current to charge or discharge the muscle membrane capacitance, and thus alter the membrane voltage, the EPSP lags behind the synaptic current (see Figure 9–10 and the Postscript at the end of this chapter).

The Neurotransmitter Acetylcholine Is Released in Discrete Packets

During their first microelectrode recordings at frog motor end-plates in the 1950s, Fatt and Katz observed small spontaneous depolarizing potentials (0.5–1.0 mV) that occurred at an average rate of about 1/s. Such spontaneous potentials were restricted to the end-plate, exhibited the same time course as stimulus-evoked EPSPs, and were blocked by curare. Hence, they were named "miniature" end-plate potentials (mEPPs, or mEPSPs in our current terminology).

What could account for the small, fixed size of the miniature end-plate potential? Del Castillo and Katz tested the possibility that an mEPSP represents the action of a *single* ACh molecule. This hypothesis was quickly dismissed, because applying very small amounts of ACh to the end-plate could elicit depolarizing responses that were much smaller than the 1.0-mV mEPSP. The low doses of ACh did produce an increase in baseline fluctuations or "noise." Later analysis of the statistical components of this noise led to estimates that the underlying unitary postsynaptic response was a depolarization of 0.3 μV in amplitude and 1.0 ms in duration. This was the first hint of the electrical signaling properties of a single ACh receptor-channel (described later).

Del Castillo and Katz concluded that each mEPSP must represent the action of a multimolecular packet or "quantum" of transmitter. Further, they suggested that the large, stimulus-evoked EPSP was made up of an integral number of quanta. Evidence for this quantal hypothesis is presented in Chapter 15.

Individual Acetylcholine Receptor-Channels Conduct All-or-None Currents

What are the properties of the ACh receptor-channels that produce the inward current that generates the depolarizing end-plate potential? Which ions move through the channels to produce this inward current? And what does the current carried by a single ACh receptor-channel look like?

In 1976, Erwin Neher and Bert Sakmann obtained key insights into the biophysical nature of ACh receptor-channel function from recordings of the current conducted by single ACh receptor-channels in skeletal muscle cells, the unitary or elementary current. They found that the opening of an individual channel generates a very small rectangular step of ionic current (Figure 12–7A). At a given resting potential, each channel opening generates the same-size current pulse. At –90 mV, the current steps are approximately –2.7 pA in amplitude. Although this is a very small current, it corresponds to a flow of approximately 17 million ions per second!

Whereas the amplitude of the current through a single ACh receptor-channel is constant for every opening, the duration of each opening and the time between openings vary considerably. These variations occur because channel openings and closings are stochastic; they obey the same statistical law that describes the exponential time course of radioactive decay. Because channels and ACh undergo random thermal motions and fluctuations, it is impossible to predict exactly how long it will take any one channel to bind ACh or how long that channel will stay open before the ACh dissociates and the channel closes. However, the average length of time a particular type of channel stays open is a well-defined property of that channel, just as the half-life of radioactive decay is an invariant property of a particular isotope. The mean open time for ACh receptor-channels is approximately 1 ms. Thus, each channel opening permits the movement of approximately 17,000 ions. Once a channel closes, the ACh molecules dissociate and the channel remains closed until it binds ACh again.

The Ion Channel at the End-Plate Is Permeable to Both Sodium and Potassium Ions

Once a receptor-channel opens, which ions flow through the channel, and how does this lead to depolarization of the muscle membrane? One important means of identifying the ion (or ions) responsible for the synaptic current is to measure the value of the chemical driving force (the chemical battery) propelling ions through the channel. Remember, the current through a single open channel is given by the product of the single-channel conductance and the electrochemical driving force on the ions conducted through the channel (Chapter 9). Thus, the current generated by a single ACh receptor-channel is given by:

$$I_{EPSP} = \gamma_{EPSP} \times (V_m - E_{EPSP}), \qquad \textbf{(12–1)}$$

where I_{EPSP} is the amplitude of current through one channel, γ_{EPSP} is the conductance of a single open channel, E_{EPSP} is membrane potential at which the net flux of ions through the channel is zero, and $V_m - E_{EPSP}$ is the electrochemical driving force for ion flux. The current steps change in

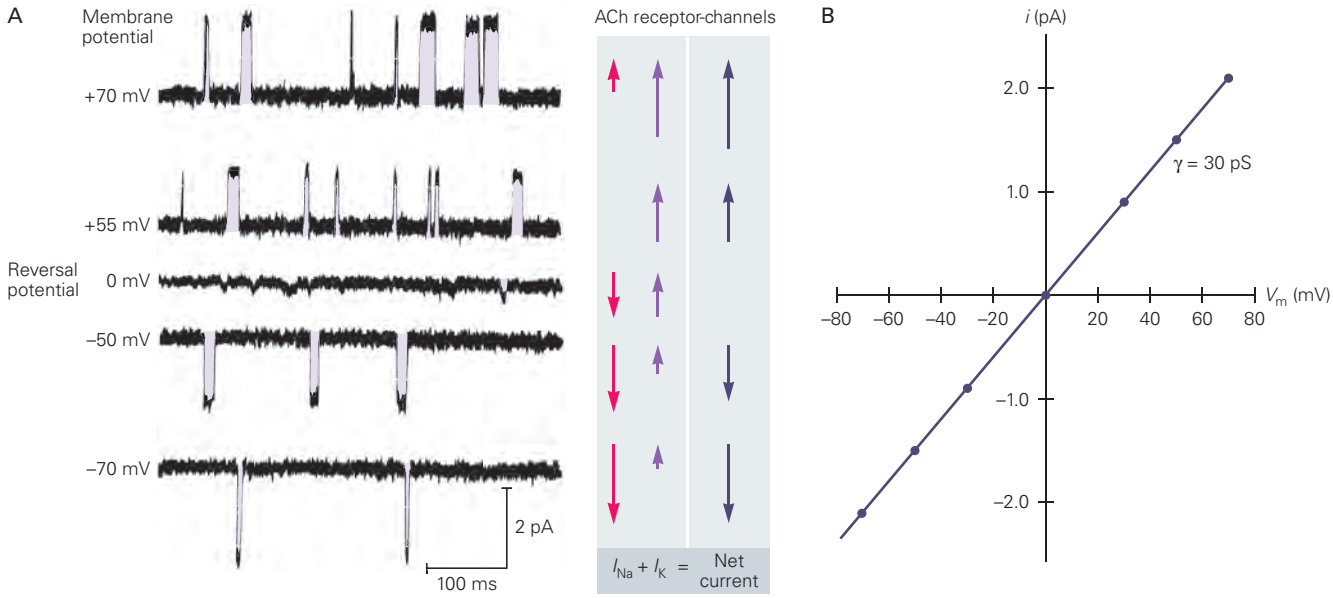

Figure 12–7 Individual acetylcholine (ACh) receptor-channels conduct an all-or-none elementary current.

A. The patch-clamp technique is used to record currents from single ACh receptor-channels. The patch electrode is filled with salt solution that contains a low concentration of ACh and is then brought into close contact with the surface of the muscle membrane (see Box 8–1). At a fixed membrane potential, each time a channel opens, it generates a relatively constant elementary current. At the resting potential of –90 mV, the current is approximately –2.7 pA (1 pA = 10^{-12} A). As the voltage across a patch of membrane is systematically varied, the resultant current varies in amplitude as a result of changes in driving force. The current is inward at voltages negative to 0 mV and outward at voltages positive to 0 mV, thus defining 0 mV as the reversal potential. The arrows on the right side of the traces illustrate the individual sodium and potassium fluxes and resultant net current as a function of voltage.

B. The linear relation between current through a single ACh receptor-channel and membrane voltage shows that the channel behaves as a simple resistor having a single-channel conductance (γ) of about 30 pS.

size as the membrane potential changes because of the change in driving force. For the ACh receptor-channels, the relationship between I_{EPSP} and membrane voltage is linear, indicating that the single-channel conductance is constant and does not depend on membrane voltage; that is, the channel behaves as a simple ohmic resistor. From the slope of this relation, the channel is found to have a conductance of 30 pS (Figure 12–7B). As we saw in Chapter 9, the total conductance, g, due to the opening of a number of receptor-channels (n) is given by:

$$g = n \times \gamma.$$

The current–voltage relation for a single channel shows that the reversal potential for ionic current through ACh receptor-channels, obtained from the intercept of the membrane voltage axis, is 0 mV, which is not equal to the equilibrium potential for Na^+ or any of the other major cations or anions. This is due to the fact that this chemical potential is produced not by a single ion species but by a combination of two species: The ligand-gated channels at the end-plate are almost equally permeable to both major cations, Na^+ and K^+. Thus, during the end-plate potential, Na^+ flows into the cell and K^+ flows out. The reversal potential is at 0 mV because this is a weighted average of the equilibrium potentials for Na^+ and K^+ (Box 12–1). At the reversal potential, the influx of Na^+ is balanced by an equal efflux of K^+ (Figure 12–7A).

The ACh receptor-channels at the end-plate are not selective for a single ion species, as are the voltage-gated Na^+ or K^+ channels, because the diameter of the pore of the ACh receptor-channel is substantially larger than that of the voltage-gated channels. Electrophysiological measurements suggest that it may be 0.6 nm in diameter, an estimate based on the size of the largest organic cation that can permeate the channel. For example, tetramethylammonium (TMA) is approximately 0.6 nm in diameter and yet still permeates the channel. In contrast, the voltage-gated Na^+ channel is only permeant to organic cations that are smaller than 0.5 × 0.3 nm in cross section, and voltage-gated K^+ channels will only conduct ions less than 0.3 nm in diameter.

The relatively large diameter of the ACh receptor-channel pore is thought to provide a water-filled

Box 12–1 Reversal Potential of the End-Plate Potential

The reversal potential of a membrane current carried by more than one ion species, such as the end-plate current through the ACh receptor-channels, is determined by two factors: (1) the relative conductance for the permeant ions (g_{Na} and g_K in the case of the end-plate current) and (2) the equilibrium potentials of the ions (E_{Na} and E_K).

At the reversal potential for the ACh receptor-channel current, inward current carried by Na^+ is balanced by outward current carried by K^+:

$$I_{Na} + I_K = 0. \quad \textbf{(12–2)}$$

The individual Na^+ and K^+ currents can be obtained from

$$I_{Na} = g_{Na} \times (V_m - E_{Na}) \quad \textbf{(12–3a)}$$

and

$$I_K = g_K \times (V_m - E_K). \quad \textbf{(12–3b)}$$

We can substitute Equations 12–3a and 12–3b for I_{Na} and I_K in Equation 12–2, replacing V_m with E_{EPSP} (because at the reversal potential $V_m = E_{EPSP}$):

$$g_{Na} \times (E_{EPSP} - E_{Na}) + g_K \times (E_{EPSP} - E_K) = 0. \quad \textbf{(12–4)}$$

Solving this equation for E_{EPSP} yields

$$E_{EPSP} = \frac{(g_{Na} \times E_{Na}) + (g_K \times E_K)}{g_{Na} + g_K}. \quad \textbf{(12–5)}$$

This equation can also be used to solve for the ratio g_{Na}/g_K if one knows E_{EPSP}, E_K, and E_{Na}. Thus, rearranging Equation 12–5 yields

$$\frac{g_{Na}}{g_K} = \frac{E_{EPSP} - E_K}{E_{Na} - E_{EPSP}}. \quad \textbf{(12–6)}$$

At the neuromuscular junction, E_{EPSP} = 0 mV, E_K = –100 mV, and E_{Na} = +55 mV. Thus, from Equation 12–6, g_{Na}/g_K has a value of approximately 1.8, indicating that the conductance of the ACh receptor-channel for Na^+ is slightly higher than for K^+. A comparable approach can be used to analyze the reversal potential and the movement of ions during excitatory and inhibitory synaptic potentials in central neurons (Chapter 13).

environment that allows cations to diffuse through the channel relatively unimpeded, much as they would in free solution. This explains why the pore does not discriminate between Na^+ and K^+ and why even divalent cations, such as Ca^{2+}, are able to pass through. Anions are excluded by the presence of fixed negative charges in the channel, as described later in this chapter. Recent X-ray crystallographic data have provided a direct view of the large pore of the ACh receptor-channel (see Figure 12–12).

Four Factors Determine the End-Plate Current

How do the rectangular current steps carried by single ACh receptor-channels produce the large synaptic current at the end-plate in response to motor nerve stimulation? Stimulation of a motor nerve releases a large quantity of ACh into the synaptic cleft. The ACh rapidly diffuses across the cleft and binds to the ACh receptors, causing more than 200,000 receptor-channels to open almost simultaneously. (This number is obtained by comparing the total end-plate current, approximately –500 nA, with the current through a single channel, approximately –2.7 pA).

The rapid opening of so many channels causes a large increase in the total conductance of the end-plate membrane, g_{EPSP}, and produces the fast rising phase of the end-plate current. As the ACh in the cleft decreases rapidly to zero (in <1 ms), because of enzymatic hydrolysis and diffusion, the channels begin to close randomly. Although each closure produces only a small step-like decrease in end-plate current, the random closing of large numbers of small unitary currents causes the total end-plate current to appear to decay smoothly (Figure 12–8).

The Acetylcholine Receptor-Channels Have Distinct Properties That Distinguish Them From the Voltage-Gated Channels That Generate the Muscle Action Potential

The ACh receptors that produce the end-plate potential differ in two important ways from the voltage-gated channels that generate the action potential in

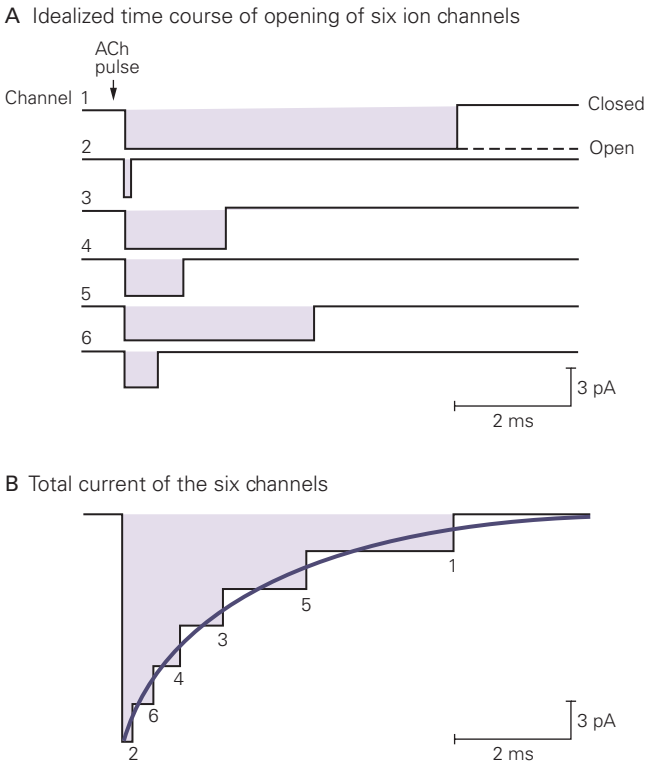

Figure 12–8 The time course of the total current at the end-plate reflects the summation of contributions of many individual acetylcholine receptor-channels. (Reproduced, with permission, from Colquhoun 1981. Copyright © 1981 Elsevier.)

A. Individual ACh receptor-channels open in response to a brief pulse of ACh. In this idealized example, the membrane contains six ACh receptor-channels, all of which open rapidly and nearly simultaneously. The channels remain open for varying times and close independently.

B. The stepped trace shows the sum of the six single-channel current records in part A. It represents the current during the sequential closing of each channel (the number indicates which channel has closed). In the final period of current, only channel one is open. In a current record from a whole muscle fiber, with thousands of channels, individual channel closings are not detectable because the scale needed to display the total end-plate current (hundreds of nanoamperes) is so large that the contributions of individual channels cannot be resolved. As a result, the total end-plate current appears to decay smoothly.

muscle. First, the action potential is generated by sequential activation of two distinct classes of voltage-gated channels, one selective for Na^+ and the other for K^+. In contrast, a single type of ion channel, the ACh receptor-channel, generates the end-plate potential by allowing both Na^+ and K^+ to pass with nearly equal permeability.

Second, the Na^+ flux through voltage-gated channels is regenerative: By increasing the depolarization of the cell, the Na^+ influx opens more voltage-gated Na^+ channels. This regenerative feature is responsible for the all-or-none property of the action potential. In contrast, the number of ACh receptor-channels opened during the synaptic potential is fixed by the amount of ACh available. The depolarization produced by Na^+ influx through the ACh-gated channels does not lead to the opening of more ACh receptor-channels and cannot produce an action potential. To trigger an action potential, a synaptic potential must recruit neighboring voltage-gated Na^+ channels (Figure 12–9).

As might be expected from these two differences in physiological properties, the ACh receptor-channels and voltage-gated channels are formed by different macromolecules that exhibit different sensitivities to drugs and toxins. Tetrodotoxin, which blocks the voltage-gated Na^+ channel, does not block the influx of Na^+ through the nicotinic ACh receptor-channels. Similarly, α-bungarotoxin binds tightly to the nicotinic receptors and blocks the action of ACh but does not interfere with voltage-gated Na^+ or K^+ channels.

Transmitter Binding Produces a Series of State Changes in the Acetylcholine Receptor-Channel

Each ACh receptor has two binding sites for ACh; both must be occupied by transmitter for the channel to open efficiently. However, during prolonged applications of ACh, the channel enters a desensitized state where it no longer conducts. The time course of desensitization of the muscle nicotinic receptor is too slow to contribute to the time course of the EPSP under normal conditions, where ACh is present in the synaptic cleft for only a very brief period of time. However, desensitization can play a more important role in determining the time course of the postsynaptic response at certain neuronal synapses, where the transmitter may persist in the synaptic cleft for more prolonged times or where the postsynaptic receptors undergo more rapid desensitization.

For example, the persistence of ACh in the synaptic cleft at cholinergic synapses in the brain may lead to significant desensitization of certain subtypes of neuronal nicotinic receptors. Heavy smokers can build up sufficient levels of nicotine to desensitize receptors in the brain. Desensitization also plays a role in the action of the drug succinylcholine, a dimer of ACh that is resistant to acetylcholinesterase and is used during general anesthesia to produce muscle relaxation. Succinylcholine does so through its ability to produce both receptor desensitization and prolonged depolarization, which blocks muscle action potentials by inactivating voltage-gated Na^+ channels.

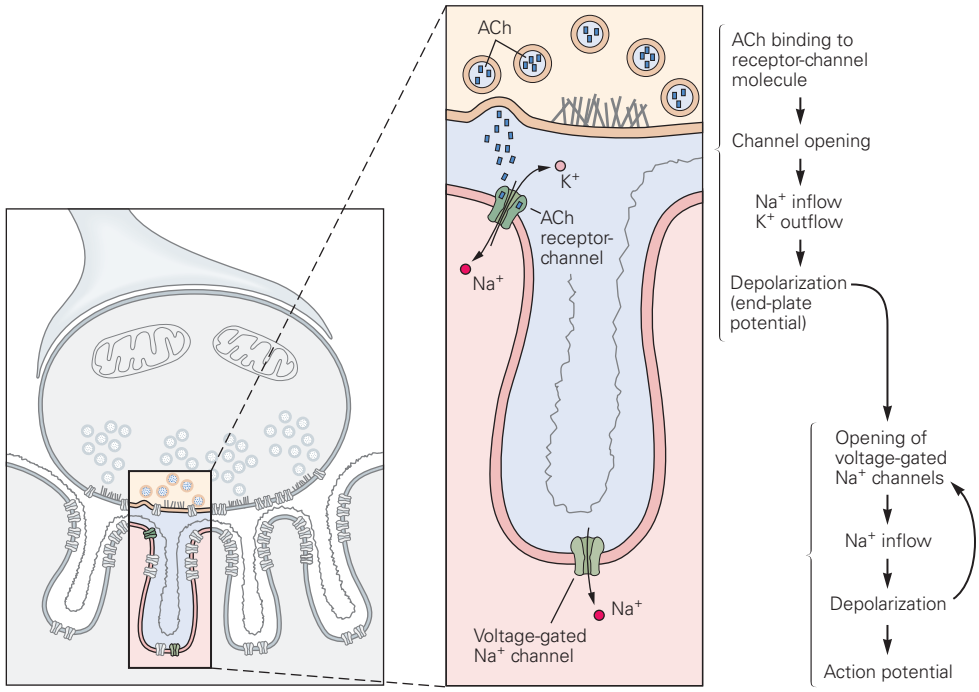

Figure 12–9 The end-plate potential resulting from the opening of acetylcholine receptor-channels opens voltage-gated sodium channels. The end-plate potential is normally large enough to open a sufficient number of voltage-gated Na^+ channels to exceed the threshold for an action potential. (Adapted from Alberts et al. 1989.)

A minimal reaction model, first proposed by Katz and his colleagues, captures many (but not all) of the key steps of ACh receptor-channel function, in which a closed receptor-channel (R) successively binds two molecules of ACh (A) prior to undergoing a rapid conformational change to an open state (R*). This is followed by a slower conformational change to the nonconducting desensitized state (D). The model also incorporates the finding that there is a small probability that an individual receptor may enter the desensitized state even in the absence of ACh. These binding and gating reactions can be summarized by the following scheme:

$$\begin{array}{ccccccc} A + R & \longleftrightarrow & AR + A & \longleftrightarrow & A_2R & \longleftrightarrow & A_2R^* \\ \updownarrow & & \updownarrow & & \updownarrow & & \updownarrow \\ A + D & \longleftrightarrow & AD + A & \longleftrightarrow & A_2D & \longleftrightarrow & A_2D^* \end{array}$$

X-ray crystal structure models have now been obtained for all three states of the ACh receptor (described later).

The Low-Resolution Structure of the Acetylcholine Receptor Is Revealed by Molecular and Biophysical Studies

The nicotinic ACh receptor at the nerve-muscle synapse is part of a single macromolecule that includes the pore in the membrane through which ions flow. Where in the molecule is the binding site located? How is the pore of the channel formed? How is ACh binding coupled to channel gating?

Insights into these questions have been obtained from molecular and biophysical studies of the ACh receptor proteins and their genes, beginning with the purification of the macromolecule from the electric ray *Torpedo marmorata* (Figure 12–2). Using different biochemical approaches, Arthur Karlin and Jean Pierre Changeux purified the receptor from electroplaques, specialized muscle-like cells whose stack-like packing enables their individual EPSPs to summate in series to generate the large voltages (>100 V) used by the electric ray to stun its prey. Their studies indicate that the mature nicotinic ACh receptor is a membrane glycoprotein formed from five subunits of similar molecular weight: two α-subunits and one β-, one γ-, and one δ-subunit (Figure 12–10).

Karlin and his colleagues identified two extracellular binding sites for ACh on each receptor protein in the clefts between each α-subunit and its neighboring γ- or δ-subunit. One molecule of ACh must bind at each of the two sites for the channel to open efficiently (Figure 12–10). Because α-bungarotoxin binds remarkably tightly to the same binding site on the α-subunit as does ACh, the toxin acts as an irreversible transmitter antagonist.

Further insights into the structure of the ACh receptor-channel come from the analysis of the primary amino acid sequence of the receptor's four different

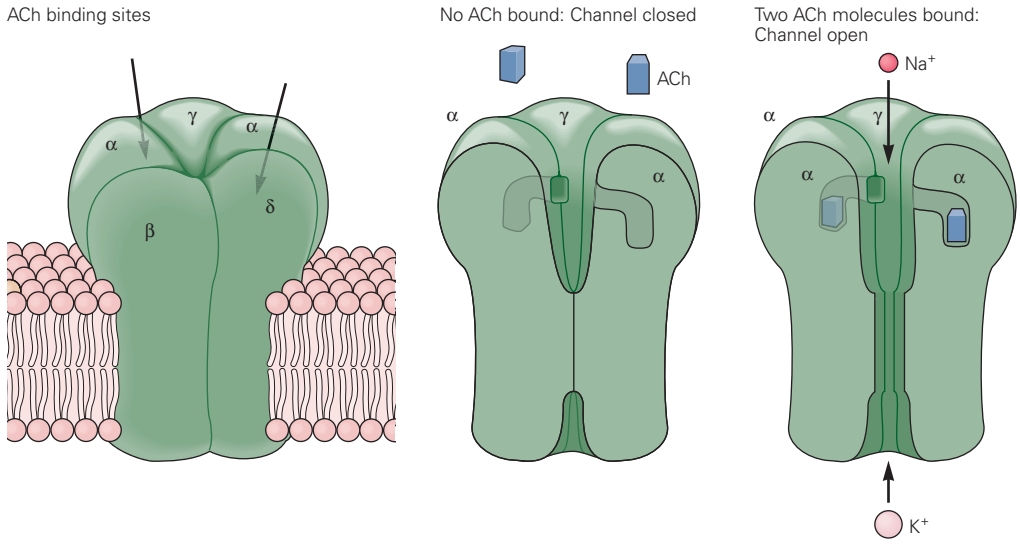

Figure 12–10 The nicotinic ACh receptor-channel is a pentameric macromolecule. The receptor and channel are components of a single macromolecule consisting of five subunits: two identical α-subunits and one each of β-, γ-, and δ-subunits. The subunits form a pore through the cell membrane. When two molecules of ACh bind to the extracellular binding sites—formed at the interfaces of the two α-subunits and their neighboring γ- and δ-subunits—the conformation of the receptor-channel molecule changes (see Figure 12–12). This change opens the pore through which K^+ and Na^+ flow down their electrochemical gradients.

subunits and from biophysical studies. Molecular cloning by Shosaku Numa and colleagues demonstrated that the four subunits are encoded by distinct but related genes. Sequence comparison of the subunits shows a high degree of similarity—one-half of the amino acid residues are identical or conservatively substituted—which suggests that all subunits have a similar structure. Furthermore, all four of the genes for the subunits are homologous; that is, they are derived from a common ancestral gene. Nicotinic ACh receptors in neurons are encoded by a set of distinct but related genes. All of these receptors are pentamers; however, their subunit composition and stoichiometry vary. Whereas most neuronal receptors are composed of two α-subunits and three β-subunits, some neuronal receptors are composed of five identical α-subunits (the α7 isoform) and so can bind five molecules of ACh.

All nicotinic ACh receptor subunits contain a highly conserved sequence near the extracellular binding site for ACh consisting of two disulfide-bonded cysteine (cys) residues with 13 intervening amino acids. The resultant 15-amino acid loop forms a signature sequence both for nicotinic ACh receptor subunits and for related receptors for other transmitters in neurons. The *cys-loop receptor family*, also known as *pentameric ligand-gated ion channels* (pLGIC), includes receptors for the neurotransmitters γ-aminobutyric acid (GABA), glycine, and serotonin.

The distribution of the polar and nonpolar amino acids of the subunits provided the first clues as to how the subunits are threaded through the membrane bilayer. Each subunit contains four hydrophobic regions of approximately 20 amino acids called M1 to M4, each of which forms an α-helix that spans the membrane (Figure 12–11A). The amino acid sequences of the subunits suggest that the subunits are arranged such that they create a central pore through the membrane (Figure 12–11B).

The walls of the channel pore are formed by the M2 membrane-spanning segment and by the loop connecting M2 to M3. Three rings of negative charges that flank the external and internal boundaries of the M2 segment play an important role in the channel's selectivity for cations. Certain local anesthetic drugs block the channel by interacting with one ring of polar serine residues and two rings of hydrophobic residues in the central region of the M2 helix, midway through the membrane.

Three-dimensional models of the entire receptor-channel complex were initially proposed by Karlin based on low-resolution neutron scattering and by Nigel Unwin based on electron diffraction images. The complex is divided into three regions: a large

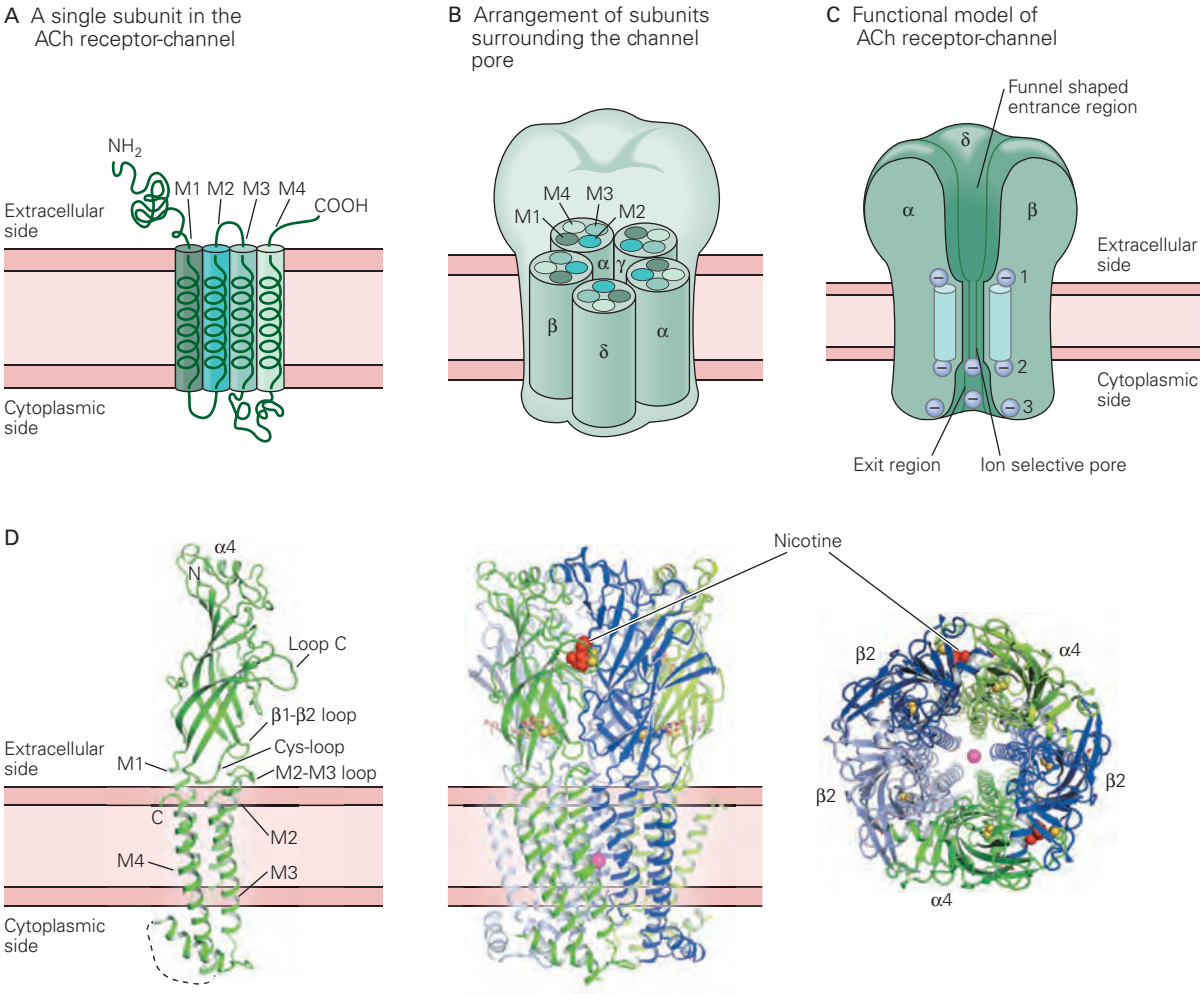

Figure 12–11 The ACh receptor subunits are homologous membrane-spanning proteins.

A. Each subunit contains a large extracellular N-terminus, four membrane-spanning α-helixes (M1–M4), and a short extracellular C-terminus. The N-terminus contains the ACh-binding site, and the membrane helixes form the pore.

B. The five subunits are arranged such that they form a central aqueous channel, with the M2 segment of each subunit forming the lining of the pore. The γ-subunit lies between the two α-subunits. (Dimensions are not to scale.)

C. Negatively charged amino acids on each subunit form three rings of negative charge around the pore. As an ion traverses the channel, it encounters these rings of charge. The rings at the external and internal surfaces of the cell membrane (**1**, **3**) may serve as prefilters that help repel anions and form divalent cation blocking sites. The central ring near the cytoplasmic side of the membrane bilayer (**2**) may contribute more importantly to establishing the specific cation selectivity of the selectivity filter, which is the narrowest region of the pore.

D. A high-resolution X-ray crystal structure model of a human neuronal nicotinic ACh receptor-channel. *Right:* A top-down view of the open channel, which is composed of two α_4-subunits and three β_2-subunits arranged around the central pore. These subunits are closely related variants of the α- and β-subunits of the muscle receptor. Two molecules of nicotine (atoms shown as **red** spheres) are bound to the receptor. A permeating cation is shown as a **pink** sphere. *Center:* A side view of the receptor showing the location of the phospholipid bilayer of the membrane and bound nicotine. *Left:* A side view of a single α_4-subunit in the plane of the membrane. The amino-terminus of the subunit consists of a large extracellular domain. Loop C helps form the ligand-binding site. The β_1-β_2 and cys-loops at the interface between the extracellular domain and the M1–M4 membrane-spanning α-helixes transmit a conformational change from the ligand-binding site to the pore to open the channel. (Reproduced, with permission, from Morales-Perez et al. 2016. Copyright © 2016 Springer Nature.)

extracellular portion that contains the ACh binding site, a narrow transmembrane pore selective for cations, and a large exit region at the internal membrane surface (Figure 12–11C). The extracellular region is surprisingly large, approximately 6 nm in length. In addition, the extracellular end of the pore has a wide mouth approximately 2.5 nm in diameter. Within the bilayer of the membrane, the pore gradually narrows.

The autoimmune disorder myasthenia gravis results from the production of antibodies that bind to the extracellular domain of the ACh receptor, leading to a decrease in the number or function of the nicotinic ACh receptors at the neuromuscular junction. If the change is severe enough, this can decrease the EPSP below the threshold for triggering an action potential, resulting in debilitating weakness. Several congenital forms of myasthenia result from mutations in nicotinic ACh receptor subunits that can also alter receptor number or channel function. For example, a mutation in an amino acid residue in the M2 segment leads to a prolonged channel open time, termed the slow channel syndrome, which results in excessive postsynaptic excitation that leads to degeneration of the end-plate (Chapter 57).

The High-Resolution Structure of the Acetylcholine Receptor-Channel Is Revealed by X-Ray Crystal Studies

A deeper understanding of the fine details of the ACh binding site initially came from high-resolution X-ray crystallographic studies of a molluscan ACh-binding protein, which is homologous to the extracellular amino terminus of nicotinic ACh receptor subunits. Remarkably, unlike typical ACh receptors, the molluscan ACh-binding protein is a soluble protein secreted by glial cells into the extracellular space. At cholinergic synapses in snails, it acts to reduce the size of the EPSP, perhaps by buffering the free concentration of ACh in the synaptic cleft.

Further insights into the structure of the complete receptor-channel have come from X-ray crystal structures of related pentameric ligand-gated channels from bacteria and multicellular animals, culminating with a recent X-ray crystal structure of a human neuronal nicotinic ACh receptor in complex with nicotine. Combined with knowledge of structures of related proteins, we now have a remarkably detailed knowledge of the structure and mechanisms underlying ligand binding, channel gating, and ion permeation of the ACh receptor-channel and related ligand-gated channels.

In the neuronal ACh receptor, two α-subunits combine with three β-subunits to form the pentamer (Figure 12–11D). The large extracellular domain of the receptor contains two ACh binding sites and forms a pentameric ring that surrounds a large central vestibule, which presumably funnels ions toward the narrow transmembrane domain of the receptor. Each α-subunit binds one molecule of nicotine at a site located at the interface with a neighboring β-subunit. Electron diffraction data from Nigel Unwin's higher-resolution structures of related cys-loop receptors and from the high-resolution structure of the desensitized state of the neuronal nicotinic receptor show that the four transmembrane segments of each subunit are indeed α-helixes that traverse the 3-nm length of the lipid bilayer (Figure 12–12). In the desensitized state, the M2 segments from the five subunits form a narrow constriction near the intracellular side of the membrane, preventing ion permeation.

Our picture of the transmembrane region of the nicotinic ACh receptor-channel in the open and closed state is still incomplete. However, by comparison with structures of related pLGICs, a coherent picture of the receptor is beginning to emerge. In the closed state, the pore-lining M2 segments lie roughly parallel to each other, forming a narrow central pore. The pore is further constricted to a diameter of 0.3 to 0.4 nm by a ring of highly conserved hydrophobic leucine residues near the middle of the M2 segment (Figure 12–12). This hydrophobic constriction is thought to provide a high-energy barrier that restricts the passage of hydrated cations whose diameter is greater than the constriction in the pore. At present the discrepancy in pore diameter inferred from electrophysiological measuements (0.6 nm) and the narrower value from the crystal structure remains unresolved.

In the open state, the M2 segments are thought to tilt outward and rotate, widening the constriction of the leucine residues in the middle of M2, thus enabling ion permeation. The narrowest constriction in the open pore lies near the intracellular mouth of the channel, where the electronegative hydroxyl side chains from one ring of threonine residues (serine and threonine residues in the muscle ACh receptor) and a second ring of negatively charged glutamate residues are thought to form the selectivity filter. In the desensitized state the M2 segments tilt further, causing the selectivity filter to constrict even more, preventing ion permeation.

A detailed picture of how ligand binding leads to channel opening is now emerging based on various structural and functional studies. Binding of ligand is thought to promote the closure of the cleft between neighboring subunits, leading to the tightening of the extracellular domain of the pentamer, similar to the closing of the petals of a flower. This results in a twisting motion that causes the bottom of the extracellular domain of the receptor to push on the M1 segment and extracellular loop connecting the M2 and

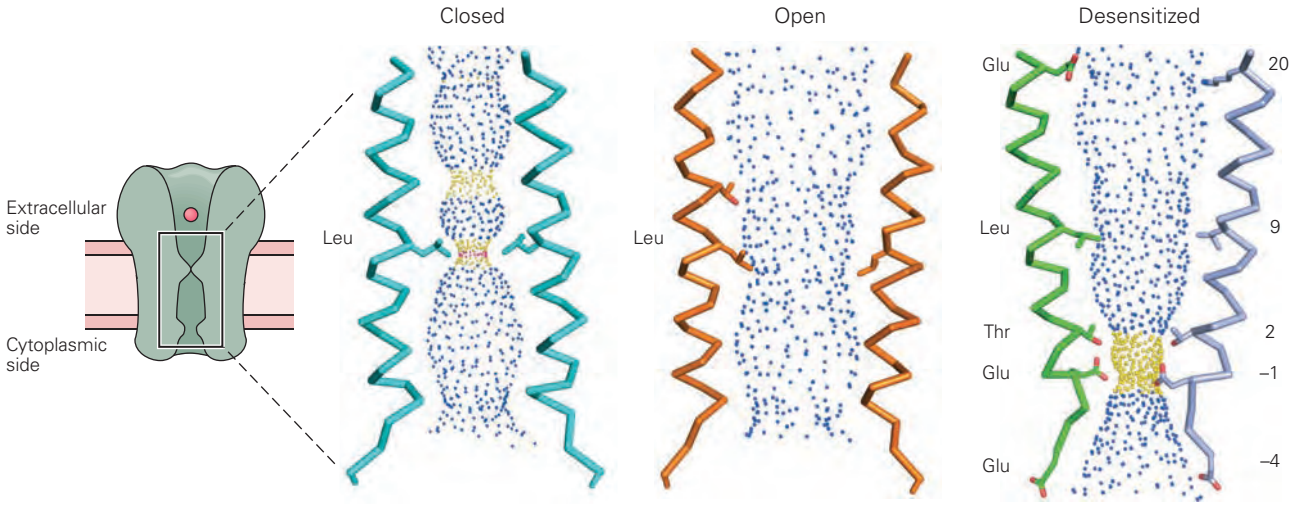

Figure 12–12 A high-resolution three-dimensional structural model of a neuronal nicotinic ACh receptor-channel. High-resolution models of the pentameric family of ligand-gated channels are shown for the closed, open, and desensitized states of the receptor-channel. Two out of five of the M2 α-helixes are shown. The desensitized structure is from the human neuronal ACh receptor. The closed and open states are based on structures of neuronal glycine receptors, which are closely related in amino acid sequence to ACh receptor subunits. Key amino acid side chains are illustrated for the desensitized ACh receptor with position numbering on the right and amino acid abbreviations on the left. According to convention, position 0 is near the intracellular surface of the phospholipid bilayer; other positions are labeled according to relative position in the primary amino acid sequence. A conserved leucine in the middle of the M2 segment (position 9) forms a gate that constricts the pore in the closed state. Ligand binding causes the subunits to tilt outward and twist, opening up the leucine gate. A further conformational change during desensitization causes the subunits to tilt inward near the bottom, constricting the pore near the intracellular side of the channel and thereby producing a nonconducting state. The negatively charged glutamates at positions 20, –1, and –4 correspond to the external (**1**), middle (**2**), and internal (**3**) rings of charge in Figure 12–11C. The negatively charged glutamate at position –1 and the electronegative threonine at position 2 form the selectivity filter of the channel. (Reproduced, with permission, from Morales-Perez et al. 2016. Copyright © 2016 Springer Nature.)

M3 transmembrane segments. This motion exerts a force on the M2 segment that leads to its rotation and tilting, thereby opening up the hydrophobic leucine gate in the middle of the pore and allowing ion permeation. Although future studies will no doubt refine our understanding of the structural bases for nicotinic receptor-channel and function, these recent advances give us an unprecedented molecular understanding of one of the most fundamental processes in the nervous system: synaptic transmission and, specifically, the signaling of information from nerve to muscle.

Highlights

1. The terminals of motor neurons form synapses with muscle fibers at specialized regions in the muscle membrane called end-plates. When an action potential reaches the terminals of a presynaptic motor neuron, it causes the release of ACh.
2. ACh diffuses across the narrow (100-nm) synaptic cleft in a matter of microseconds and binds to nicotinic ACh receptors in the end-plate membrane. The energy of binding is translated into a conformational change that opens a cation-selective channel in the protein, allowing Na^+, K^+, and Ca^{2+} to flow across the postsynaptic membrane. The net effect, due largely to the influx of Na^+ ions, produces a depolarizing synaptic potential called the end-plate potential.
3. Because the ACh receptor-channels are concentrated at the end-plate, the opening of these channels produces a local depolarization. This local depolarization is large enough (75 mV) to exceed the threshold for action potential generation by a factor of three to four.
4. It is important that the safety factor of nerve-muscle transmission be at a high level, as it determines our ability to move, breath, and escape from danger. Decreases in ACh receptor number or function as a result of autoimmune disease or genetic mutations can contribute to neurological disorders.
5. Patch-clamp recordings have revealed the step-like increase and decrease in current in response to the opening and closing of single ACh receptor-channels. A typical excitatory postsynaptic current