

PRINCIPLES OF NEURAL SCIENCE

THIRD EDITION

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An Overall View

Neuronal signaling depends on rapid changes in the electrical potential difference across nerve cell membranes. During an action potential the membrane potential changes quickly, up to 500 volts per second. These rapid changes in potential are made possible by *ion channels*, a class of integral proteins that traverse the cell membrane. These channels have three important properties: (1) they conduct ions, (2) they recognize and select among specific ions, and (3) they open and close in response to specific electrical, mechanical, or chemical signals.

Ion channels in nerve and muscle conduct ions across the cell membrane at extremely rapid rates of up to 100,000,000 ions per second, thereby providing a large flow of ionic current. This current flow causes the rapid changes in membrane potential required for signaling, as will be discussed in Chapters 8 and 10. The high rate of ionic flow in channels is extraordinary—the turnover rates of even the most active enzymes are slower by several orders of magnitude.

In addition to having a high permeation rate, ion channels are highly selective for one or more types of ions. For example, the membrane potential of nerve cells at rest is largely determined by ion channels that are selectively permeable to K^+ . Typically, these channels are a hundred-fold more permeable to K^+ than to other cations, such as Na^+ . During the action potential, however, ion channels selective for Na^+ are activated; these channels are 10- to 20-fold more permeable to Na^+ than to K^+ . Thus, a key feature of neuronal signaling is the activation of different classes of ion channels, each of which is selective for specific ions.

Finally, channels involved in neuronal signaling are also *gated*: They open and close in response to various stimuli. Nongated channels that are always open contrib-

ute significantly to the resting potential (see Chapter 6). In contrast, gated channels that can open or close rapidly in response to different signals are very useful for rapid neuronal signaling. Three major signals can gate ion channels: voltage (voltage-gated channels), chemical transmitters (transmitter-gated channels), and pressure or stretch (mechanically gated channels). Individual channels are usually most sensitive to only one type of signal.

In this chapter we consider four questions: Why do cells have channels? How do channels conduct ions at such high rates and yet remain selective? How are channels gated? How are the properties of these channels modified by various intrinsic and extrinsic signals? Later, in Chapters 6 and 8, we shall consider how nongated channels generate the resting potential and how voltage-gated channels generate the action potential. In Chapters 9 to 11 we shall examine how transmitter-gated channels produce synaptic potentials.

Ions Cross the Cell Membrane Through Channels

To appreciate why cells need channels, we need to understand the nature of the plasma membrane and the physical chemistry of ions in solution. The plasma membrane of all cells, including nerve cells, is about 6–8 nm thick and consists of a mosaic of lipids and proteins. The surface of the membrane is formed by a double layer of lipids. Embedded within this continuous lipid sheet are proteins, including ion channels. The lipids within the membrane are hydrophobic—they are immiscible with water. In contrast, the ions of the extracellular and intracellular space are hydrophilic—they attract water molecules strongly.

Although the net charge on a water molecule is zero, charge is separated within the molecule: Water molecules are *dipolar*. The oxygen atom in a water molecule tends to attract electrons and so bears a small net negative charge, whereas the hydrogen atoms tend to lose electrons and have a small net positive charge. As a result of this distribution of charge, water creates a polar environment. Cations are strongly attracted electrostatically to the oxygen atom of water, and anions to the hydrogen atoms. Because they attract water, ions become surrounded by electrostatically bound water, called the *waters of hydration* (Figure 5–1). For an ion to move from water into the nonpolar hydrocarbon tails of the lipid bilayer in the membrane, a large amount of energy has to be supplied to overcome the attractive forces between the ions and the surrounding water molecules. For this reason, it is extremely unlikely for an ion to move from solution into the lipid bilayer, and therefore the bilayer itself is almost completely impermeable to ions. Ions cross the membrane only through specialized proteins such as ion channels, where, as we shall see, the energetics favor ion movement.

The fact that ion channels are made up of protein and are not simply holes in the lipid membrane has been known with certainty for only about 15 years. The idea of ion channels, however, dates to the end of the nineteenth century. At that time, physiologists knew that cells are

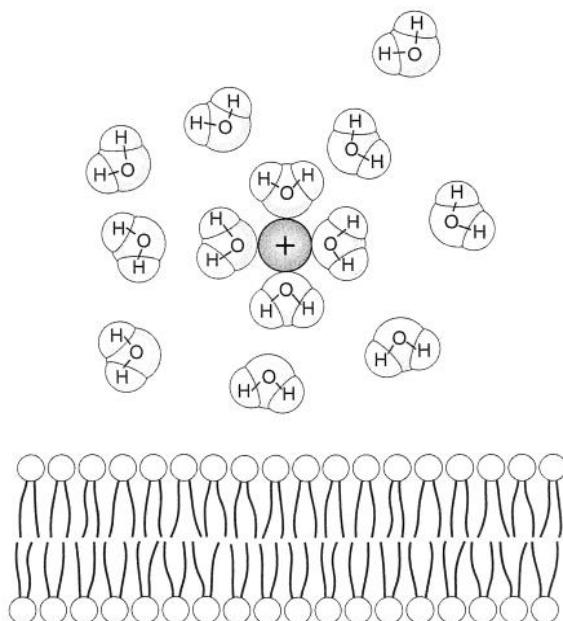
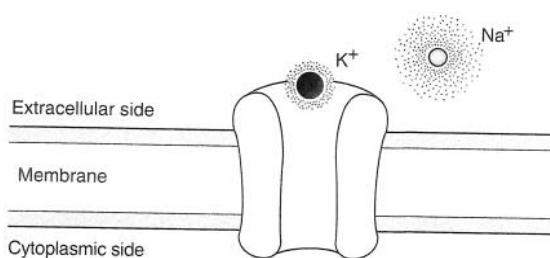


FIGURE 5–1

Ions in solution are surrounded by a cloud of water molecules (waters of hydration) that are attracted by the net charge of the ion. This cloud is carried along by the ion as it diffuses through the solution, adding to its effective size. It is extremely energetically unfavorable, and therefore improbable, for the ion to leave this polar environment to enter the nonpolar environment of the lipid bilayer.

permeable to many small solutes, including some ions, despite the barrier that the cell membrane presents. To explain osmosis (water flow) across biological membranes, the Viennese physiologist Ernst Brücke proposed that membranes contain channels that allow water to flow across membranes but exclude larger solutes. Later, William Bayliss, a British physiologist, suggested that a water-filled channel would also permit ions to cross membranes since the ions would not need to be stripped of their waters of hydration.

The idea that ions move through channels leads to a question: How can a water-filled channel conduct at high rates and yet be selective? How does a channel allow K^+ to pass while excluding Na^+ ions? The explanation cannot be based solely on ionic diameter, because K^+ has a crystal radius of around 0.133 nm, which is larger than the Na^+ crystal radius of 0.095 nm. As we have seen, however, an ion in solution is surrounded by the waters of hydration. Thus, the ease with which an ion moves in solution (its mobility or diffusion constant) is not related simply to the size of an isolated ion; rather it is determined by the size of the shell of water surrounding the ion. The smaller an ion, the more highly localized its charge and the stronger its electric field. As a result, a smaller ion such as Na^+ has a stronger effective electric field surrounding it than a larger ion like K^+ , and thus exerts a stronger attraction on its waters of hydration. As Na^+ moves through solution,

**FIGURE 5–2**

A model for K^+ selectivity based on ion diffusion in a water-filled pore. Although a Na^+ ion is smaller than a K^+ ion, its effective diameter in solution is larger because its local field strength is more intense, causing it to attract a larger cloud of water molecules. Thus, a K^+ -selective channel can, in principle, select for K^+ over Na^+ by excluding hydrated ions larger than a given diameter (determined by the pore diameter).

its extra electrostatic attraction for water tends to slow it down relative to K^+ ; thus, Na^+ behaves as if it has a larger water shell. In fact, there is an inverse relation between the size of an ion and its mobility in solution. We therefore can model a channel selective for K^+ simply on the basis of interactions of the ion with water in a water-filled channel (Figure 5–2).

Whereas this idea provides a possible explanation for how a channel can select K^+ and exclude Na^+ , it cannot explain how a channel could select Na^+ and exclude K^+ . The difficulty in explaining a Na^+ -selective channel led many physiologists in the 1930s and 1940s to abandon the channel theory in favor of the idea that ions cross cell membranes by first binding to a specific carrier protein that then transports the ion through the membrane. In this carrier model, selectivity is achieved through a specific chemical binding between the ion and the polar or charged amino acid residues of the carrier protein, not on the basis of mobility in solution. In fact, we now know that ions can cross membranes by means of carriers, or transport proteins, the Na^+-K^+ pump being a well-characterized example (see Chapter 3).

However, many observations on ion conductance across the cell membrane do not fit the carrier model. One of the most telling pieces of evidence is the rate of ion transfer across membranes. This was first examined in the early 1970s in acetylcholine-activated ion channels located in the membrane of skeletal muscle at the synapse between nerve and muscle (see Chapter 10). Using measurements of membrane current noise (small statistical fluctuations in the mean ionic current induced by acetylcholine), Bernard Katz and Ricardo Miledi, and later Charles Anderson and Charles Stevens, inferred that a single acetylcholine-activated channel can transport 10^7 ions per second. In contrast, the Na^+-K^+ pump can transport at most 10^3 ions per second. If the acetylcholine receptor acted as a carrier, it would have to shuttle an ion across the membrane in $0.1\ \mu s$, a physically implausible rate. Therefore, the acetylcholine receptor (and similar li-

gand-gated receptors) must conduct ions through a protein channel. Later measurements on many voltage-gated channels selective for K^+ , Na^+ , and Ca^{2+} demonstrated similar large unitary conductances, indicating that they too are channels.

But we are still left with the crucial problem: How does a channel achieve ion selectivity? To explain selectivity, the original pore theory was extended first by Loren Mullins, and later by George Eisenman and Bertil Hille, who proposed that channels have a narrow region that acts as a molecular sieve (Figure 5–3). At this *selectivity filter*, an ion sheds most of its waters of hydration and forms a weak chemical bond (electrostatic interaction) with charged or polar amino acid residues that line the walls of the channel. Since the shedding of waters of hydration is energetically unfavorable, an ion will permeate a channel only if the energy of interaction with the selectivity filter compensates for the loss of waters of hydration. Permeant ions remain bound to the selectivity filter for a short time (less than $1\ \mu s$), after which the electrochemical gradient propels the ion through the channel. In some channels the pore diameter is large enough to accommodate several water molecules. An ion traversing such a channel need not be stripped completely of all of its water shell. Thus, a variety of physical interactions between the ion and the channel molecule produces a wide range of ion selectivities (Figure 5–3).

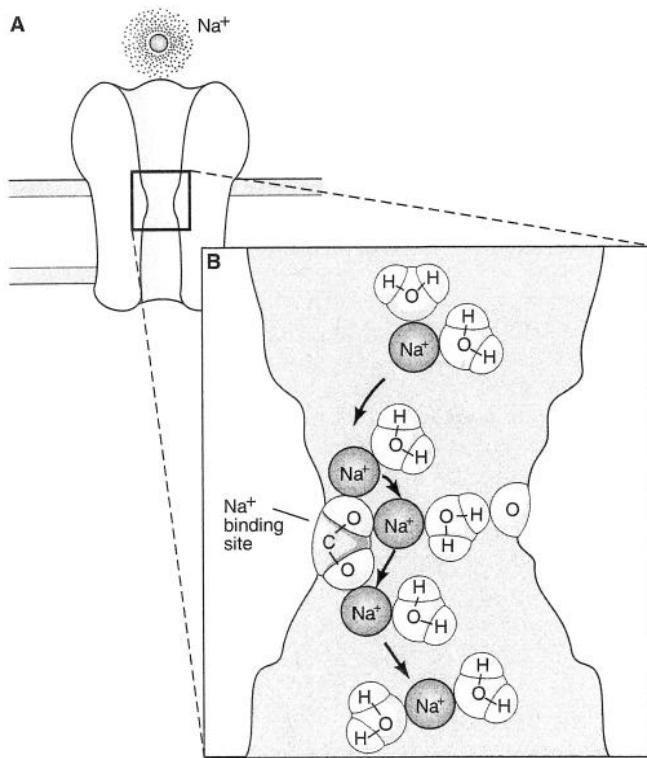
These interactions in voltage-gated and transmitter-gated ion channels are described in detail in Chapters 8 and 10.

Ion Channels Can Now Be Investigated by Functional and Structural Methods

To understand fully how channels work, we ultimately will need three-dimensional structural information that has proven so informative in the study of enzymes and other cytoplasmic proteins. So far X-ray crystallographic and other structural analyses have not been generally applied to integral membrane proteins, such as ion channels, because their hydrophobic regions make them difficult to crystallize. However, two other powerful methods, single-channel recording and gene cloning, have taught us a good deal about ion channels.

Single-Channel Recording Can Measure the Activity of a Single Protein Molecule

Before it became possible to resolve the small unitary currents through single ion channels in biological membranes, it was already possible to study channel function in artificial planar lipid bilayers. In the early 1960s, Paul Mueller and Donald Rudin developed a technique for making functional lipid bilayers by painting a thin drop of lipid over a small hole in a nonconducting chamber that separates two salt solutions. Because lipids are impermeable to ions, these lipid membranes have a very low con-

**FIGURE 5–3**

Sodium channels have a selectivity filter somewhere along the length of the channel, with a site that weakly binds Na^+ ions. (From Hille, 1984.)

A. Schematic diagram of the Na^+ channel.

B. Schematic diagram of the site within the channel that selects which ions will permeate. According to the hypothesis developed by Bertil Hille and colleagues, as a Na^+ ion moves through the filter it binds transiently at the active site. Here, the positive charge of the ion is stabilized by a hydrophilic (polar) amino acid residue lining the channel and also by a water molecule that is attracted to a second polar amino acid residue lining the other side of the channel wall. It is thought that, for steric reasons, a K^+ ion with its associated water molecules cannot be stabilized as effectively and therefore will be excluded from the filter. (From Hille, 1984.)

ductance to ions (high resistance). When Mueller and Rudin added certain bacterial proteins to the salt solution in the bath, the membrane underwent a dramatic increase in ion conductance.

Based on this remarkable finding, Stephen Hladky and Dennis Haydon, in 1970, studied in detail the conductance changes produced by the antibiotic gramicidin A, a peptide only 15 amino acids long that consists of alternating hydrophobic *d* and *l* amino acids. Surprisingly, Hladky and Haydon, followed by Olaf Anderson and his colleagues, found that when they applied a low concentration of gramicidin A to the planar bilayer, the antibiotic induced small unitary, step-like changes in current flow across the membrane (Figure 5–4A). These reflected the

all-or-none opening and closing of an ion channel formed by the peptide.

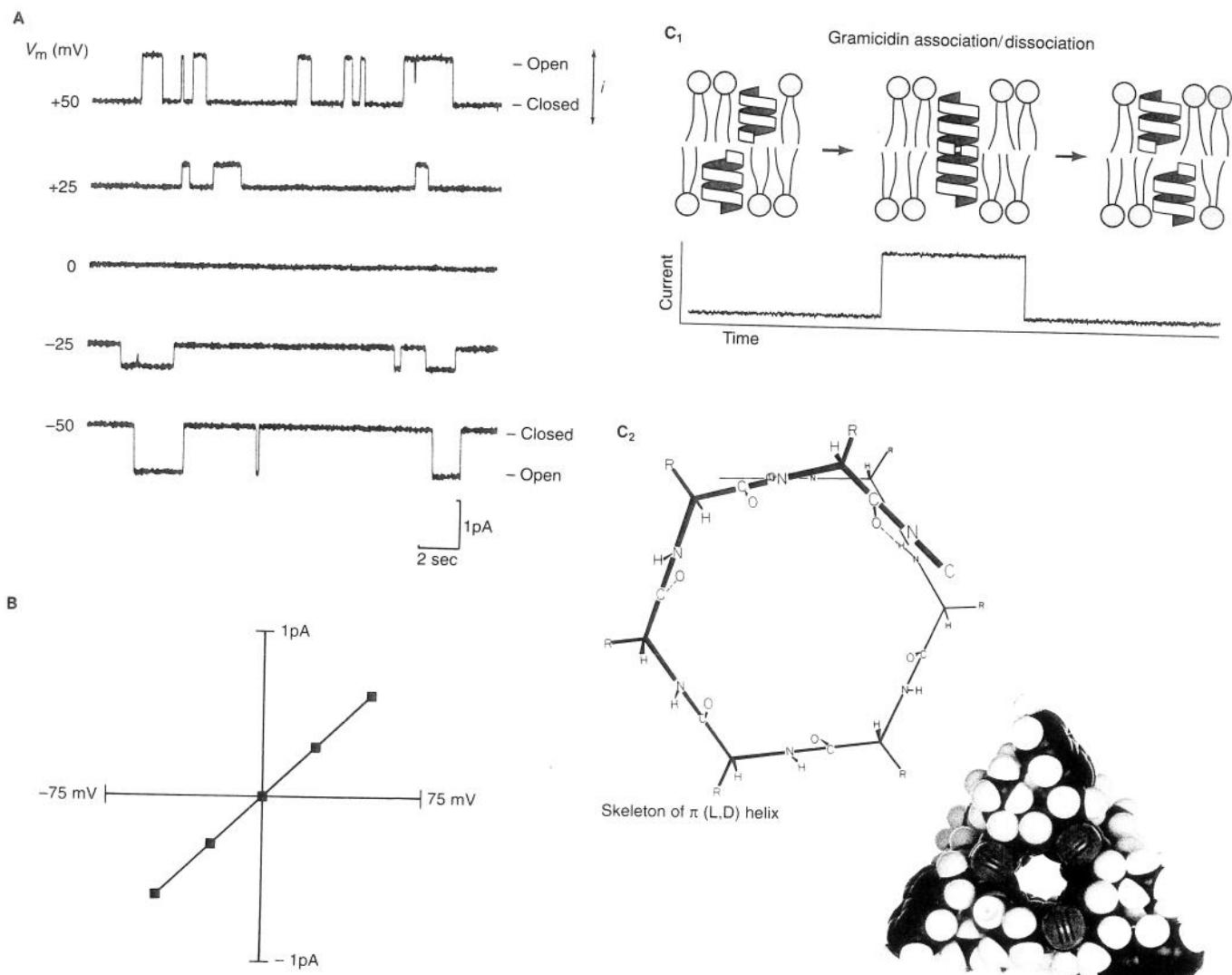
The unitary current depends on membrane potential in a linear manner (Figure 5–4A, B). Thus, the channel behaved as a simple resistor; the amplitude of the single channel current could be obtained from Ohm's law, $I = V/R$. The slope of the relation between current (I) and voltage (V) yielded a value for the resistance of a single open channel of around 8×10^{10} ohms (Figure 5–4B). However, in dealing with channels, we generally speak of its *conductance*, the reciprocal of *resistance*, which provides an electrical measure of ion permeability. The unitary conductance of the gramicidin A channel is around 12×10^{-12} siemens or 12 picosiemens (pS), where 1 siemen = 1/ohm.

Biochemical and X-ray crystallographic analyses have shown that the unusual alternating *d* and *l* amino acid composition of gramicidin allows the peptide to form a β -helical structure (Figure 5–4C2). The polar carbonyl oxygen atoms of the peptide bonds (with a slight negative charge) all tilt inward toward the center of the helix, where they form the walls of the channel and interact with the permeant cations. The hydrophobic amino acid side chains all point outward from the center of the helix and interact with the lipid membrane. Two gramicidin peptides are thought to form a channel by dimerizing end-to-end (Figure 5–4C1). The opening and closing of the gramicidin channel corresponds to the dimerization and dissociation of the gramicidin monomers, respectively.

Although such artificial systems provided the first insights into the basic principles of channel properties, these principles had yet to be demonstrated in biological membranes. In 1976 Erwin Neher and Bert Sakmann developed the patch-clamp technique for recording current flow from single channels in biological membranes (Box 5–1). Neher and Sakmann used the same frog skeletal muscle preparation that Katz and Miledi examined using noise analysis. A glass micropipette containing acetylcholine, the neurotransmitter that activates channels in the membrane of skeletal muscle, was pressed tightly against the muscle membrane. Small unitary current events, representing the opening and closing of single acetylcholine-activated ion channels, were observed in the area of the membrane under the pipette tip. As with the gramicidin A channels, these acetylcholine receptor-channels also displayed a linear relation between current and voltage and had a single-channel conductance of around 25 pS.

Ion Channels Can Now Be Studied Through Molecular Biological Approaches

What do biological channels look like? How does the channel protein span the membrane? What happens to the structure of the channel when it changes conformation from its closed to its open state? Where along the length of the channel do drugs and transmitters bind? Definitive answers to these questions will require X-ray crystallographic analysis of purified ion channel proteins. How-

**FIGURE 5-4**

Characteristics of the current that flows through gramicidin channels.

A. Channels formed by a few gramicidin molecules in a lipid bilayer open and close in an all-or-none fashion, resulting in brief current pulses of quantal size through the membrane. If the electrical potential (V_m) across the membrane is varied, the current through the channels changes proportionally due to the altered electrical driving force.

B. A plot of the current through the channel versus the potential difference across the membrane reveals that the current is

ever, over the past several years biochemical and molecular biological approaches have resulted in considerable progress in understanding channel structure and function.

Ion channels are large integral membrane glycoproteins, ranging in molecular weight from 25,000 D to 250,000 D. All channels have a central aqueous pore that spans the entire width of the membrane. Many ion channels are made up of two or more subunits, which may be identical or distinct (Figure 5-6).

linearly related to the driving force, i.e., the channel behaves as an electrical resistor that follows Ohm's law ($I = V/R$). (Data in A and B courtesy of Olaf Anderson and Lyndon Providence.)

C. Proposed structure of the gramicidin A channel. 1. A functional channel is formed by end-to-end dimerization of two gramicidin peptides. (From Sawyer et al., 1989.) 2. The helical structure of gramicidin A peptide. The carbonyl and amide groups of the peptide backbone form the hydrophobic channel lining. The hydrophobic side chains (R) point outward into lipid. The space-filling model shows how the pore is formed in the center of the helix. (From Urry, 1971.)

The genes for six or seven major classes of ion channels have now been cloned and sequenced. The primary amino acid sequence of the channel inferred from the nucleotide sequence has been used to suggest the structure of different channel proteins. These models rely on computer programs that predict regions of secondary structure (α -helices or β -sheets) that are likely to correspond to transmembrane domains of the channel, based on existing information on proteins whose actual three-dimensional structure is known from electron and X-ray diffraction

Recording Current Flow from Single Ion Channels

The patch-clamp technique was developed in 1976 by Erwin Neher and Bert Sakmann to record current flow from single ion channels. This technique is a refinement of voltage clamping (see Box 8–1). A small fire-polished glass micropipette with a tip diameter of around 1 μm is pressed against the membrane of a frog skeletal muscle fiber that has been treated with proteolytic enzymes to remove connective tissue from the muscle surface. The pipette is filled with a physiological salt solution. A metal electrode in contact with the electrolyte in the micropipette connects it to a special electrical circuit that measures the current that flows through channels in the membrane under the pipette tip.

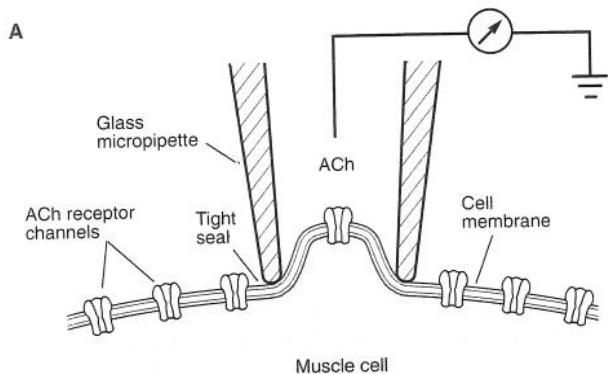


FIGURE 5–5A
Patch-clamp setup. (Adapted from Alberts et al., 1989.)

In 1980 Neher discovered that applying a small amount of suction to the patch pipette greatly tightens the seal between the pipette and the membrane. The result is a seal with extremely high resistance between the inside and outside of the pipette. This dramatically lowers electronic noise and extends the utility of the technique to the whole range of channels involved in electrical excitability, including those with small conductance. Since this discovery, Neher and Sakmann, and many others, have used the patch-clamp technique to study all three major classes of ion channels—voltage-gated, transmitter-gated, and mechanically gated channels—in a variety of neurons and other cells.

analysis. The first membrane protein whose structure was well understood is the bacterial photo-pigment bacteriorhodopsin (Figure 5–7A). This protein has a molecular weight of 25,000. It contains regions with polar (hydrophilic) amino acids, such as the acidic amino acids glutamate and aspartate, and basic amino acids, such as lysine, and regions with nonpolar or uncharged (hydrophobic) amino acids, such as glycine, alanine, and phenylalanine.

BOX 5–1

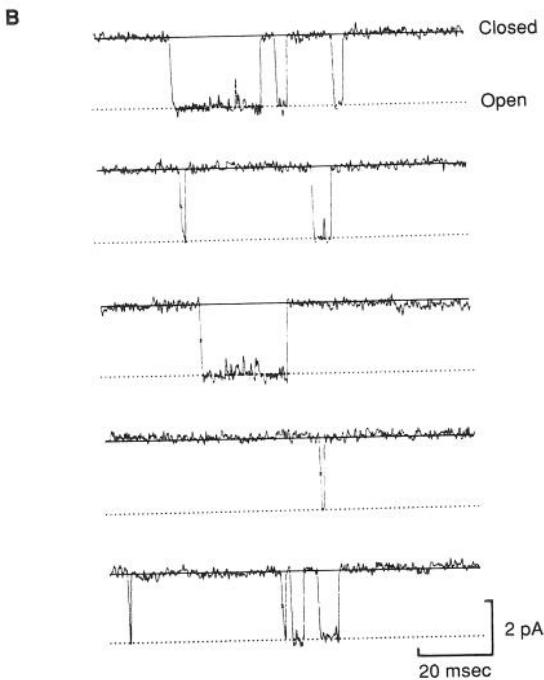
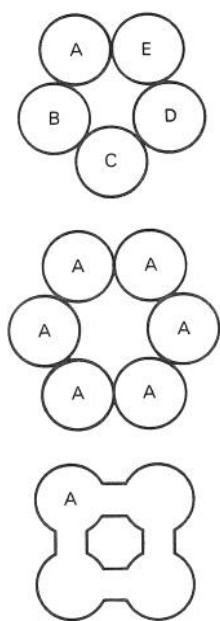


FIGURE 5–5B
Record of the current flowing through a single ion channel as the channel switches between closed and open states. (Courtesy of B. Sakmann.)

Independently Christopher Miller developed a method for incorporating channels from biological membranes into planar lipid bilayers. With this technique, biological membranes are first homogenized and a membrane vesicle fraction is isolated by differential centrifugation. Under appropriate ionic conditions these vesicles fuse with a planar lipid membrane. Any ion channel in the vesicle will thus be incorporated into the planar membrane. This technique has two experimental advantages. First, it allows ion channels to be studied from regions of cells that are inaccessible to patch clamp. For example, Miller has successfully studied a K^+ channel isolated from the internal membrane of skeletal muscle sarcoplasmic reticulum. Second, it allows the study of how the composition of the membrane lipids influences channel function.

There are, in all, seven hydrophobic regions. Each hydrophobic region is about 15–20 amino acids long, has an α -helical secondary structure, and spans the membrane. These membrane-spanning regions are in turn linked by three cytoplasmic and three extracellular hydrophilic loops.

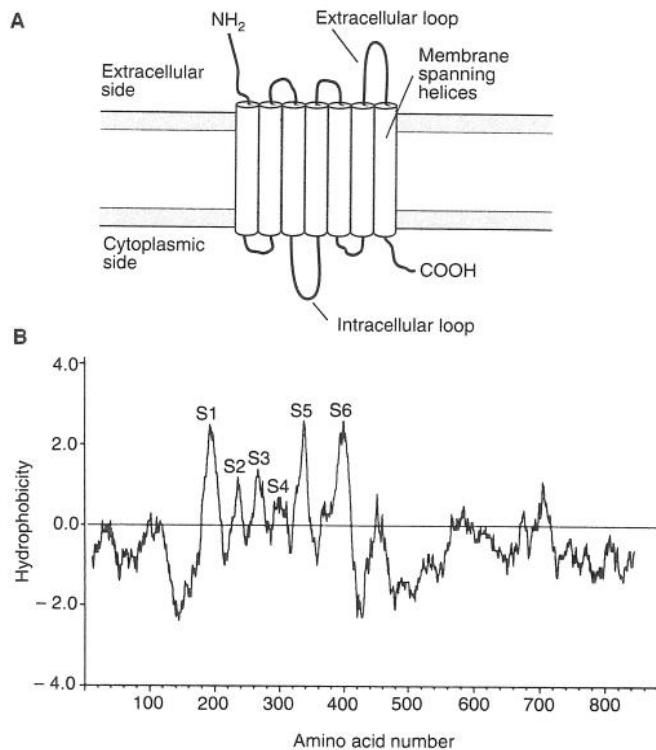
Efforts to understand the secondary structure of channels are based largely on information from bacterio-

**FIGURE 5–6**

Subunit basis of channel structure. Channels can be constructed as hetero-multimers from distinct subunits (top), homo-oligomers from a single type of subunit (middle), or from a single polypeptide chain organized into repeating motifs that act as pseudo-subunits (bottom).

orhodopsin and more recent information from the X-ray diffraction studies of the photosynthetic reaction center (an important plant membrane protein). Regions of a protein that are nonpolar can be identified using a *hydrophobicity plot*, in which each amino acid residue is assigned a hydrophobicity index based on the nature of its side chain. Amino acids with hydrophobic side chains are given large positive numbers; amino acids with hydrophilic side chains are given large negative numbers. The hydrophobic index for several amino acids around a given residue is then averaged and this number is plotted as a function of position in the primary sequence (Figure 5–7B). Since an α -helix made of 15–20 amino acids can span the lipid bilayer of a biological membrane (with a thickness of 4 nm), a stretch of 15–20 or more amino acids with a large hydrophobic index is a candidate for a membrane-spanning region. To form a complete channel whose walls completely surround an aqueous pore, four to six transmembrane α -helices are required.

In principle, a membrane-spanning α -helix could also be constructed from an amphipathic peptide consisting of alternating polar and nonpolar amino acids. If the polar amino acids are placed at every third or fourth position, all the polar side chains will line up on one side of that helix (which makes a complete turn every 3.5 amino acid residues). This is an attractive model for an ion channel, since the polar side chains could form the walls of the water-filled pore while the nonpolar side chains would face the lipid bilayer or hydrophobic interior of the protein.

**FIGURE 5–7**

Secondary structure of membrane-spanning proteins.

A. A proposed secondary structure for bacteriorhodopsin. Each cylinder represents a membrane-spanning α -helix containing around 20 hydrophobic amino acid residues. The membranes are connected by segments (loops) of hydrophilic residues. (From Huang et al., 1982.)

B. The membrane-spanning regions of an ion channel can be identified using a hydrophobicity plot. A running average of the hydrophobicity is plotted for the entire amino acid sequence for a K^+ channel from rat brain. Each point in the plot represents the average hydrophobic index of a 19-amino-acid-long window plotted at the amino acid residue position corresponding to the midpoint of this window. This plot is based on the inferred amino acid sequence obtained from the nucleotide sequence of the cloned K^+ channel gene. (From Frech et al., 1989.)

Additional insight into channel structure and function can be obtained by comparing the primary amino acid sequence of related channels from different species and identifying regions with high degrees of sequence homology. The fact that such regions have been highly conserved through evolution points to the importance of that region in channel structure and function. Further insight into structure-function relationships can be obtained from sequence homologies among different, but related, channels. Such homologous regions are likely to underlie a common biophysical function shared by the different channels. For example, all voltage-gated channels contain a putative α -helix membrane-spanning domain that contains positively charged amino acids (lysine or arginine) spaced at every third position along the α -helix. The fact that this motif is observed in all voltage-gated Na^+ , K^+ , and Ca^{2+}

channels, but not in ligand-gated channels, lends support to the view that this charged region may play an important role in voltage-dependent gating (see Chapter 8).

Once a structure for a channel has been proposed, it can be tested in several ways. First, antibodies can be raised against synthetic peptides corresponding to different hydrophilic regions in the protein sequence. Using immunocytochemistry, one can then determine whether the antibody binds to the extracellular or cytoplasmic surface of the membrane, thus defining whether a particular region of the channel is extracellular or intracellular.

Second, genetic engineering can be used to produce chimeric channels, channels with selected parts derived from the genes of different species. This technique takes advantage of the fact that channels in different species have somewhat different properties. For example, the bovine acetylcholine-gated receptor channel has a slightly higher single-channel conductance than the same channel in electric fish. By comparing the properties of a chimeric channel to those of the original channels, it is possible to assess which regions of the channel are involved in different functions. For example, Sakmann and Shosaku Numa and their colleagues have been able to identify a specific membrane-spanning segment of the acetylcholine-gated channel as the region that forms the lining of the pore (see Chapter 10). Finally, the roles of different amino acid residues or stretches of residues can also be tested using *site-directed mutagenesis*, a type of genetic engineering in which specific amino acid residues are substituted or deleted.

Ion Channels Share Several Characteristics

All cells make use of local intercellular signaling processes, but only nerve and muscle cells are specialized for rapid signaling over long distances. Although nerve and muscle cells have a particularly rich variety and high density of membrane ion channels, their channels do not appear to differ fundamentally from those in other cells in the body. In this section we describe the general properties of ion channels found in a wide variety of cell types.

Ion Channels Facilitate the Passive Flux of Ions Across the Cell Membrane

The flux of ions through ion channels is passive, requiring no expenditure of metabolic energy. The direction and eventual equilibrium for this flux is determined not by the channel itself, but rather by the electrochemical driving force across the membrane.

Ion channels select the types of ions that they allow to cross the membrane. Each channel type discriminates between possible permeant ions on the basis of ionic charge, allowing either cations or anions to permeate. Some cation-permeable channel types are relatively nonselective for the cations present in extracellular fluid—they will pass Na^+ , K^+ , Ca^{2+} , and Mg^{2+} . However, most cation-selective channels are more selective; each one is permeable primarily to a single type of ion, either Na^+ , K^+ , or Ca^{2+} . All known types of anion-selective channels are permeable to only one physiological ion, Cl^- .

The kinetics of ion flow through a channel are characterized by the size and voltage-dependence of the channel's conductance. The kinetic properties of ion permeation are best described by the channel's conductance, which is determined by measuring the current (ion flux) that flows through the open channel in response to a given electrochemical driving force. The net electrochemical driving force is determined by two factors—the electrical potential difference and the concentration gradient of the permeant ions across the membrane. Changing either one can change the net driving force (see Chapter 6). In some channels, the current through the open channel varies linearly with driving force, i.e., the channels behave as simple resistors. In others the current flow through the open channel is a nonlinear function of driving force. This type of channel behaves like a rectifier—it conducts ions more readily in one direction than in the other when the direction of the driving force is reversed. It is customary to characterize the conductance of a rectifying channel not by a single value but rather by plotting current versus voltage for the channel over the physiological voltage range (Figure 5–8).

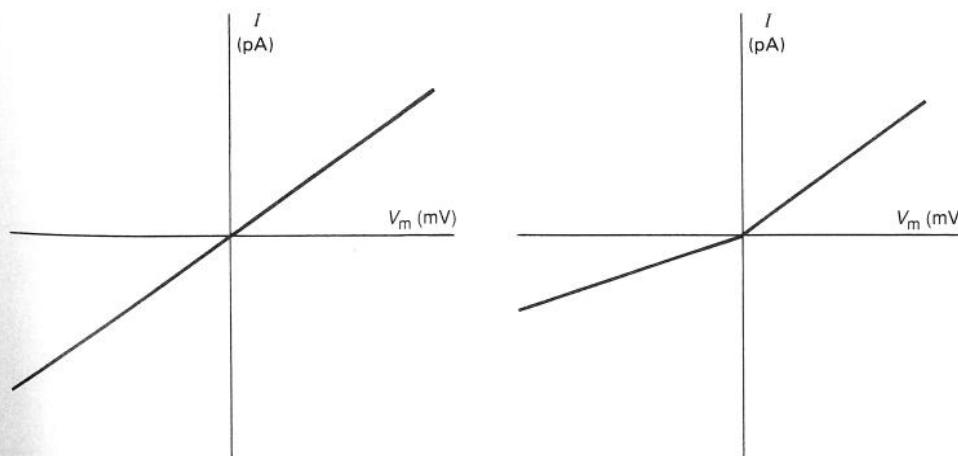
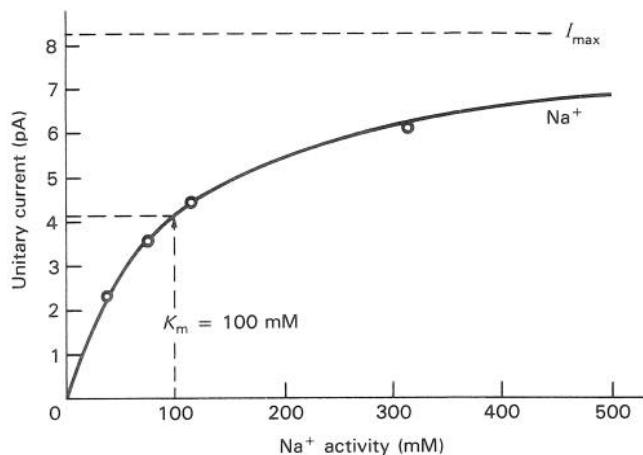


FIGURE 5–8
Single-channel current–voltage relationships. In many channels the relation between current flow through an open channel (I) and the applied membrane voltage (V_m) is linear, as illustrated in the plot at left. Such channels are said to be ohmic, as they follow Ohm's law, $I = V_m/R$. In other channels the relation between current and membrane potential is nonlinear, as shown on the right. This kind of channel is said to rectify.

**FIGURE 5-9**

The relation between single-channel current and ionic concentration saturates. Here the size of the outward ionic current through an ACh-activated channel is plotted as a function of internal Na⁺ concentration (actually Na⁺ activity is plotted). The data (open points) are fitted by the equation for a simple one-to-one binding relation, for which a dissociation constant (K_m) of 100 mM defines the affinity of the channel for Na⁺, at which concentration the binding sites are half-occupied. (Adapted from Horn and Patlak, 1980.)

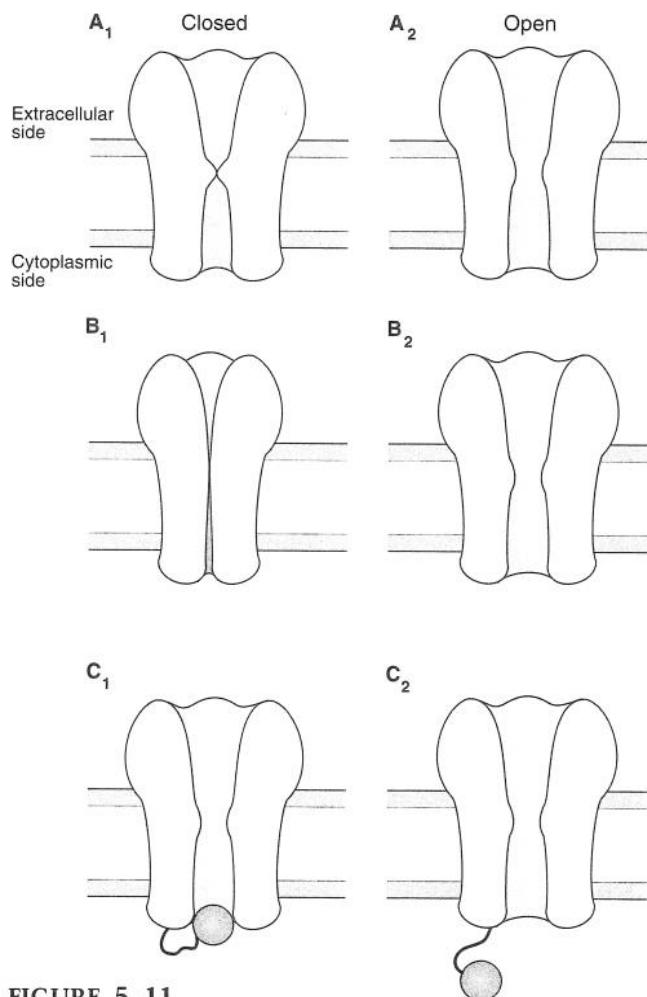
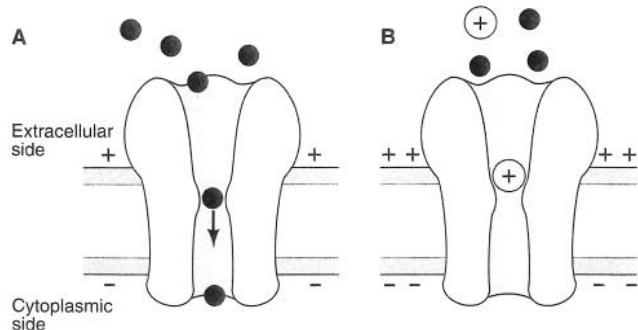
Current flow through an ion channel may saturate. The rate at which ions flow through a channel (i.e., current) varies with the concentration of the ions in the surrounding solution. At low concentrations the current increases almost linearly with concentration. At higher ion concentrations the current tends to saturate, even though the electrochemical driving force is greater (Figure 5-9).

This saturation effect is consistent with the idea that ion permeation involves binding of ions to specific polar sites within the pore of the channel, rather than obeying

FIGURE 5-10

Ion channels can be blocked.

- A. Permeating ions readily pass through the selectivity filter.
- B. Blocking particles (larger circles) enter the mouth of the channel but become stuck, as they are too wide to pass through the selectivity filter. If the blocking particle entering the membrane is electrically charged, its binding kinetics will be influenced by the membrane potential. For example, a positively charged blocker is forced toward the binding site when the inside of the cell is made more negative.

**FIGURE 5-11**

Three different physical models for channel gating.

- A. A discrete conformational change occurs in one region of the channel.
- B. A generalized conformational change results in changes in structure along the length of the channel.
- C. A blocking particle swings into and out of the channel mouth.

the laws of electrochemical diffusion in free solution. A simple electrodiffusion model would predict that the ionic current should continue to increase as the ionic concentration is increased—the more charge carriers in solution, the greater the current flow. But with nearly all channels, current flow begins to saturate at high ionic concentrations. The relation between current and ionic concentration for a wide range of ion channels is often well fitted by a simple one-to-one binding equation (rectangular hyperbola), suggesting that a single ion binds to a channel during permeation. The ionic concentration at which current flow is half-maximal defines the dissociation constant for ion binding in the channel. One striking feature of these plots is that the dissociation constant is typically quite high—around 100 mM—indicating a weak binding compared with the dissociation constants for typical enzyme-substrate interactions. This weak interaction indicates

that the bonds between the ion and the channel are rapidly formed and broken. In fact, an ion typically stays bound in the channel for less than 1 μ s. This rapid off-rate for ion binding ensures that channels achieve a very high conduction rate (on the order of 10^7 ions per second).

Permeation through the ion channel can be inhibited by blocking access to or plugging the pore. Permeation through an ion channel can be inhibited by a blocking molecule that binds either to a site at the mouth of the pore or somewhere within the pore (Figure 5–10). If the inhibitor is an ionized molecule that binds to a site within the pore, binding will be influenced by membrane potential, because the charged inhibitor molecule will sense the membrane electric field as it enters the channel. For example, if a positively charged channel blocker enters the channel from outside the membrane, making the membrane potential more negative will drive the blocker into the channel, increasing the degree of the block. Although most blocking molecules are typically exogenous drugs or toxins, some are present under physiological conditions. For example, common ions such as Mg^{2+} , Ca^{2+} , and Na^+ can act as channel blockers in certain types of channels.

The Opening and Closing of a Channel Involves Conformational Changes

All ion channels so far studied that open and close are *allosteric proteins*. Each channel protein has two or more conformational states that are relatively stable. Each of these stable conformations represents a different functional state. For example, each allosteric channel has at least one open state and one closed state, and may have more than one of each. The transition of a channel between closed and open states is called gating.

Relatively little is known about gating mechanisms, other than that they involve a conformational change in channel structure. Although the picture of a gate swinging open and shut is a convenient image, it probably is accurate only for certain channels (for example, the inactivation of Na^+ and K^+ channels, which we shall consider in Chapter 8). More commonly, channel gating involves widespread changes in channel conformation. For example, evidence from high-resolution electron microscopy and image analysis of the gap junction type of ion channel, which we shall consider in Chapter 9, suggests that the opening and closing of this channel involves a concerted twisting and tilting of the six subunits that make up the channel. Three general physical models of channel gating are illustrated in Figure 5–11.

Because the primary function of ion channels in neurons is to mediate rapid signaling, several specialized allosteric control mechanisms have evolved that influence the amount of time a channel spends in each of its different conformations. Some ion channels are regulated by the noncovalent binding of chemical ligands. These ligands may be neurotransmitters or hormones in the extracellular environment that bind to the extracellular side of the channel (Figure 5–12A), or they may be intracellular second messengers that are activated by transmitters. As

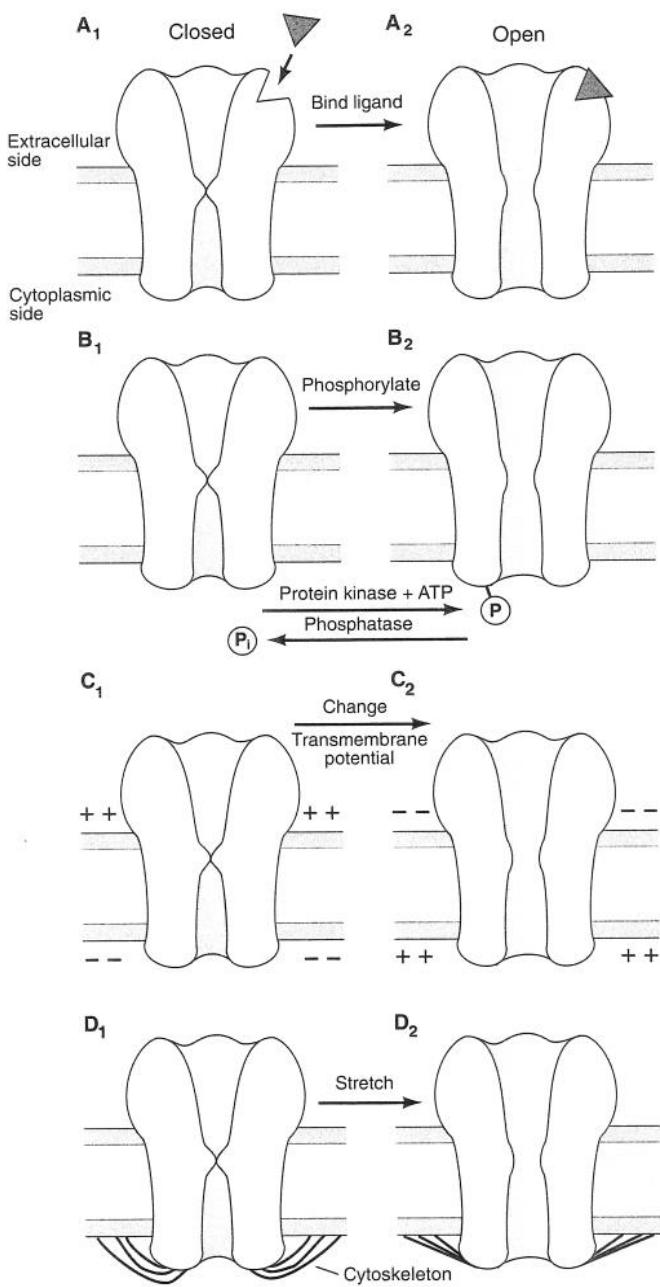


FIGURE 5–12

Channel gating is controlled by several types of stimuli.

- A. Ligand-gated channels open in response to binding of the ligand to its receptor. The energy from ligand binding drives channel gating toward an open state.
- B. Protein phosphorylation and dephosphorylation regulate the opening and closing of some channels. The energy for channel opening comes from the transfer of the high-energy phosphate, P.
- C. Changes in membrane voltage can open and close some channels. The energy for channel gating comes from changes in the electrical potential difference across the membrane.
- D. Other channels are activated by stretch or pressure. The energy for gating may come from mechanical forces due to channel–cytoskeleton interactions.

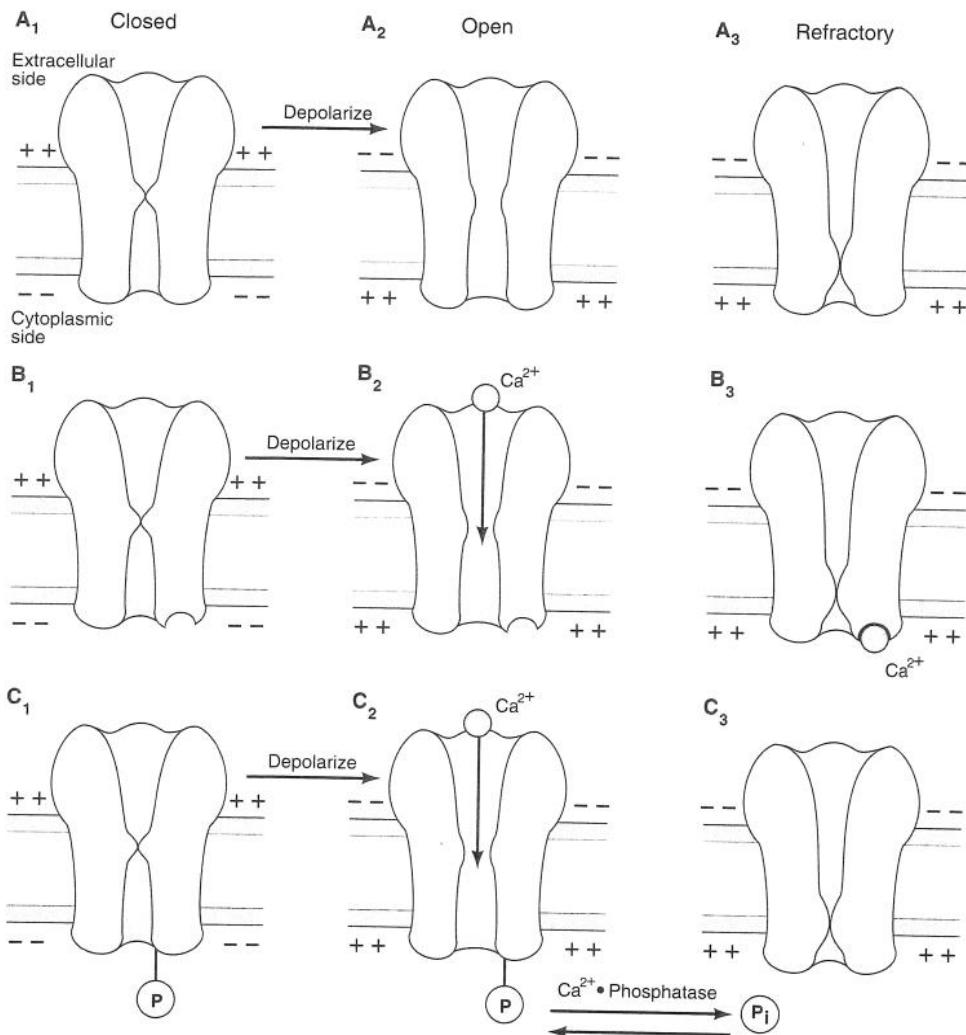


FIGURE 5–13

Three mechanisms by which channels can enter refractory states in which they are closed and incapable of being activated.

A. Voltage-gated channels often respond to a change in membrane potential by first going from a closed resting state (**1**) to a transient open state (**2**). The channel then enters a prolonged refractory or inactivated state (**3**). Only after the potential difference across the membrane is restored to its original value can the channel recover from inactivation, returning to the resting state (**1**).

B. Intracellular Ca^{2+} causes inactivation in some channels by directly binding to the channel. The internal Ca^{2+} level rises as

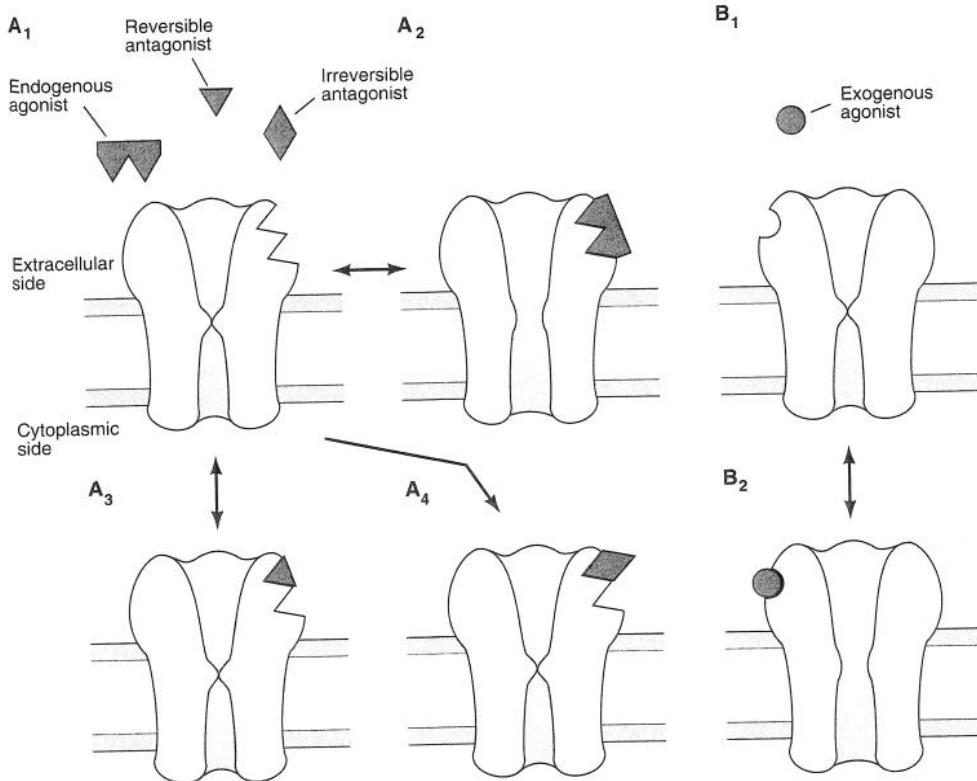
a result of the opening of voltage-dependent Ca^{2+} channels in response to depolarization. The internal Ca^{2+} then can act in a *cis* manner, inactivating the channel that permitted its entry. Alternatively, internal Ca^{2+} can act in a *trans* fashion, causing inactivation of other types of ion channels.

C. An increase in internal Ca^{2+} concentration may also activate phosphatases (calcineurin) and produce inactivation through dephosphorylation of Ca^{2+} channels. At high concentrations, Ca^{2+} may even produce an irreversible, nonspecific inactivation of channels due to recruitment of Ca^{2+} -activated proteases.

we shall consider in more detail in Chapter 12, the second messenger may act on the inside of the channel either directly, by binding to the channel, or indirectly, by initiating protein phosphorylation that is mediated by enzymes called *protein kinases* (Figure 5–12B). This covalent modification of the channel is reversed by dephosphorylation, a reaction catalyzed by protein phosphatases. Covalent modification results in relatively long-lasting changes in the functional states of ion channels called *modulatory changes*. Because ion channels are integral membrane proteins, some are subject to the influence of two other classes of allosteric regulators: the electric field

across the membrane and the mechanical stretch of the membrane (Figure 5–12C, D). Under the influence of allosteric regulators, ion channels can enter one of three functional states: closed and activatable (resting); open (active); closed and nonactivatable (refractory).

How does a given stimulus, such as a voltage change or ligand binding, produce a change in conformation of a channel? For voltage-gated channels, such as the Na^+ channel, the opening and closing is associated with a movement of a charged region of the channel through the electric field of the membrane. Changes in the membrane voltage tend to move this charged region back and forth

**FIGURE 5–14**

The binding of exogenous ligands to a channel can bias the channel to either an open or a closed state by a variety of mechanisms.

A. For a channel that normally is opened by the binding of an endogenous ligand (1, 2), a drug or toxin may block the binding of the activator by either a reversible (3) or irreversible (4) reaction.

B. Some exogenous regulators can bias a channel to the open state by binding to a regulatory site.

through the electric field, and thus drive the channel between closed and open states. For transmitter-gated channels, the change in free energy of the ligand bound to its site on the channel as compared to the ligand in solution leads to channel opening. For mechanically activated channels the energy associated with membrane stretch is thought to be transferred to the channel through the cytoskeleton (Figure 5–12).

The rates at which transitions occur between open and closed states of a channel depend on the signals that gate the channel. For a voltage-gated channel, the rates are steeply dependent on membrane potential. Although these rates can vary from the microsecond to minute time scale, on average they tend to require a few milliseconds. Thus, once a channel opens, it stays open for a few milliseconds before closing, and after it closes it stays closed for a few milliseconds before opening again. This time scale of gating is much slower than the rate of ion permeation through an open channel, which occurs in less than a microsecond. Once a transition between an open and closed state begins, it proceeds virtually instantaneously (in less than 10 μ sec, the present limits of experimental measurements), giving rise to abrupt, all-or-none step-like changes in single-channel current as the channel goes from a fully closed to a fully open state.

Ligand-gated and voltage-gated channels enter refractory states through different processes. Ligand-gated channels can enter the refractory state when they are exposed to a high concentration of the ligand. This process is called *desensitization*. At present, desensitization is not com-

pletely understood. In some channels it appears to be an intrinsic property of the channel, whereas in others it is due to phosphorylation of the channel molecule by a protein kinase. Many, but not all, voltage-gated channels can enter a refractory state following activation. This process is termed *inactivation*. Inactivation of voltage-gated Na^+ and K^+ channels is thought to be due to a conformational change in the channels, controlled by a subunit or region of the channel separate from that which controls activation. For example, intracellular application of certain proteolytic enzymes can eliminate the ability of voltage-gated Na^+ channels to inactivate without affecting the ability of the channel to be activated. In contrast, inactivation of certain voltage-gated Ca^{2+} channels is thought to be a consequence of Ca^{2+} influx. In this case, an increase in internal Ca^{2+} concentration inactivates the Ca^{2+} channel either directly, by binding to an allosteric control site on the inside of the channel, or indirectly, by activating an intracellular enzyme that inactivates the channel by protein dephosphorylation (Figure 5–13).

Exogenous factors, such as drugs and toxins, can modulate the allosteric control sites of an ion channel. Most of these agents bias the channel toward the closed state (Figure 5–14). Some compounds act as competitive inhibitors, binding to the same site at which the normal gating ligand binds. This binding may be either of low energy and reversible—as in the blockade of the nicotinic acetylcholine (ACh) receptor-channel by the poison curare—or of high energy and not reversible—as in the blockade of the ACh receptor-channel by the snake venom poison α -bunga-

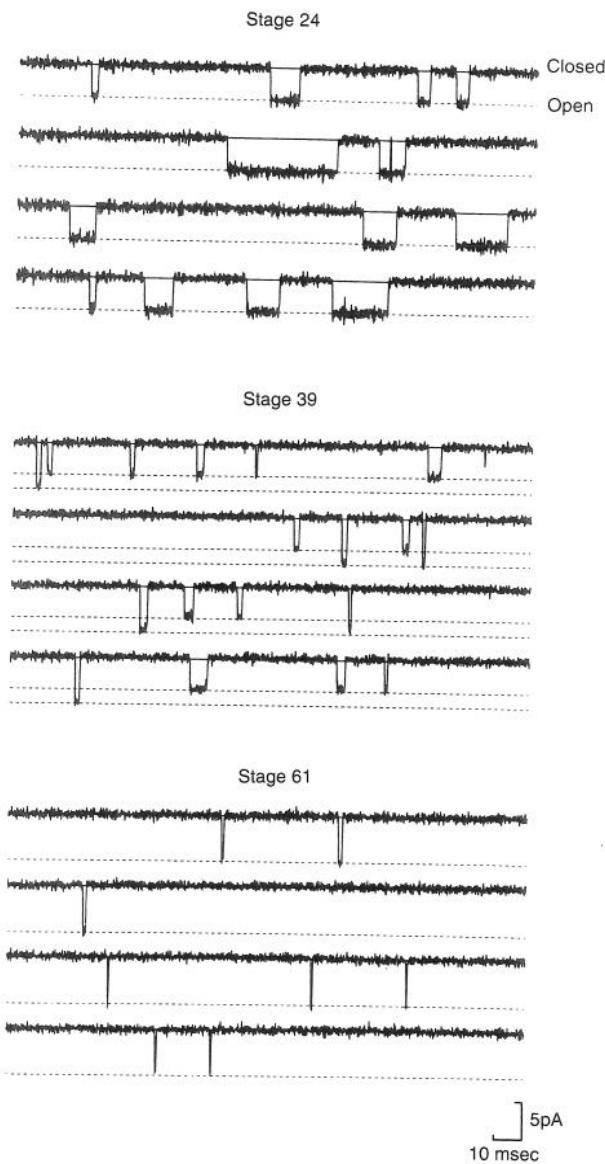


FIGURE 5–15

The functional properties of ion channels can change over the course of development. These examples of conductance in individual ACh-activated channels were recorded from *Xenopus* myotomal muscle at early, intermediate, and late stages of development. In immature muscle the single channels have a small conductance and a relatively long open time. In mature muscle the channel conductance is larger and the average open time is smaller. At intermediate stages of development the population of channels is mixed, exhibiting both types of gating behavior and both classes of conductance. (From Owens and Kullberg, 1989.)

rotoxin. Other exogenous substances act in a noncompetitive or allosteric manner and affect the normal gating mechanism only indirectly. This type of inhibition can work not only on gating transitions normally controlled by ligand binding, but also on those controlled by voltage and by stretch. A few exogenous allosteric modulators bias the channel to the open state.

Variants of Each Type of Ion Channel Are Found in Different Tissues

More than a dozen basic channel types are known to exist in neurons, and each type includes several closely related isoforms that differ in their rate of opening or closing and sensitivity to different regulators of gating. This variability is generated either by differential expression of two or more homologous genes, or by alternative splicing of the mRNA from the same gene. As with isozymes of a particular enzyme, variants of a channel type are expressed at different developmental stages, in different cell types and even in different regions within a cell (Figure 5–15). These subtle variations in structure and function of an ion channel type are presumed to adapt the channel to its specific function. The rich variety of cell-specific subtypes of ion channels may make it possible to develop drugs that can activate or block channels in selected regions of the nervous system. Such drugs can, in principle, be selected to have maximum therapeutic effectiveness with a minimum of side effects.

Genes That Encode Ion Channels Can Be Grouped into Families

Three gene families encode most of the ion channels that have been described to date. The members of a given gene family show substantial amino acid sequence homology with one another. Each family is thought to have evolved from a common ancestral gene by gene duplication and divergence. The genes that encode voltage-gated ion channels, selective for either Ca^{2+} , Na^+ , or K^+ , belong to one of these families. Similarly, transmitter-gated ion channels that are sensitive to either ACh, γ -aminobutyric acid (GABA), or glycine belong to another. Like the family of voltage-gated ion channels, the members of the transmitter-gated ion channel family can differ from each other in ion selectivity. The genes coding for the different gap junction channels, specialized channels that bridge the cytoplasm of two cells, form the third class of channel gene families (see Chapter 9). Because the genes for only a few ion channels have been sequenced, it remains to be seen how many additional channel families exist.

An Overall View

Ion channels are an important class of membrane-spanning glycoproteins that exist in all cells and govern the flow of ions across the membrane. In nerve and muscle cells they are important for controlling the rapid changes in membrane potential associated with the action potential and postsynaptic potentials. The Ca^{2+} influx controlled by these channels can alter many metabolic processes within cells, leading to activation of various enzymes and proteins. As described in Chapter 13, Ca^{2+} influx also acts as a trigger for the release of neurotransmitter.

Channels can be distinguished from each other on the basis of their ion selectivity and the factors that control their opening and closing, or gating. Ion selectivity is

achieved through physical-chemical interaction between the ion and various amino acid residues that line the walls of the channel pore. Gating involves a conformational change of the channel in response to various external stimuli, including voltage, ligands, and stretch or pressure.

Two methodological advances in the past several years have greatly increased our understanding of channel function. First, the patch-clamp technique has made it possible to measure directly the activity of single ion channel molecules by recording the unit current flow through single open channels. Second, gene cloning and sequencing have determined the primary amino acid sequences of many ion channels. From these results, many of the channels described so far can be grouped into two gene families: the voltage-gated channels (including channels selective for Na^+ , K^+ , and Ca^{2+}) and the transmitter-gated channels.

The activity of channels can be modified by cellular metabolic reactions, including protein phosphorylation, by various ions that act as blockers, and by toxins, poisons, and drugs. Channels are also important targets in various diseases. Certain autoimmune neurological disorders, such as myasthenia gravis and the Lambert-Eaton syndrome (which we will discuss in Chapter 16), are thought to result from the actions of specific antibodies interfering with channel function. Cystic fibrosis involves a genetic defect in a certain type of chloride channel. With our increasing understanding of channel structure and function it seems likely that other diseases of channel function will soon be identified. Through a detailed knowledge of the genetic basis of channel structure and function, it may one day be possible to devise new pharmacological therapies for certain neurologic and psychiatric disorders.

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