons have roughly the following millimolar concentrations of ions in the cytosol and in the external medium. (The concentration gradients for the much-studied squid axon are substantially higher.[149,482]) The membrane potentials that could arise from each one of these concentrations, according to Eq. 8-2, are also given.[149] In a resting

	Cytosol	Extracellular	E_m (mV)
K^+	150	5.5	–90
Na^+	15	150	+60
Ca^{2+}	10^{-4}	1.5	+270
Cl^-	9	12.5	–70

neuron the K^+ potential dominates with an observed membrane potential of ~–80 mV. Some K^+ channels are open and the K^+ and Cl^- concentrations are nearly in Donnan equilibrium across the membrane. The Na^+ and Ca^{2+} channels are closed, and the sodium and calcium pumps keep the internal concentrations of these ions low.

When an action potential is propagated, a wave of depolarization moves along the axon, changing the membrane potential suddenly to a less negative value. When it reaches ~50 mV the Na^+ channels open, allowing sodium ions to flow into the cell causing further propagation of the wave of depolarization. After ~1–2 ms the Na^+ channels begin to deactivate. At the same time the slower K^+ channels open allowing potassium ions to flow out and to repolarize the membrane, the membrane potential sometimes transiently reaching more negative values (hyperpolarization) than the ~80 mV resting potential. Action of the Na^+,K^+-ATPase then restores the original state. The finely tuned properties and sequential opening and closing of the channel proteins are essential to the conduction of nerve impulses.

The existence of voltage-gated ion channels in bilayers are not limited to nerve membranes. They are present to some extent in all cell membranes. Even the paramecium has at least seven kinds of Na^+, K^+, and Ca^{2+} channels.[483] Channels may also be formed by many peptide antibiotics. Among them are the human defensins (Chapter 31) and the ~20-residue **alamethicin**. Six to eleven of the mostly helical monomers of that antibiotic assemble to form a single voltage-dependent channel.[484,484a] The bacterial toxin colicin E1 (Box 8-D) forms voltage-dependent channels within bacterial membranes.[485]

Receptor-associated ion channels. Many neurotransmitters, including acetylcholine and glutamate, act to open ion channels that are part of the receptor protein or of a tight complex of proteins.[149,486] Such **ionotropic receptors** are responsible for most rapid neuronal action. For example, binding of acetylcholine to its receptor in the neuromuscular junction causes the release of Ca^{2+} ions from the exterior into the muscle fibers. Binding of glutamate to its ionotropic receptor in a synaptic ending of a dendrite causes an influx of ions into the cytoplasm, initiating an action potential in the dendrite. In most instances the properties of the receptor channel favor the rapid flow of Ca^{2+} ions into the cytoplasm.

Many other receptors are 7-helix transmembrane proteins, which activate guanine nucleotide G proteins (Chapter 11, Section D, 3). The G proteins couple some receptors directly to Ca^{2+} channels; they couple other receptors to adenylate cyclase and cyclic AMP-activated channels and yet others via phospholipase C to K^+ channels and indirectly to Ca^{2+} channels (Fig. 30-19). All of these G protein coupled receptors are referred to as **metabotropic receptors**. A single synapse often contains both ionotropic receptors and metabotropic receptors. The ionotropic receptors induce a rapid (<1 ms) response, while the metabotropic receptors act more slowly. However, in most cases the final effect is the release of calcium ions into the cytoplasm

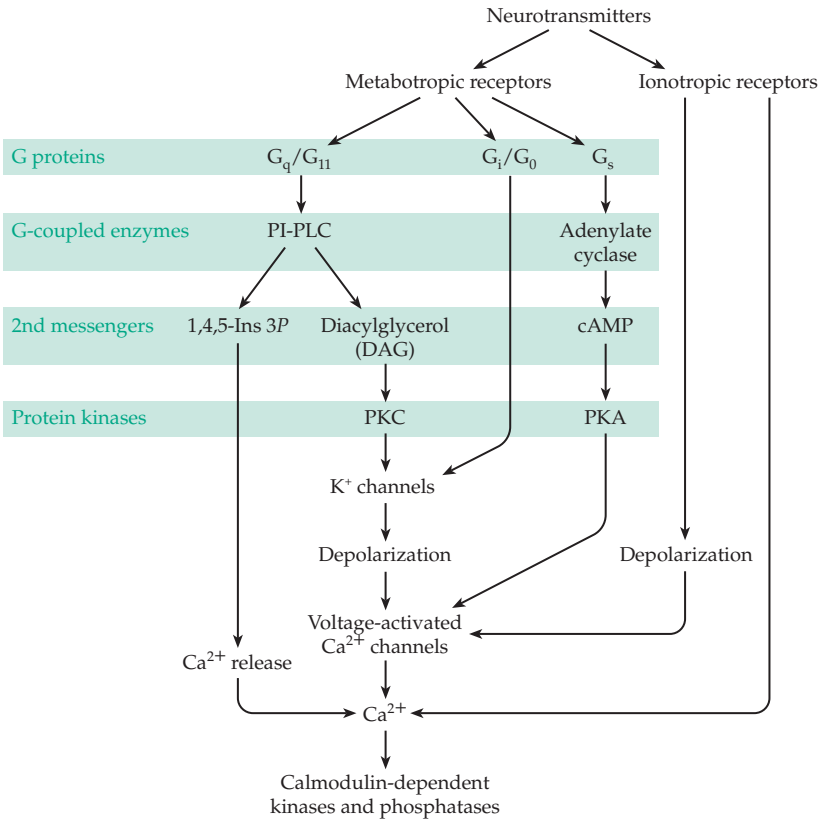


Figure 30-19 Major signaling pathways from metabotropic and ionotropic receptors in neurons. Various G proteins control the signaling from mutabotropic receptors using phosphatidylinisitol-specific phospholipase C (PI-PLC) and adenylate cyclase or acting directly on K^+ ion channels. Adapted from Fig. 5.1 of Nicholls' *Proteins, Transmitters, and Synapses*.[149]

(Fig. 30-19). The rapid response may be initiation of an action potential, while the slow response may be activation of calmodulin-dependent kinases and phosphatases.[149]

6. A Plethora of Neurotoxins

Bacteria, protozoa, and venomous animals synthesize numerous toxins that are used to kill their prey or to defend themselves. Sea anemones, jellyfish, cone snails, insects, spiders, scorpions, and snakes all make potent and highly specific neurotoxins. Plants form a host of alkaloids and other specialized products, some of which are specifically neurotoxic and able to deter predators. More than 500 species of marine cone snails of the genus *Conus* synthesize a vast array of polypeptide toxins (**conotoxins**),[487–489] some with unusual posttranslational modifications.[490,491] The slow-moving snails are voracious predators that use their toxins, which they inject with a disposible harpoon-like tooth,[492] to paralyze fish, molluscs, or worms.[493]

The targets for natural biological toxins include ion channels and receptors for transmitters. At least four parts of the voltage-gated sodium channels are binding sites for extremely toxic natural products.[494–499] **Tetrodotoxin** (Fig. 30-16),[496,497] which is found in the puffer fish, certain newts,[429a] and venom of the blue-ringed octopus, and also the shellfish poison **saxitoxin** (Fig. 30-16) block the entry of sodium ions into the channels.[498] **Bactrachotoxin** (Fig. 22-12) and related lipophilic compounds such as **veratridine** *increase* sodium permeability by blocking the channels permanently open. **Pyrethroid insecticides** (p. 1237) prolong the time that the sodium channels stay open after excitation. Some **scorpion toxins** (Fig. 30-16),[494,499] which all have a hydrophobic core made from a short α helix and a three-strand antiparallel β sheet,[500–502] and **sea anemone toxins**[495,503–505] also stabilize the open conformation of the Na^+ channels. Other smaller ~4-kDa scorpion toxins block K^+ or Cl^- channels or other receptors.[500,506,507] Some are most toxic to insects and others to mammals.[500] Although their three-dimensional structures resemble those of scorpion toxins, the amino acid sequences of anemone toxins show no homology.[505] The most potent poison produced by the red tide organism, the dinoflagellate *Gymnodinium breve* (Fig. 1-9), is **brevetoxin A** (Fig. 30-16).[436,508] It selectively opens one class of sodium channels.[495]

Venoms of **cobras**, **sea snakes**, and pit vipers contain several 6- to 7-kDa proteins that bind to acetylcholine receptors (Fig. 30-23) of the postsynaptic neurons, preventing binding of the neurotransmitter and opening of the ion channels.[509,510] All of these toxins contain four disulfide bridges and share with certain plant proteins a folding pattern that has been called the **toxin-agglutinin fold**[511,512] (Fig. 30-16). These toxins include **erabutoxin a** (Fig. 30-16) from a sea snake[513,514] as well as the 74-residue toxin **bungarotoxin a** (from the banded Krait). This toxin, which has been used to titrate acetylcholine receptors in neuromuscular junctions, is a member of the *long neurotoxin* group, which contains 71–74 residues and five disulfide bonds.[515] Other *short neurotoxins* are 60–62 residues in length with four disulfide bridges.[516] Cobra toxins contain both neurotoxins and **cardiotoxins**, which have somewhat similar structures but quite different modes of action.[517,518] In contrast, **crotoxin**

from the venom of a South American rattlesnake[510,510a] and **β-bungarotoxin**[519] consist of 13-kDa phospholipases A_2 complexed with smaller 7.5-kDa proteins. They act at the presynaptic membranes of selected neurons by blocking neurotransmitter release.[520]

The seven types of **botulinum toxin**[521–523a] and the **tetanus toxin**[524] are the most neurotoxic substances known. Only 10^8 molecules are sufficient to kill a mouse. Both toxins are zinc proteases, which block presynaptic transmitter release by cleaving specific synaptic vesicles proteins (see p. 1780 and Fig. 30-20).[522,523,525–528] They bind initially to ganglioside in the neuromuscular junction, one subunit then being internalized as with the diphtheria toxin (Box 29-A). Botulinum toxins specifically enter motor neurons,[521,528a] while tetanus toxin is taken up via synaptic vesicle endocytosis[529] by both peripheral and central neurons. Retrograde axonal transport carries the toxin into the central nervous system and across synaptic clefts into cholinergic interneurons, which are poisoned.

The black widow spider produces the 130-kDa **α-latrotoxin,** which causes massive release of acetylcholine, norepinephrine, dopamine, and GABA from synaptosomal endings.[530,531] The small **anatoxin-a** or "very fast death factor" (Fig. 30-22), which is synthesized by various cyanobacteria, antagonizes both muscarinic and nicotinic acetylcholine receptors.[532] Cone snails synthesize mixtures of the 13- to 17-residue conotoxins (Fig. 30-16).[493] They cause rapid paralysis of fish permitting the snails to prey on the much faster fish. They bind to a variety of targets, which include Na^+, K^+, and Ca^{2+} channels,[435,492] and acetylcholine,[533,534] and glutamate[490] receptors. One of the toxins is a 17-residue peptide containing five residues of γ-carboxyglutamate and is also notable for the fact that intercerebral injection of less than one microgram of the toxin induces a prolonged sleeplike state in mice.[490,493] The venom of *Conus geographicus* is so toxic that two-thirds of human stinging cases are fatal.

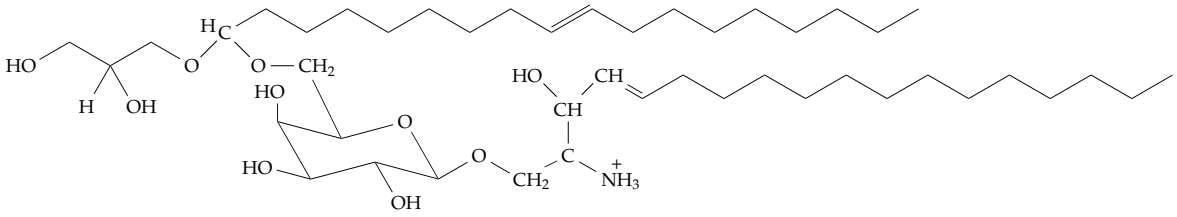
The most deadly nonproteinaceous toxin known, **palytoxin**, is also the most complex structure ever established without the aid of X-ray crystallography.[535,536] It is produced by marine zoanthids of the genus *Palythoa* and has the molecular formula $C_{129}H_{223}N_3O_{54}$.

7. Neuronal Metabolism

The brain has a very high rate of metabolism. Although accounting for only 1/50 of the body mass its utilization of energy amounts to 1/5 of the basal metabolism. This is ~20 watts and is nearly constant day and night. It reflects the unusually active metabolism of neurons, a major part of which can be attributed to the sodium–potassium ion pumps in the membranes and to the maintenance of the excitable state.[536a] The source of energy for these processes is the ATP that is utilized to drive the ion pumps and thereby to maintain the membrane potential needed to drive the action potentials. The ATP is formed largely by oxidative metabolism of glucose and, to a lesser extent, of acetoacetate. The large surface area of the axons as well as the frequency with which they transmit nerve impulses accounts for the high rate of metabolism.

Another factor peculiar to neurons doubtless contributes also to their rapid metabolism. The nucleus and most of the ribosomes are found in the cell body. Although few ribosomes are seen in axons and dendrites[536b], many proteins are needed in high concentrations within the axons and synaptic endings. Among these are enzymes catalyzing synthesis and catabolism of neurotransmitters and membrane proteins. If an axon is cut, the separated synaptic endings soon atrophy, an observation that long ago suggested that essential materials, which may include mRNAs,[537] might flow from the cell body. It has now been established experimentally that many materials do move at the rate of 0.3–3 mm / day from the cell body down the axon.[538] More remarkable is **fast axonal transport** by which proteins and other materials move at rates of up to 5 μm / s (0.4 m / day). This transport is specifically blocked by vinblastine (Box 7-D) and batrachotoxin (Fig. 22-12). As has been pointed out in Chapter 20, an ATP-hydrolyzing protein chemically related to the myosin heads functions together with microtubules to provide a kind of miniature railway that moves materials along the microtubules. Transport is sometimes in the opposite direction, i.e., from the synaptic endings to the cell body. This **retrograde axonal transport** may be of importance in altering neuronal properties in response to electrical activity at synaptic endings. It also provides a means of recycling materials originally sent in the other direction.

Brain cells appear to transcribe an unusually large fraction of the genome.[539–541] About 20% of the DNA of human brain was found to hybridize with mRNA formed by brain cells. In other tissues about half this amount of DNA appears to be transcribed. A related observation that seems surprising is the absence of common electrophoretic variants of enzymes in the brain.[539] However, brain cells synthesize specialized isoforms of many proteins, e.g., of the G proteins (p. 558), the cytoskeletal protein 4.1 (Fig. 8-14),[542] and transglutaminase.[543] Unusual lipids, such as the cationic acetal of a galactosylcerebroside shown above,[544] are also formed. Adult rat brain contains about 30,000 different kinds of polyadenylated messenger RNA,[540] much of which lacks the poly(A) tail.[513] Many of these mRNAs contain a specific 82-nucleotide sequence within at least one of their introns. Sutcliffe *et al.* suggest that this is an **identifier sequence** instructing brain cells to express these genes.[540,545] However, the sequence is also found in genes transcribed in other tissues, and its significance is not clear.[546,547]



8. Synapses and Gap Junctions

Like the micro-transistors in a computer chip, synapses are the devices by which the brain operates. Synapses process and integrate information from many input channels, send signals on to other neurons, and store information. The information is not stored in digital form, but as chemical alterations in the synapses themselves.[482,548,549] Synapses are formed when axons, growing in response to a chemical trail, reach their destinations and send out branches, each with a bulbous terminal knob (**bouton**). When these boutons meet receptive regions on dendrites of another axon, synapses are formed.[550] The synapse is a very firm connection with a thin, tight synaptic cleft through which signaling takes place. It is surrounded in part by astrocytes or other glial cells (Fig. 30-20A,C).

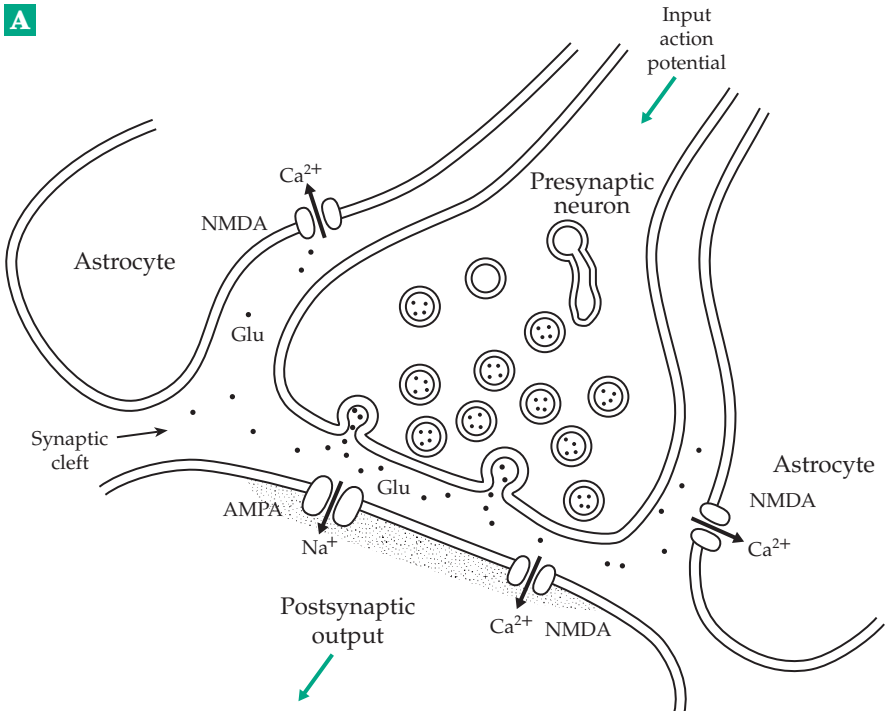
With the advent of electron microscopy, the fine structure of synaptic contacts became evident. The synaptic knobs were often found to contain vesicles of ~30–80 nm diameter, which were later shown by chemical analysis and staining procedures to contain the neurotransmitters (Fig. 30-10). In the case of the acetylcholine-releasing synapses (**cholinergic synapses**) each 80-nm vesicle contains ~40,000 molecules of acetylcholine,[551] the concentration in the vesicle being of the order of 0.5 M. To show that the acetylcholine released at a synapse stimulated the postsynaptic membrane to initiate an impulse, the technique of **electrophoretic injection** or **microiontophoresis** was developed.[552] By using ultramicrocapillaries a small pulse of current, e.g., 3 x 10^8 amp for 1 ms, can be used to inject electrically a compound directly into a synaptic cleft. The results may be observed with separate recording electrodes, one of which is inserted into an axon or a muscle fiber. By this means it was shown that amounts of acetylcholine comparable to those released at the large synapses of the neuromuscular junction do cause muscles to contract.

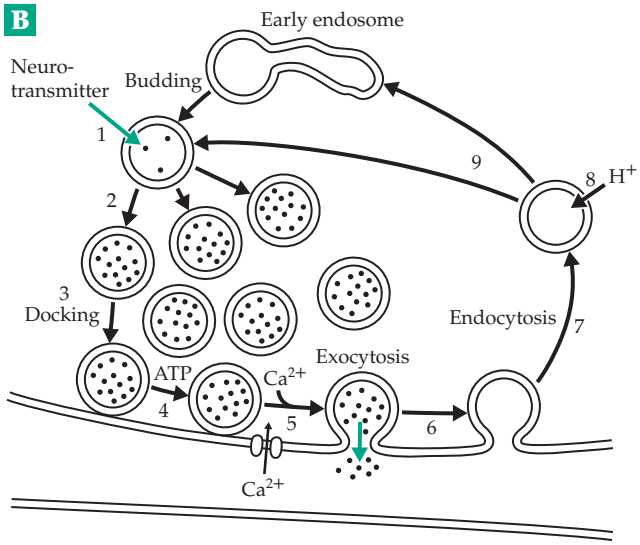
How does the release of neurotransmitter occur? That the release is "quantal," i.e., involving the entire content of a vesicle, was established from the observation of **miniature end-plate potentials**. These are fluctuations in the postsynaptic potential observed under conditions of weak stimulation of the presynaptic neuron. They reflect the randon release of neurotransmitter from individual vesicles.[553] Normally, a strong impulse will release on the order of 100–200 quanta of transmitter, enough to initiate an action potential in the postsynaptic neuron.

A synaptic vesicle cycle. The number of synaptic vesicles in a single synapse in the brain varies from fewer than 100 to several hundred. In specialized synapses there may be thousands. However, at any moment only a fraction of the total are in the "active zone," often aligned along the presynaptic membrane (Fig. 30-20A) or in specialized ribbons such as those in Fig. 30-10B. The vesicles are normally reused repeatedly, undergoing a cycle of filling with neurotransmitter, translocation to the active zone, ATP-dependent priming, exocytosis with release of the neurotransmitter into the synaptic cleft, coating with clathrin, endocytosis, and acidification as outlined in Fig. 30-20B.[554–557] The entire cycle may be completed within 40–60 s to avoid depletion of active vesicles.[558,559] A key event in the cycle is the arrival of an action potential at the presynaptic neuron end.

The accompanying depolarization of the membrane at the synaptic ending permits a rapid inflow of calcium ions through a voltage-gated calcium channel.[444,560] Within less than 0.1 ms the transient increase in intracellular [Ca^{2+}] triggers the release of the contents of the vesicles. About four calcium ions are needed to release one clathrin-coated vesicle (Fig. 30-20A,B). The membrane fusion required for transmitter release involves cytoskeletal proteins of the synaptic endings as well as specialized proteins that are present in the membranes of the synaptic vesicles (Table 30-6). In fact, every step in the cycle depends upon specialized proteins.[387]

Synaptic vesicles can be isolated in large quantities. Their composition is well known, and the proteins have been studied intensively. Indeed, much of what we know about exocytosis and vescular transport has been learned from investigation of synaptic vesicles.[554,561,562] A small synaptic vesicle of 35 nm diameter will contain ~10,000 phospholipid molecules in its membrane and only about 200 protein molecules, at least one of which must be a 13-subunit vacuolar type proton pump (Fig. 18-14). This pump acidifies the vacuole, allowing uptake of a neurotransmitter. Although many different proteins may be found in synaptic membranes, only about 15, which are listed in Table 30-6, are found in all synaptic vesicles and appear essential to function.





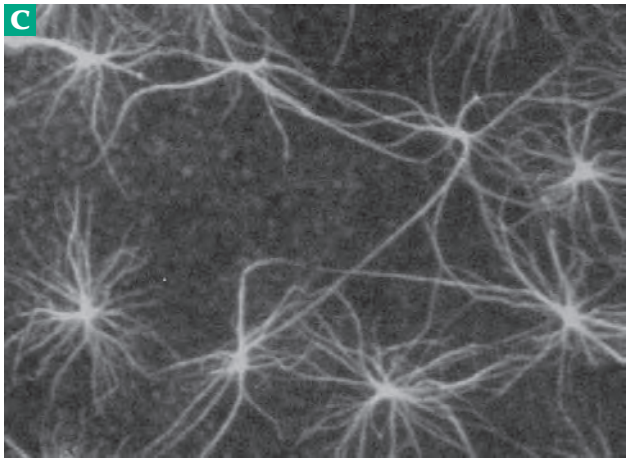
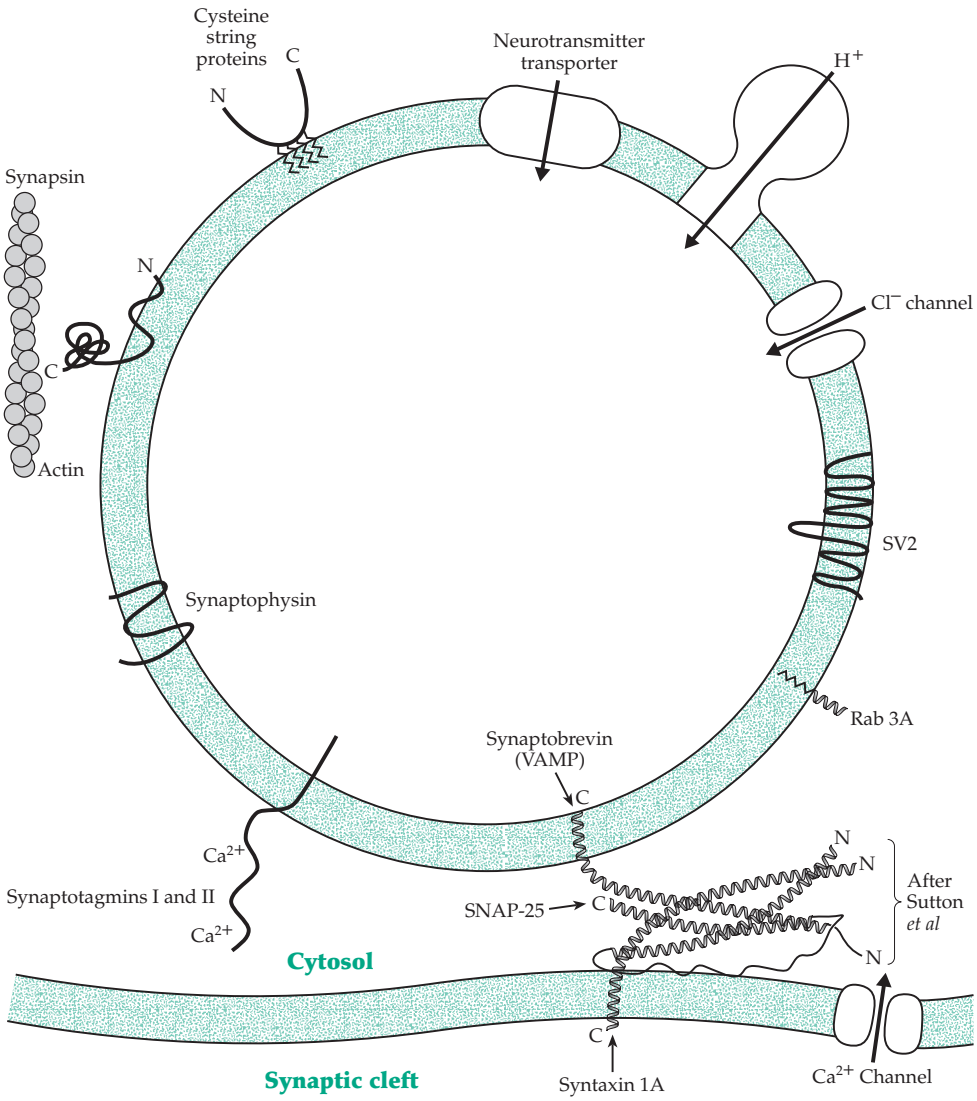


Figure 30-20 (A) Schematic drawing of a fast glutamatergic synapse. An action potential arrives at the synapse, depolarizing the presynaptic membrane and allowing calcium ions to enter the cytoplasm via voltage-gated Ca^{2+} channels. The Ca^{2+} ions induce exocytosis of small synaptic vesicles from the "active zone" near the membrane, releasing glutamate into the synaptic cleft. After diffusing rapidly across the narrow ~50-nm synaptic gap the glutamate binds to its receptors on the ending on a dendrite from a second (postsynaptic) neuron. Glutamatergic synapses usually have two types of receptor, NMDA and AMPA (see Fig. 30-24 and text). Both are ligand-gated ion channels, which release Ca^{2+} and Na^+ into the cytosol of the postsynaptic ending depolarizing its membrane and possibly initiating an action potential. (B) The synaptic vesicle cycle. The synaptic vesicles, which are formed by budding from an early endosome, are filled with neurotransmitter (1). The filled vesicles are then transported to the active zone near the presynaptic membrane (2), are "docked" on the membrane surface (3), and undergo ATP-dependent priming (4). Binding of four Ca^{2+} ions induces exocytosis and rapid release of the neurotransmitter (5). The empty vesicles receive a clathrin coat (6) and undergo endocytosis (7) and uptake of protons (8) to acidify the content in preparation for a second round of neurotransmitter uptake. Alternatively the vesicle can fuse with an endosome as part of the cycle. After Südhof and Scheller.[554] (C) Small section of brain stained to reveal the astrocytes whose extensions form synapses not only with neurons, as in (A), but also with capillary blood vessels.[149] From Kimelberg and Norenberg.[564] Micrograph from Andreas Karschin, Heinz Wässle, and Jutta Schnitzer. (D) Illustration of some proteins essential to the synaptic vesicle cycle. Several are integral membrane proteins. Synaptotagmins contain Ca^{2+}-binding domains and may serve as calcium sensors. The vesicle is portrayed as if docked to the presynaptic membrane by interaction of the SNARE proteins synaptobrevin, syntaxin, and synaptotagmin. The 4-helix bundle is as portrayed by Sutton *et al.*[563]



The synaptic vesicles, which are formed by budding from early endosomes, take up neurotransmitters using one of the transporters (step 1 in Fig. 30-20B). Transmitter uptake is G-protein dependent[565] and is driven by the proton electrochemical gradient generated by a vacuolar type (V-type) ATPase (Chapter 18).[149,566] The filled vesicles move into the active zone where they undergo an ATP-dependent priming of uncertain nature.[555,567] Exocytosis (step 5 in Fig. 30-20B) requires membrane fusion, and it is possible that partial fusion occurs during the priming steps. Priming is also thought to involve interaction between vesicle-associated v-SNARES and synaptic membrane-associated t-SNARES (p. 521).[556,563] A major v-SNARE has been identified as **synaptobrevin**, which is also known as **VAMP** (vesicle-associated membrane protein).[563,568,568a] The C-terminal-anchored synaptobrevin is inserted into the plasma membrane of neuronal and neuroendocrine cells prior to endocytosis and budding of the synaptic vesicles.[568] The target t-SNARES have been identified as the synaptic plasma membrane proteins **syntaxin**[568b] and **SNAP-25**.[569–573] Syntaxin is an integral membrane protein, whereas SNAP-25 is anchored by palmitoylation.[571] These proteins bind together to form a synaptobrevin•syntaxin•SNAP-25 complex, which forms a four-helix bundle as shown in Fig. 30-20. Synaptobrevin and syntaxin each contribute one helix, while SNAP-25 provides two; all four have a mutually parallel orientation.[563,574,574a] The helix bundle is so tight that it has a high melting temperature and is resistant to proteolytic cleavage. Nevertheless, the helical domains of both synaptobrevin and syntaxin are sites of very specific cleavage by the zinc proteases of tetanus and botulinin toxins.[527,563,570] Cutting of the protein chains by these toxins prevents proper formation of the four-helix bundle and prevents release of neurotransmitter. It is thought that the complex, which probably forms at several points on the periphery of the docked synaptic vesicle, is essential for membrane fusion.

Other proteins are also needed. All cell fusion processes seem to require regulatory proteins that are essential to neurotransmission in the nematode *C. elegans*. Two of these are encoded by the nematode genes *unc*-13 and *unc*-18. The corresponding mammalian proteins **munc-13** and **munc-18** interact with syntaxin and are essential for exocytosis of synaptic vesicles.[572,575] An ATPase is also needed for correct functioning of the SNARE complex[574] as are other additional proteins.[570]

Details of the control of exocytosis are also uncertain. **Synaptotagmin I**, which contains two Ca^{2+}-binding domains, is probably the sensor that detects the rapid influx of Ca^{2+} that initiates exocytosis.[576–578b] It binds several Ca^{2+} ions via a β-sandwich motif that contains five aspartate side chains at its tip. This motif is conserved in a large family of synaptotagmins. A possibility is that Ca^{2+}–synaptotagmin complexes may self-associate to form a protein ring around the site where the fusion pore forms.[576] Synaptotagmin I also interacts with both syntaxin and with **neurexins**, proteins related to laminin (Fig. 8-33) and present in numerous variant forms in nerve endings. Neurexins are also targets for the α-lathrotoxin of the black widow spider.[531,579] Other proteins that may participate in membrane fusion include the unique **cysteine string proteins**, which in *Drosophila* contain 13 cysteine residues, 11 of which are palmitoylated.[580,581] Nitric

TABLE 30-6
Some Proteins Important to the Formation and Functioning of Synaptic Vesicles[a]

1. Synaptic vesicle proteins	
Synapsins Ia, Ib, IIa, IIb	Peripheral, abundant
Rab3, rabphilin	Rab 3 has lipid anchor
Cysteine string proteins (CSP)	Ca^{2+}-binding
Synaptotagmins	Single transmembrane helix; Ca^{2+} receptor N terminus in vesicle
Synaptobrevins (VAMPs)[b]	SNARE proteins, C termini in vesicle
Synaptophysins, synaptogyrin	Integral membrane protein
SV2 A, B, C	Integral membrane protein, Cl^- transporter
SCAMPS 1 and 4	Integral membrane protein
SVOP	Integral membrane protein
Vacuolar H^+ pump	13 subunits
Cytochrome 561	H^+ generator
Neurotransmitter transporters	For acetylcholine, glutamate, GABA/glycine, catecholamines, ATP
Ancillary transporters	Zn^{2+}, Cl^-
2. Presynaptic membrane proteins	
Syntaxin [b]	t-SNARE
SNAP-25[b]	t-SNARE
Munc-13	
Ca^{2+} channel	
Agrin	
Neurexin	
Actin and microtubules	In dendrites
3. Postsynaptic specializations	
Receptors	e.g., NMDA, AMPA

[a] Based on data of Südhof and Scheller: Südhof, T. C., and Scheller, R. H. (2000) in *Synapses* (Cowan, W. M., Südhof, T. C., and Stevens, C. F., eds), pp. 177–215, Johns Hopkins Univ. Press, Baltimore, Maryland and Südhof, T. C. (1995) *Nature (London)* **375**, 645–653.

[b] Targets for clostridial toxins, tetanus, botulinin.

oxide NO may be involved in a late stage of exocytosis,[392] and phospholipase D1 may also be required.[582]

Presynaptic nerve terminals may contain as few as a hundred vesicles, which must be recycled rapidly after exocytosis in order to allow for repetitive firing.[558,559,583] Several proteins are needed for endocytosis (step 7 in Fig. 30-20). These include **endophilin I**,[584] the vesicle transport ATPase **NSF**,[574] GTPases,[565] and the soluble NSF attachment protein α-SNAP (which is not related to SNAP-25).[585]

Functions of some other abundant proteins of synaptic vesicles have not yet been accurately defined. The **synapsins** are abundant peripheral membrane ATP-binding proteins with multiple phosphorylation sites and variable C-terminal domains that interact with cytoskeletal proteins such as actin microtubules, microfilaments, and spectrin.[554,561,586,587] Another abundant protein is **synaptophysin**, an integral membrane protein found in all synaptic vesicles.[554,561,588] Other proteins are discussed by Südhof and Scheller.[554] The small G protein **rab 3** together with the Ca^{2+}-binding protein **rabphilin** participate in a G-protein cycle that helps to drive exocytosis.[554] Synaptotagmin, as well as clathrin assembly proteins bind inositol hexaphosphate (InsP_6; Fig. 11-9), which undergoes active turnover in synapses. This suggests a role for InsP_6 in the endocytosis steps of the synaptic vesicle cycle.[589] The brain is rich in zinc ions. Much of the Zn^{2+} is bound into zinc finger domains of transcriptional regulators, but much is also present in a relatively free form within synapses of the hippocampus, cerebral cortex, and other regions.[590,591] Zinc ions may function as a neuromodulator in glutamatergic synapses.[591]

What does a neurotransmitter do at the postsynaptic membrane? In the case of acetylcholine in neuromuscular junctions the principal action appears to be one of opening sodium channels and thereby depolarizing the postsynaptic membrane. If enough nerve impulses arrive, an action potential will be initiated in the postsynaptic neuron. In other cases, the first response may be activation of a protein kinase either directly or by opening a channel for Ca^{2+}, which indirectly regulates protein kinases and phosphatases.[592] Thus, a complex cascade may be activated. See also Fig. 30-19.

The postsynaptic nerve ending, which is usually the tip of an axonal dendrite, has its own set of proteins, which varies to some extent with the nature of the neurotransmitter. In excitatory cells the plasma membrane of the postsynaptic neuron is thickened to ~30–40 nm to form the "**postsynaptic density**," a disc-like structure of clustered receptors of two types, which extends ~ 30 nm into the cytosol.[593,594] Only single receptor channels are indicated in Fig. 30-20, but many receptors are present in the clusters[594,595] as are other specialized proteins. One of these, designated PSD-95, was found to associate with the NMDA receptor using the yeast two-hybrid system (Box 29-F).[594] Neuronal nitric oxide synthase may also be present.

The large neuromuscular junctions, which contain clusters of acetylcholine receptors, have wider synaptic clefts (> 40 nm), which contain basal lamina, a dense network of collagen fibrils together with the heparan sulfate proteoglycan **agrin** (p. 437). Agrin activates a muscle-specific kinase MusK, which phosphorylates the acetylcholine receptors inducing clustering of the receptors together with other proteins embedded in the plasma membrane and binding to the cytosolic protein **rapsyn** (see Fig. 30-23B).[596,597] Agrin is also a component of **immunological synapses,** which are important in lymphocyte development (Chapter 31).[596,598,599] The neuromuscular junction is formed between two cell types, a neuron and a muscle myotube. Both contribute proteins, which include a muscle-specific laminin.[600]

Astrocytes and other glia. Although the glial cells greatly outnumber neurons, they were long regarded simply as glue, as implied by the name glia. We know now that the several types of glial cells have functions in many different aspects of brain chemistry.[149,564,601–605] The oligodendrocytes generate myelin sheaths around many brain neurons. Macrophages that invade the brain differentiate into **microglia** that serve as part of the innate immune system (Chapter 31). **Bergmann glia** of the cerebellum help guide axons during brain development. The astrocytes have many processes, which not only contact synapses directly (Fig. 30-20A,D) but also form contacts with capillary blood vessels. They often contain receptor ion channels of the same types as are found in postsynaptic membranes (see Fig. 30-20A) and respond to Ca^{2+} influx as do neurons.[602–603a] Glia often take up neurotransmitters and ions from synapses in order to prepare for consecutive nerve impulses. Glia may also control the number of synapses formed,[604–604b] and they may have other roles in brain development. For example, an iodothyronine deiodinase (Eq. 15-60) is expressed primarily in neonatal brain, where it supplies thyroid hormone essential to brain development.[605]

Gap junctions in synapses. Not all neurons communicate via chemical synapses. Gap junctions, which are found in both neurons, astrocytes, and other cells, serve as **electrical synapses**. Thus, heart cells are all electrically coupled together by gap junctions.[606–608] Gap junctions are formed with the aid of hexameric **connexons,** which are present in each of the opposed membranes and are aligned one with the other (Fig. 1-15F,G).[607,609,610] There may be thousands of connexons in a single gap junction, which resemble ion channels in appearance but contain pores ~1.5 nm in diameter. They are formed from 26- to 43- kDa

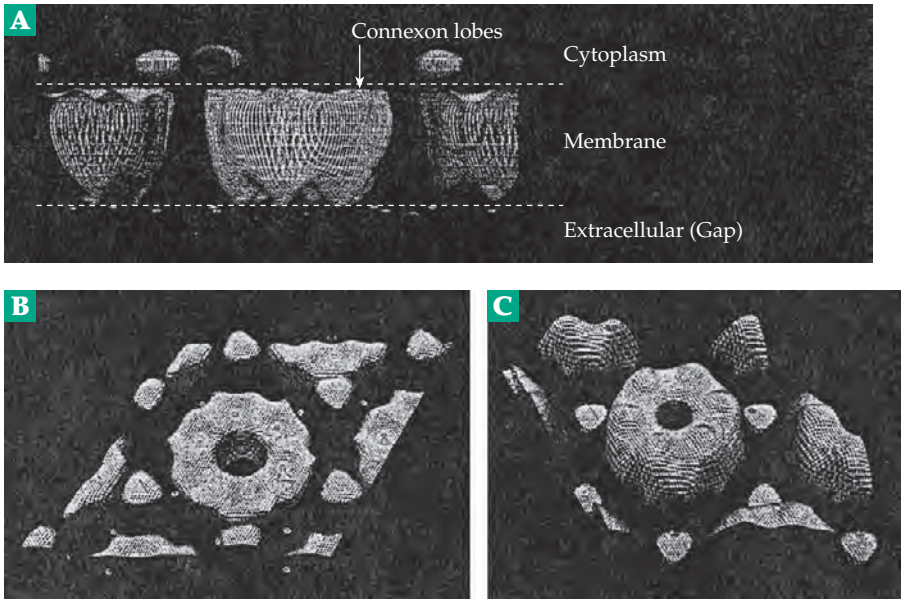


Figure 30-21 Images of gap junction connexins obtained by electron crystallographic methods at a resolution of 1.6 nm. (A) Cross-section. The thinkness of the (43 x 6) kDa hexameric connexin is 5.0 mm. (B) View of the connexin from the cytoplasmic side. (C) View from the extracellular side. From Perkins, Goodenough, and Sosinsky.[609] Courtesy of Guy Perkins.

protein subunits of the multigene family of **connexins**.[610–611a] Each gap junction consists of a pair of hexameric rings of connexins (Fig. 30-21), one ring from each of the two juxtaposed membrane surfaces.[609] Defects in connexins cause inherited deafness, neuropathy, malignancy, and cataract formation.[612–613a] The connexin subunits each contain four transmembrane helices and are related structurally to the peripheral myosin protein 22, the myelin proteolipid (p. 1767), and the protein stargazin (p. 1901), which is involved in synapse formation in the brain.[428a]

Another type of channel has been recognized quite recently. An ion channel, which regulates Mg^{2+} ion transport in kidney tubules, forms within the tight junctions that seal the extracellular space between cells (Fig. 1-15B). A protein **paracellin** forms channels through the tight junction protein complexes that surround the cells.[614,615]

9. Neurotransmitters

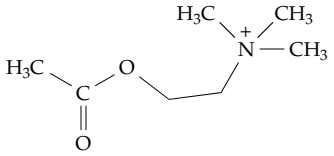
Studies of neuromuscular junctions of the autonomic nervous system as early as 1904 led to the suggestion that adrenaline might be released at the nerve endings. Later it was shown that, while adrenaline does serve as a transmitter at neuromuscular junctions in amphibians, it is primarily a hormone in mammals. Nevertheless, it was through this proposal that the concept of chemical communication in synapses was formulated. By 1921, it was shown that acetylcholine is released at nerve endings of the parasympathetic system, and it later became clear the motor nerve endings of the somatic system also release acetylcholine.

Acetylcholine is an established neurotransmitter because it meets five important criteria: (1) a synthetic mechanism exists within the presynaptic neuron; (2) a mechanism of storage (in vesicles) is evident; (3) the transmitter is released in proportion to the strength of the stimulus (frequency of firing); (4) postsynaptic action of the transmitter has been demonstrated directly by microiontophoresis; and (5) an efficient means for inactivation of the transmitter is present. The same five criteria must be met by other compounds if they are to be considered as transmitters.

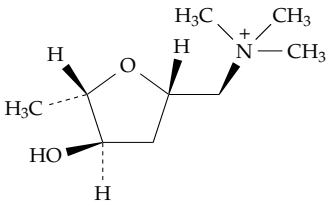
At present, in addition to acetylcholine, glutamate, and γ-aminobutyrate (GABA), glycine, noradrenaline (norepinephrine), and dopamine and 5-hydroxytryptamine (serotonin) are regarded as established transmitters. Other probable (**putative**) or possible **candidate transmitters** are also known. Aspartate, taurine, and a large number of peptides (Tables 30-1, 30-4) are under consideration.

Some transmitters, including noradrenaline, dopamine, serotonin, and various neuropeptides, are sometimes called **neuromodulators** rather than neurotransmitters. These compounds may not initiate a nerve impulse but may act on adenylate cyclase to increase or decrease cAMP levels and protein kinase activity. They may also diffuse through the extracellular space to influence a region of the brain greater than a single synaptic cleft. However, the distinction between transmitters and modulators is not exact.

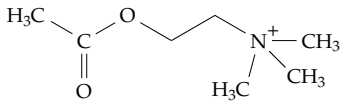
For many years it was assumed that a single neuron released only a single transmitter. We know now that this is incorrect.[616] For example, enzymes in neuromuscular junctions synthesize not only acetylcholine but also catecholamines, taurine, and GABA.[617] Some synapses in the central nervous system release both glycine and GABA.[618]



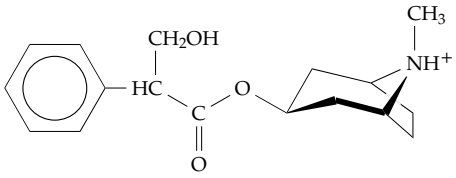
Acetylcholine in a skewed conformation



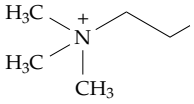
L(+)-Muscarine, a cholinergic agonist from the mushroom *Amanita muscaria*

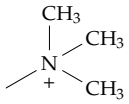


Acetylcholine in extended conformation

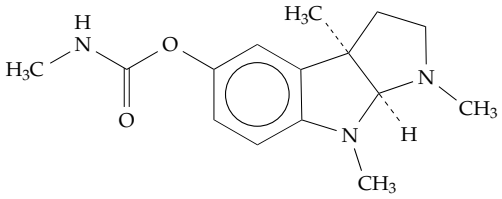


Atropine

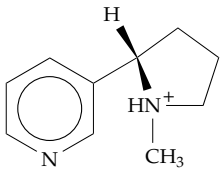




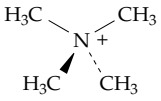
Decamethonium, a synthetic drug with potent curare-like activity



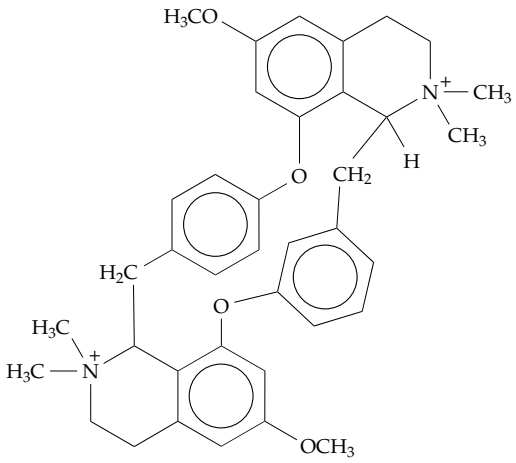
Physostigmine, an acetylcholinesterase inhibitor widely used in treatment of glaucoma. Compare with other carbamate esters (Box 12-E)



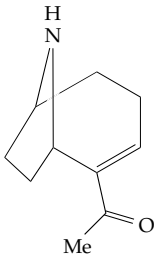
Nicotine (protonated)



Tetramethylammonium ion



D-Tubocurarine, the principal ingredient of South American arrow poisons. Blocks cholinergic receptors in skeletal muscle



Anatoxin-a (very fast death factor) from the cyanobacterium *Anabaena*

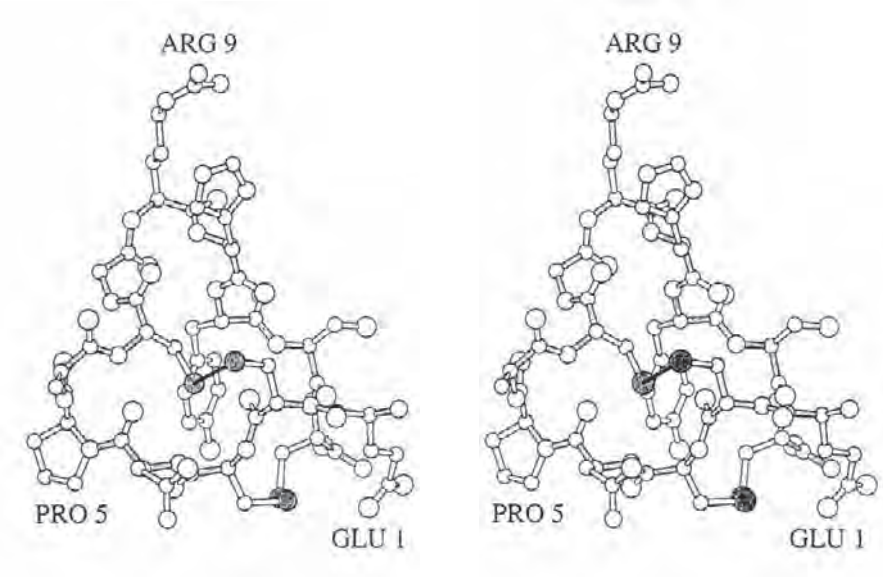


Figure 30-22 Some inhibitors of cholinergic synapses. The structure of conotoxin GI is from Guddat *et al.*[533] Courtesy of A. B. Admundson.

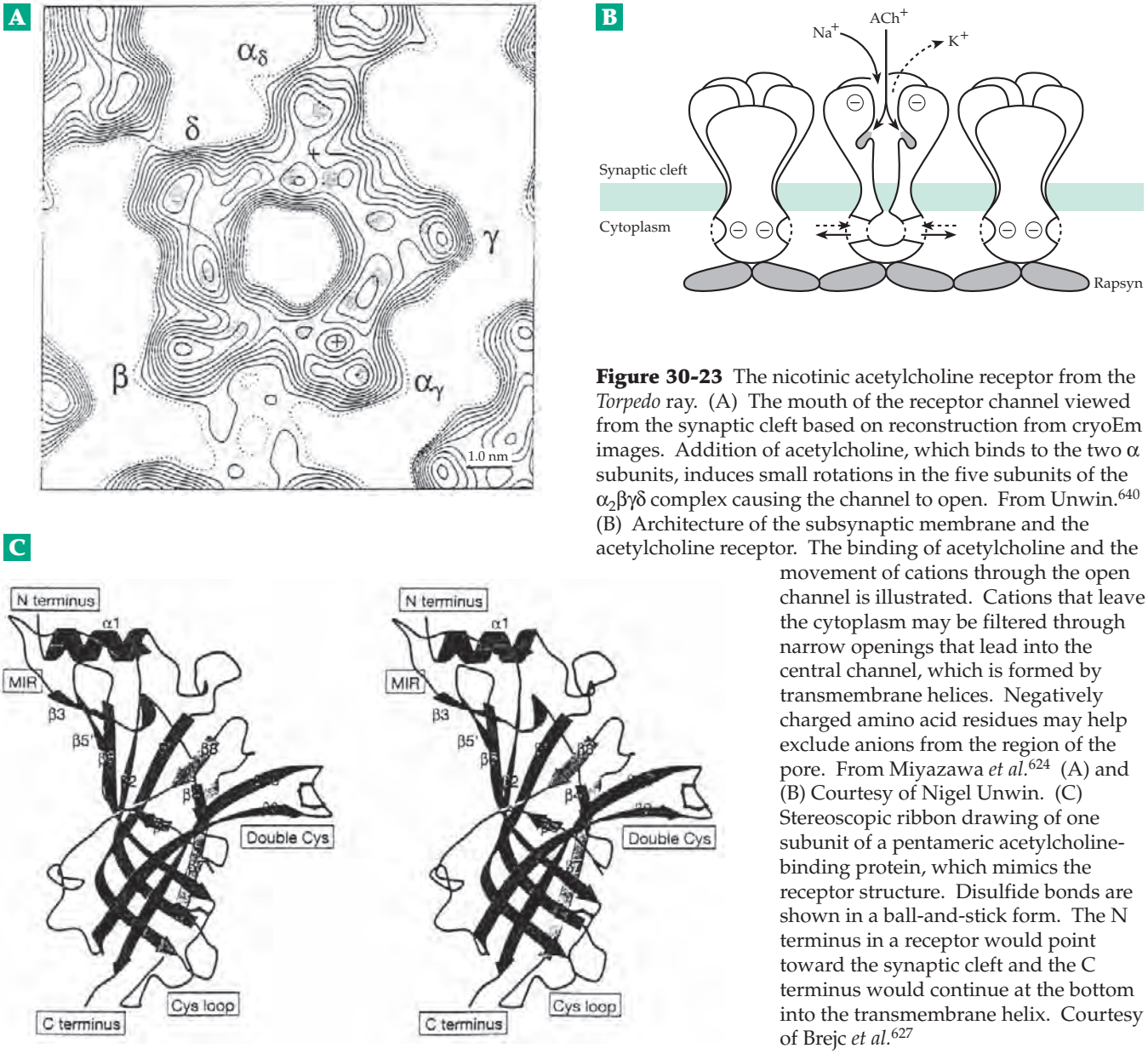


Figure 30-23 The nicotinic acetylcholine receptor from the *Torpedo* ray. (A) The mouth of the receptor channel viewed from the synaptic cleft based on reconstruction from cryoEm images. Addition of acetylcholine, which binds to the two α subunits, induces small rotations in the five subunits of the $\alpha_2\beta\gamma\delta$ complex causing the channel to open. From Unwin.[640] (B) Architecture of the subsynaptic membrane and the acetylcholine receptor. The binding of acetylcholine and the movement of cations through the open channel is illustrated. Cations that leave the cytoplasm may be filtered through narrow openings that lead into the central channel, which is formed by transmembrane helices. Negatively charged amino acid residues may help exclude anions from the region of the pore. From Miyazawa *et al.*[624] (A) and (B) Courtesy of Nigel Unwin. (C) Stereoscopic ribbon drawing of one subunit of a pentameric acetylcholine-binding protein, which mimics the receptor structure. Disulfide bonds are shown in a ball-and-stick form. The N terminus in a receptor would point toward the synaptic cleft and the C terminus would continue at the bottom into the transmembrane helix. Courtesy of Brejc *et al.*[627]

Cholinergic receptors and their agonists and antagonists. Among the acetylcholine-releasing (cholinergic) neurons are the motor neurons that form synapses at neuromuscular junctions, the preganglionic neurons of the entire autonomic system, and the postganglionic neurons of the parasympathetic system. There are also many cholinergic synapses within the brain. In contrast, in insects neuromuscular transmission is mediated by glutamate while acetylcholine is the principal neurotransmitter in the central nervous system.[619]

Important in the study of neurotransmitters is the identification of specific agonists, which mimic the action of a transmitter, and of antagonists, which block the action of the transmitter. Two groups of compounds influence acetylcholine-secreting neurons, leading to the classification of these neurons either as **muscarinic** (activated by muscarine; Fig. 30-22) or **nicotinic** (stimulated by nicotine). The muscarinic receptors, which are found in many autonomic neurons, are specifically inhibited by **atropine** and **decamethonium** (Fig. 30-22). The nicotinic synapses occur in ganglia and skeletal muscle. They are inhibited by curare and its active ingredient **D-tubocurarine** (Fig. 30-22) and by the protein snake venom **α-bungarotoxin**. This toxin has been used to titrate the number of acetylcholine receptors in the motor end plate of the rat diaphragm. About 4×10^7 receptors per end plate (or 13,000 / μm^2) were found.[620]

Nicotinic receptors (nAChRs; Fig. 30-23) of the type found in neuromuscular junctions are most frequently isolated from the electric organs of the electric

eel *Electrophorus* or from electric fish of the genus *Torpedo*. They have been studied more intensively than any other receptor.[621–626a] They contain four kinds of subunit with a stoichiometry $\alpha_2\beta\gamma\sigma$ and molecular masses of 39, 48, 58, and 64 kDa respectively. The amino acid sequences of the four proteins contain homologous regions, some of which are thought to represent membrane-spanning segments of the peptides. These receptors are ligand-gated ion channels and are closely similar to $GABA_A$ and $GABA_C$ receptors, to glycine receptors, and to 5-hydroxytryptamine (serotonin) receptors of the 5-HT_3 type. Parts of their amino acid sequences are also homologous to those of both the voltage-gated Na^+ channels and gap junctions,[433,627] suggesting that the transmembrane domain may resemble that of Fig. 30-18.[628] However, notice the difference in symmetry. Acetylcholine binds to the two α subunits (Fig. 30-23). Neurotoxins may bind at several sites.[629] Some indication of the possible function of the various subunits comes from studies of the neuromuscular junction in which the different subunits are degraded at different rates with half-lives of from one to ten days. During development fetal ε subunits are replaced by adult γ subunits. Perhaps more rapid changes in receptor composition are sometimes needed.[630]

Similar nAChRs are also found in the brain.[621,631,632] However, they are not identical but have at least 17 differing amino acid sequences (α1–α10, β1–β4, γ, δ, and ε). The neuromuscular junction receptor (muscle type) from fish is described as $(\alpha1)_2 \bullet \beta1 \bullet \gamma / \varepsilon \bullet \delta$.[626] The brain contains homopentamers of subunits α7, α8, and α9 as well as various heteropentamers. The various forms possess different affinities for acetylcholine and for antagonists such as nicotine.[633,634] In the brain the highest affinity for nicotine is shown by an α4β2 form, which represents over 80% of the nAChR in mammalian brain.[634,635] Knockout mice in which the β2 subunit gene has been deleted lose their sensitivity to nicotine.

Conductance measurements showed that the nicotinic receptors contain channels permeable to Na^+ and other cations and that they are acetylcholine-gated ion channels. Construction of a three-dimensional image from electron micrographs at various angles of tilt shows a tube with approximate pentagonal symmetry and a narrow channel through the center (Fig. 30-23).[622,624,636] Acetylcholine binds to sites on the two α subunits ~3 nm away from the ion channel. An allosteric change opens the channel, allowing cations (largely Na^+) to flow out, depolarizing the membrane. There are at least four structural states in the channel opening-and-closing cycle.[637,638] The three-dimensional structure has been modeled using an acetylcholine-binding protein of known structure from a snail[626,627,639] as a mimic of the cytoplasmic nicotine-binding domain of the receptor. The structure of one subunit of the binding protein is shown in Fig. 30-23C. This protein, which is secreted into synapses by glial cells, may provide a buffering action by binding the acetylcholine. Although the most rapid effect of acetylcholine binding to the nicotinic receptor is depolarization of the postsynaptic membrane, other slower effects follow. Thus, protein kinases are activated and phosphorylate the receptor as well as other proteins.[641]

After a pulse of transmitter is released, it must be removed or inactivated quickly to prepare the synapse for arrival of a new nerve impulse. This is accomplished in two ways in cholinergic synapses. The first is via hydrolytic destruction by acetylcholinesterase[642–645] (pp. 634–637; Eq. 12-25). This esterase and the related butyrylcholinesterase[646] are present in the synaptic membrane itself. The second mechanism is energy-dependent transport of acetylcholine into the neuron for reuse. Since much of the transmitter is hydrolyzed, new acetylcholine is synthesized by transfer of an acetyl group of acetyl-CoA to choline.[647]

In the central nervous system muscarinic acetylcholine receptors are more abundant than nicotinic receptors. They consist of single-chain proteins of mass ~70 kDa. They are not ion channels but are 7-helix receptors homologous in sequence with β-adrenergic receptors (Fig. 11-6) and with rhodopsin.[648] Five different subtypes (M1–M5) have been characterized. The M1, M3, and M5 receptors are coupled to the G_q/G_{11} family of G proteins (pp. 557–558), and M2 and M4 are coupled to G_i/G_o proteins.[649–651] Their effects are slower and of longer duration than those of the nicotinic receptors. It has been difficult to assign functions to the individual types. Most regions of the brain contain more than one type, but they are thought to be involved in locomotion, learning, memory, thermoregulation, and cardiac and pulmonary functions. Many drugs, some of which are used in treatment of Parkinson and Alzheimer diseases, epilepsy, and asthma, affect muscarinic receptors. The M2 receptors predominate in the heart where they help to regulate the beating frequency and atrial contractility. Sudden infant death may sometimes result from a defect in muscarinic receptors.[652] Knockout mice lacking M2 receptors also have problems with movement control, body temperature, and pain responses.[651] Mice lacking M3 receptors are lean with very low levels of serum leptin and insulin.[653] Many of the muscarinic receptors activate adenylate cyclase, while others are coupled to the phosphoinositide cascade. Some indirectly activate K^+ channels.[654] Muscarinic receptors are also studied in insects, but it is difficult to correlate the insect and mammalian receptors.[655]

Amino acids as neurotransmitters. The concentrations of **glutamate** and of its decarboxylation product **γ-aminobutyrate** (GABA) are high in all regions

of the brain. The two compounds are generated sequentially in the γ-aminobutyrate shunt, a pathway that accounts for a quantitatively significant part of the total metabolism of the brain (Fig. 17-5). Because they are present in all parts of the brain in high concentrations, there was initially reluctance to accept glutamate and GABA as neurotransmitters. However, it is now accepted that L-glutamate is the major excitatory transmitter in the central nervous system.[656–658] It seems to be responsible for nearly all of the very fast acting nerve impulses in the brain. At the same time GABA is recognized as the most important inhibitory transmitter. The role of glutamate as an excitatory transmitter was first established for the neuromuscular junction of arthropods.[659] Although it is a constituent of all animal tissues, the concentration of glutamate is much higher in brain than in other tissues, and it is higher in neurons than in glia. Microiontophoretic application of either glutamate or aspartate to the brain cortex leads to very strong excitatory responses.

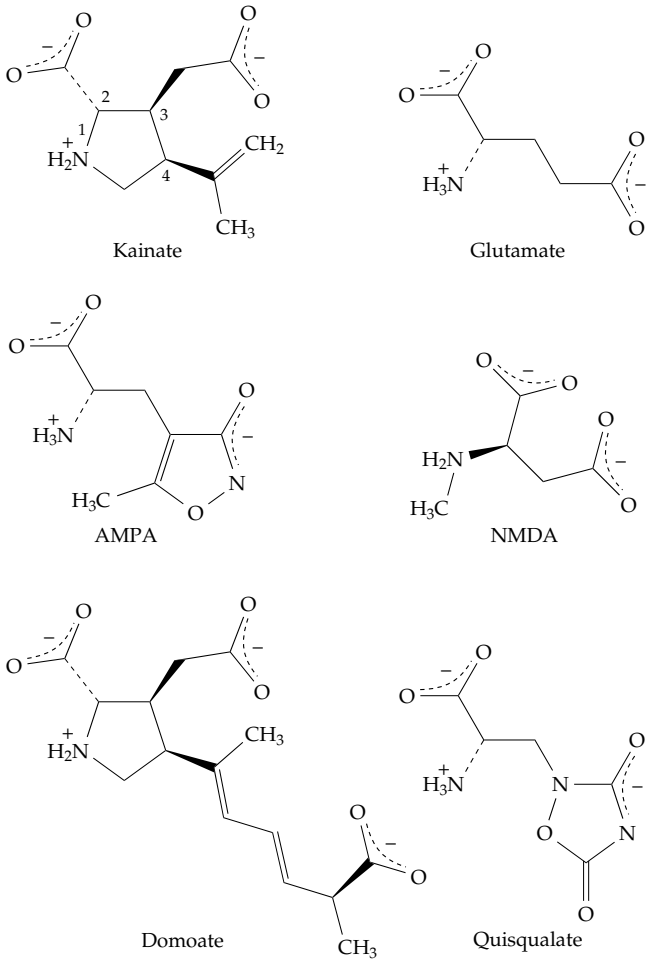


Figure 30-24 Chemical structures of some agonists of ionotropic glutamate receptors (iGluR).

Three subtypes of ionotropic glutamate receptors (iGluR) are named for the specific agonists **α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid** (AMPA), ***N*-methyl-D-aspartate** (NMDA), and **kainate**. The receptors resemble the acetylcholine receptor in containing a cation channel.[149,660–662] In addition, there are 7-helix **metabotropic glutamate receptors,** which are coupled to G proteins.[663,664] The AMPA receptors were in the recent past called **quisqualate** receptors, because they are also activated by the agonist with that name. The toxic domoate (Fig. 30-24) also binds to kainate receptors. Both domoic acid and kainic acid are terrible convulsant toxins. They are formed by two different red algae. Domoic acid accumulates in contaminated mussels and causes shellfish poisoning. The ionotropic glutamate receptors, which may be stimulated by either glutamate or aspartate, are directly linked to the opening of cation channels. Their activation may also induce the inositol phosphate cascade and slower Ca^{2+}-dependent changes. A peculiarity of the high-conductance NMDA channels is that they are blocked by Mg^{2+} in a voltage-dependent manner. They do not open unless the frequency of nerve impulses is high or some other factor causes membrane depolarization.[656]

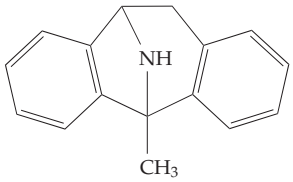
The AMPA receptors, which are thought to be the predominant mediators of fast excitatory transmission in the brain,[665] are oligomers (probably tetramers[666,666a]) of 950- to 1500-residue protein subunits. These subunits have large N-terminal domains in the synaptic cleft. There are probably three transmembrane helices and a membrane-associated loop similar to those depicted in Fig. 30-17A. A long C-terminal tail protrudes into the cytosol, while a large loop between transmembrane regions extends from the outer membrane surface, joining with the N-terminal domain to form the ligand-binding site, the structure of which resembles those of bacterial periplasmic binding proteins.[661,665,667] Four related AMP receptors, designated GluR1, 2, 3, and 4, have been identified. Related kainate receptors, whose properties overlap those of AMPA receptors, are designated GluR5, 6, and 7.[662] Although AMPA receptors are essential for fast signal transmission they lose sensitivity rapidly (on a millisecond time scale) as a result of conformational alterations.[667a] Many factors, including inhibition by polyamines,[667b] affect these receptors. However, brief high-frequency activation of some AMP receptors leads to a long-lasting increase in efficiency, termed LTP, which is important to learning (see p. 1801).[666a]

The NMDA receptors are heterooligomers with two type of subunits. The NR1 (or ζ) subunits exist as a series of at least eight splice variants. The NR2A, B, C, and D (ε series) are encoded by four different genes.[668,669] NR1 is regarded as the principal subunit ard NR2 as a regulatory subunit. As with the AMPA receptors[670] the oligomeric NMDA receptors are

anchored at appropriate locations in the postsynaptic membrane by scaffolding proteins containing PDZ domains (Table 7-3).[671] The C-terminal domains of the ε subunits are unusually long and participate in anchoring. NMDA receptors are found not only in neurons but also in astrocytes (Fig. 30-20), where they are thought to have important signaling functions.[672,673] These include regulation of Ca^{2+} flow, in part via gap junctions.[603a]

The N-terminal domain of the NR1 subunit of the NMDA receptor contains a glycine-binding site.[674] Full activity of the receptor requires a **coagonist** bound in this site. Surprisingly, **D-serine** seems to be the normal coagonist, at least in some sites.[675,676] This newly recognized neurotransmitter is synthesized from L-serine by a pyridoxal phosphate-dependent recemase and is destroyed by the flavoprotein D-amino acid oxidase. Associated with NMDA receptors are clusters of **ephrin receptors**, proteins that bind the glycosylphosphatidylinositol (GPI)-anchored proteins known as ephrins in presynaptic membranes. Binding of ephrins to their postsynaptic receptors activates tyrosine kinases and enhances the influx of Ca^{2+} ions.[676a,b]

Specific inhibitors of NMDA channels include a 27-residue "spasmotic" conotoxin,[490] 2-amino-4-phosphonobutyrate, related longer chain aminophosphonates, and the following potent anticonvulsant drug, which is able to penetrate the blood–brain barrier.[677]



(+)5-Methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine

Metabotropic glutamate receptors have been classified into eight types (mGluRs1–8).[678–680a] Group I (mGluRs1–5) are selectively activated by 3,5-dihydroxyphenylglycine; Group II (mGluR2 and mGluR3) are activated by L-2-(carboxycyclopropyl)glycine; and Group III (mRluR4 and mGluR 6–8) are activated by L-2-aminophosphonobutyrate. They are all 7-helix G-protein-coupled receptors with external ligand-binding domains that resemble those of bacterial periplasmic binding proteins.[680] Splice variants for at least mGlnR1 are known.[678] Metabotropic glutamate receptors are neuromodulary but nevertheless play essential roles in the cerebellum and other parts of the brain. For example, mice deficient in the mGluR1 protein have severe problems with motor coordination and learning.[681,682] Metabotropic glutamate receptors may participate in calcium sensing and signaling.[683,684]

Synaptosomal particles have a high-affinity proton-dependent uptake system for glutamate.[685] Glutamate and aspartate may also be taken up from the synaptic cleft by neurons or by glial cells, which then transfer the glutamate into neurons for reuse.[686,687] Five distinct mammalian transporter genes have been cloned.[688] They are driven by concentration gradients of Na^+ and K^+ across the membrane.[689,690] However, some serve as glutamate-gated chloride ion channels.[691,691a]

Excitotoxicity. As essential as glutamate is for brain function it is toxic in excess. Excessive stimulation of the NMDA receptors, which occurs during convulsions, strokes, or traumatic injury and which can accompany anoxia or hypoglycemia, causes neuronal death.[660,692–694] Blocking these receptors with the above-mentioned anticonvulsant drug or aminophosphonates has a remarkable protective effect against the neurotoxicity of the accumulating glutamate.[658,677] Vitamin E and **tocotrienols** (Fig. 15-24) may also be protective.[695]

The inhibitory neurotransmitter gamma-aminobutyrate (GABA). Glutamate, aspartate, and cysteic acid are all potent excitors, but their decarboxylation products γ-aminobutyrate (**GABA**), β-alanine, and taurine are inhibitors as is also glycine. Of these GABA is the most important.[696] Its concentration in the brain is high and varies at least threefold in different parts of the brain. It is hardly present elsewhere in the body. GABA and GABA-binding sites are found in 30–50% of the nerve endings. The function as an inhibitory transmitter has also been demonstrated in inhibitory neurons present in the peripheral nervous system of arthropods. Virtually every neuron in the brain is to some extent subject to inhibition by GABA.[697,698] Glial cells also have GABA receptors.

The receptors for GABA are divided into type A, which are blocked by **bicuculline**,[699] and type B, which are stimulated by **baclofen** (Fig. 30-25).[698] The $GABA_A$ receptors are the major sites of fast synaptic inhibition in the central nervous system.[700] They are structurally related to the nicotinic acetylcholine, glycine, and serotonin type 3 ($5\text{-}HT_3$) receptors. Cloning has revealed 16 different mammalian subunits: α1–α4, β1–β3, γ1–γ3, δ, ε, π, and Φ.[701–704a] The oligomeric receptors are ligand-gated chloride ion channels[481,705] as are also glycine receptors. These receptors are clustered in synaptic membranes, apparently anchored in part by their β subunits[706] and scaffold proteins such as the microtubule-binding **gephyrin** (from the Greek word for bridge)[701,707] and a small ~14-kDa GABA receptor-associated protein.[708] A novel serine protein kinase is also associated with GABA receptors.[703]

Whereas excitatory transmitters lead to depolarization of the postsynaptic membrane, inhibitory transmitters cause **hyperpolarization**, apparently by increasing the conductance of K^+ and Cl^-. The result is

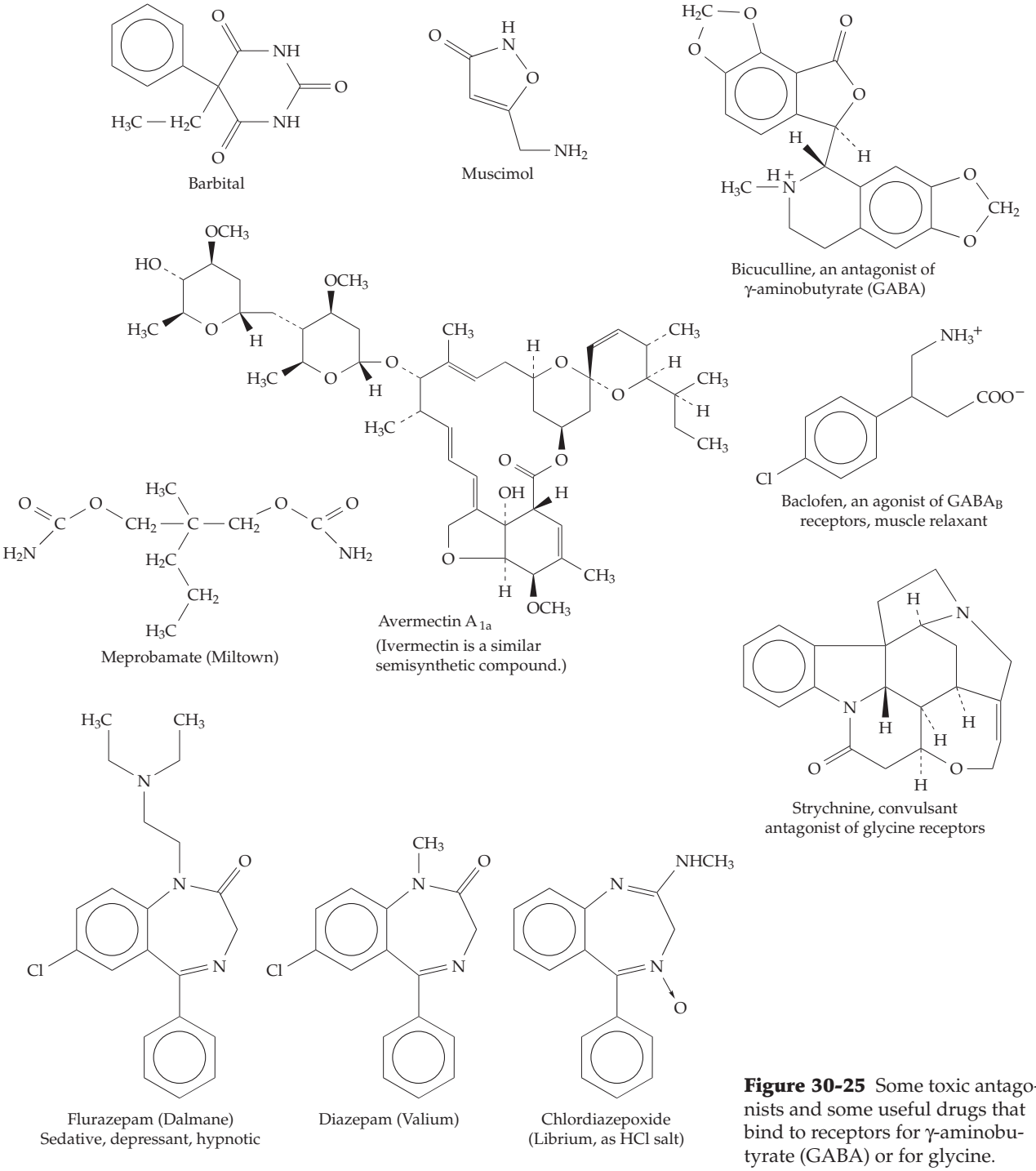


Figure 30-25 Some toxic antagonists and some useful drugs that bind to receptors for γ-aminobutyrate (GABA) or for glycine.

that it is more difficult to excite the postsynaptic membrane in the presence of, than in the absence of, these transmitters. GABA-dependent interneurons also contain the calcium-binding **parvalbumin** (Fig. 6-7), which suggests that a Ca^{2+}-dependent process is involved.[709]

The GABA$_B$ receptors resemble metabotropic glutamate receptors.[710,711] They are 7-helix G-protein coupled proteins, which activate adenylate cyclase. They tend to dimerize, and maximum activity is observed for heterodimers of GABA$_B$1 and GABA$_B$2 receptors.[712,713] They are often coupled to inward rectifying K^+ channels.[714]

The GABA receptors provide binding sites for a great variety of toxins and drugs.[481] These include barbiturates, anesthetics, antianxiety drugs, and the insecticides such as toxaphine, cyclodienes, and pyrethroids.[481] **Diazepam**, **chlordiazepoxide**, and

flurazepam[700,702,715–717] (Fig. 30-25) are antianxiety drugs and muscle relaxants, which, during the 1970s, were the most frequently prescribed drugs in the United States.[716] Binding of benzodiazepines to GABA receptor-chloride channels enhances the effect of GABA. The drugs induce relaxation but can interfere with memory, reduce concentration, and cause physical clumsiness. They may also intensify the effects of alcohol and can be addictive.[718]

Specific antagonists for $GABA_A$ receptors include the alkaloid convulsants bicuculline (Fig. 30-25)[699] and **picrotoxin** (Fig. 22-4) and the convulsant terpenoid compound **thujone** (Fig. 22-3), which is present in the wormwood plant *Artemesia absinthium*. Thujone is present in the liqueur absinthe, which was the national drink of France in the late 19th century but, because of its toxicity, has been illegal in most countries since ~1915.[719]

GABA enters synaptic vesicles via a vesicular GABA transporter, an integral membrane protein whose gene has been found in *Caenorhabditis elegans*.[720] Termination of GABA neurotransmission is accomplished by rapid Na^+-dependent uptake into neurons for reuse and uptake into glial cells.[721,722] Excess GABA is continuously oxidized to succinic semialdehyde by GABA aminotransferase[723] in the GABA cycle of Fig. 17-4. Notice the manner in which this cycle incorporates synthesis of both of the neurotransmitters glutamate and GABA. Glutamine also functions in neurons, perhaps serving as a buffer for glutamate.

The hereditary triple-repeat disease Huntington's chorea (**Huntington disease**), with an incidence of 5–10 per 100,000 persons, affects principally persons of age over 40 and is associated with a deficiency of GABA in basal ganglia.[724] The cortex is also affected. Severe neurologic symptoms arise as a result of premature death of neurons in the basal ganglia. Convulsions may also arise because of a deficiency of GABA in the brain.

Glycine. Glycine appears to be the most important neuroinhibitor in the spinal cord and brainstem. It is present at concentration of 3–5 mM in the spinal cord and in the medulla but is low in the cerebral cortex. **Strychnine** (Fig. 30-25) is a specific antagonist of glycine receptors in spinal synapses.[725] Ivermectin (Fig. 30-25) also blocks glycine Cl^- channels.[726] A mutant mouse called *spastic* is deficient in glycine receptor function. A small dose of strychnine produces an effect on a normal mouse that resembles the effect of this mutation.[727,728] A similar disorder affects some Hereford calves.[729] Strychnine-binding studies have suggested a deficit of glycine receptors in human spasticity and in the loss of motor control associated with **Parkinson disease** and **amyotrophic lateral sclerosis**.[725] A human **startle disease**, which causes an exaggerated muscular response to unexpected stimuli, also results from reduced glycinergic neurotransmission.[730]

Most glycine receptors are Cl^- ion channels that open in response to transmitter binding.[725] The strychnine-binding subunit shows significant homology with the nAChR proteins,[725] and the overall structures resemble those of GABA receptors and of nAChRs.[731,732,732a] Human α1–α4 and β subunits have been identified.[733,734] Two integral membrane glycine transporters are known.[735–737]

Anesthetics. Several types of neurotransmitter receptors provide binding sites for anesthetics. Some anesthetics are molecules of moderate size, e.g., **barbiturate** derivatives, while others, such as **diethyl ether** or **halothane** ($CF_3CHClBr$), are very small. The latter is one of the most widely used inhalation anesthetics. Both Mg^{2+} and Mn^{2+} are also powerful CNS depressants and can cause general anesthesia. It has often been proposed that the effectiveness of anesthetics is related to solubility in lipids, but it has been difficult to pinpoint a site of action. Now it is clear that specific synaptic proteins often provide the binding sites for anesthetics. Important among these are the glycine receptors.[715,738,739] GABA receptors[740,740a] and kainate glutamate receptors may also bind anesthetics.[741]

Adrenergic synapses: the catecholamines. The three closely related tyrosine metabolites, **dopamine**, **noradrenaline**, and **adrenaline**, known collectively as catecholamines, are important products of neuronal metabolism.[149,393] Dopamine and noradrenaline serve as neurotransmitters. Catecholamine-containing neurons are found throughout the brain, including the cortex and cerebellum regions. Very large dopamine-containing neurons are present in the brains of gastropod molluscs.[742] In the human brain a prominent series of dopamine neurons run from the substantia nigra to the caudate nuclei and putamen of the striatum, the **nigrostriatal** pathway (Fig. 30-12).[149,743,743a] In many invertebrates **octopamine**,[744–746] which is synthesized via tyramine (Fig. 30-26), apparently functions in place of noradrenaline. Note the precursor–product relationship between dopamine, noradrenaline, and adrenaline. The synthetic pathways to these neurotransmitters involve decarboxylation and hydroxylation, types of reaction important in formation of other transmitters as well. The most important process for terminating the action of released catecholamine transmitters is reuptake by the neurons. High-affinity uptake systems transport the catecholamine molecules back into the neurons and then into the synaptic vesicles. The uptake is specifically blocked by the drug **reserpine** (Fig. 25-12).[746a] The dopamine transporter is a major binding site for cocaine (see Fig. 30-28).[747–751] Catecholamine transmitters are catabolized by two enzymes. One is the