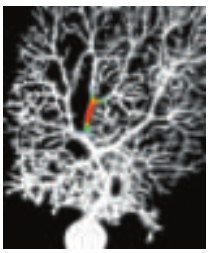
Chapter 4



Channels and Transporters

Overview

The generation of electrical signals in neurons requires that plasma membranes establish concentration gradients for specific ions and that these membranes undergo rapid and selective changes in the membrane permeability to these ions. The membrane proteins that create and maintain ion gradients are called active transporters, whereas other proteins called ion channels give rise to selective ion permeability changes. As their name implies, ion channels are transmembrane proteins that contain a specialized structure, called a pore, that permits particular ions to cross the neuronal membrane. Some of these channels also contain other structures that are able to sense the electrical potential across the membrane. Such voltage-gated channels open or close in response to the magnitude of the membrane potential, allowing the membrane permeability to be regulated by changes in this potential. Other types of ion channels are gated by extracellular chemical signals such as neurotransmitters, and some by intracellular signals such as second messengers. Still others respond to mechanical stimuli, temperature changes, or a combination of such effects. Many types of ion channels have now been characterized at both the gene and protein level, resulting in the identification of a large number of ion channel subtypes that are expressed differentially in neuronal and non-neuronal cells. The specific expression pattern of ion channels in each cell type can generate a wide spectrum of electrical characteristics. In contrast to ion channels, active transporters are membrane proteins that produce and maintain ion concentration gradients. The most important of these is the Na^+ pump, which hydrolyzes ATP to regulate the intracellular concentrations of both Na^+ and K^+. Other active transporters produce concentration gradients for the full range of physiologically important ions, including Cl^-, Ca^{2+}, and H^+. From the perspective of electrical signaling, active transporters and ion channels are complementary: Transporters create the concentration gradients that help drive ion fluxes through open ion channels, thus generating electrical signals.

Ion Channels Underlying Action Potentials

Although Hodgkin and Huxley had no knowledge of the physical nature of the conductance mechanisms underlying action potentials, they nonetheless proposed that nerve cell membranes have channels that allow ions to pass selectively from one side of the membrane to the other (see Chapter 3). Based on the ionic conductances and currents measured in voltage clamp experiments, the postulated channels had to have several properties. First, because the ionic currents are quite large, the channels had to be capable of allowing ions to move across the membrane at high rates. Second, because

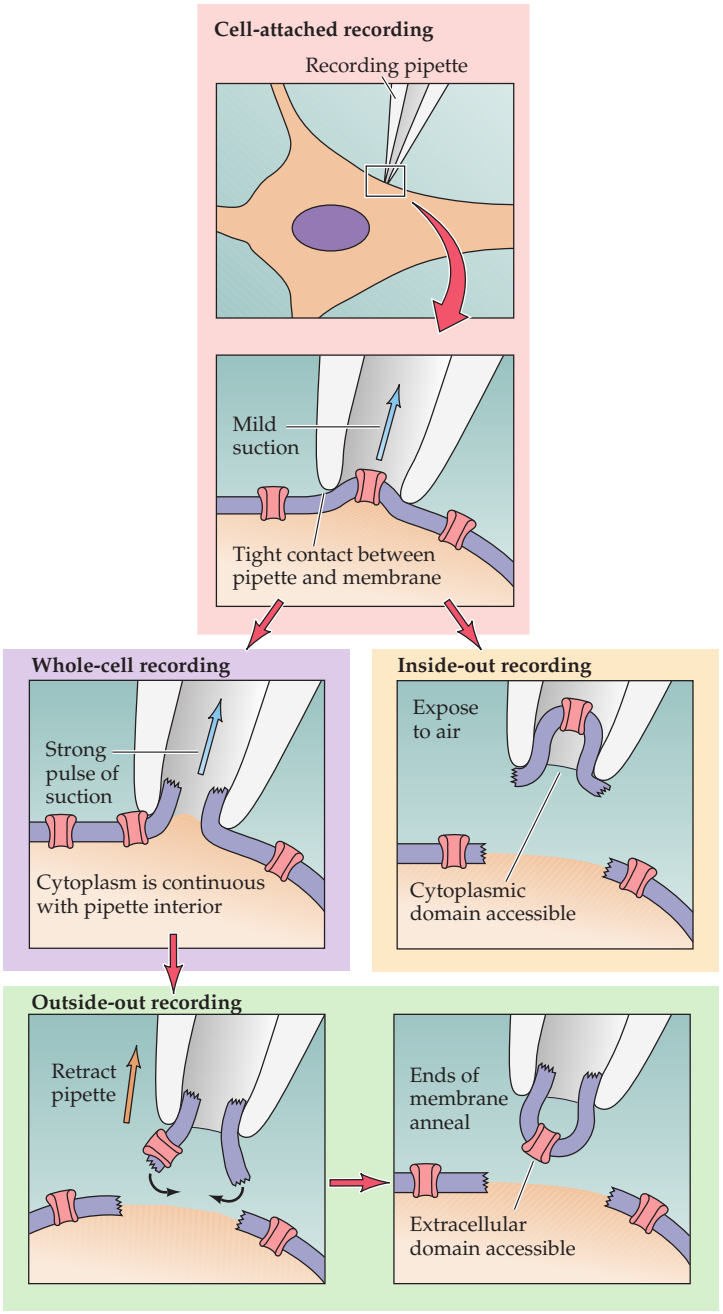
Box A

The Patch Clamp Method

A wealth of new information about ion channels resulted from the invention of the patch clamp method in the 1970s. This technique is based on a very simple idea. A glass pipette with a very small opening is used to make tight contact with a tiny area, or patch, of neuronal membrane. After the application of a small amount of suction to the back of the pipette, the seal between pipette and membrane becomes so tight that no ions can flow between the pipette and the membrane. Thus, all the ions that flow when a single ion channel opens must flow into the pipette. The resulting electrical current, though small, can be measured with an ultrasensitive electronic amplifier connected to the pipette. Based on the geometry involved, this arrangement usually is called the *cell-attached patch clamp recording method*. As with the conventional voltage clamp method, the patch clamp method allows experimental control of the membrane potential to characterize the voltage dependence of membrane currents.

Although the ability to record currents flowing through single ion channels is an important advantage of the cell-attached patch clamp method, minor technical modifications yield still other advantages. For example, if the membrane patch within the pipette is disrupted by briefly applying strong suction, the interior of the pipette becomes continuous with the cytoplasm of the cell. This arrangement allows measurements of electrical potentials and currents from the entire cell and is therefore called the *whole-cell recording method*. The whole-cell configuration also allows diffusional exchange between the pipette and the cytoplasm, producing a convenient way to inject substances into the interior of a "patched" cell.

Two other variants of the patch clamp method originate from the finding that once a tight seal has formed between the membrane and the glass pipette, small pieces of membrane can be pulled away from the cell without disrupting the seal; this yields a preparation that is free of the complications imposed by the rest of the cell. Simply retracting a pipette that is in the cell-attached configuration causes a small vesicle of membrane to remain attached to the pipette. By exposing the tip of the pipette to air, the vesicle opens to yield a small patch of membrane with its (former) intracellular sur-



Four configurations in patch clamp measurements of ionic currents.

face exposed. This arrangement, called the inside-out patch recording configuration, allows the measurement of single-channel currents with the added benefit of making it possible to change the medium to which the intracellular surface of the membrane is exposed. Thus, the inside-out configuration is particularly valuable when studying the influence of intracellular molecules on ion channel function. Alternatively, if the pipette is retracted while it is in the whole-cell configuration, a membrane patch is produced that has its extracellular surface exposed. This arrangement, called the outside-out recording configuration, is optimal for studying how channel activity is influenced by extracellular chemical signals, such as neurotransmitters (see Chapter 5). This range of possible configurations makes the patch clamp method an unusually versatile technique for studies of ion channel function.

References

HAMILL, O. P., A. MARTY, E. NEHER, B. SAKMANN AND F. J. SIGWORTH (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch. 391: 85–100.

LEOIS, R. A. AND J. L. RAE (1998) Low-noise patch-clamp techniques. Meth. Enzym. 293: 218–266.

SIGWORTH, F. J. (1986) The patch clamp is more useful than anyone had expected. Fed. Proc. 45: 2673–2677.

the ionic currents depend on the electrochemical gradient across the membrane, the channels had to make use of these gradients. Third, because Na^+ and K^+ flow across the membrane independently of each other, different channel types had to be capable of discriminating between Na^+ and K^+, allowing only one of these ions to flow across the membrane under the relevant conditions. Finally, given that the conductances are voltage-dependent, the channels had to be able to sense the voltage drop across the membrane, opening only when the voltage reached appropriate levels. While this concept of channels was highly speculative in the 1950s, later experimental work established beyond any doubt that transmembrane proteins called voltage-sensitive ion channels indeed exist and are responsible for all of the ionic conductance phenomena described in Chapter 3.

The first direct evidence for the presence of voltage-sensitive, ion-selective channels in nerve cell membranes came from measurements of the ionic currents flowing through individual ion channels. The voltage-clamp apparatus used by Hodgkin and Huxley could only resolve the *aggregate* current resulting from the flow of ions through many thousands of channels. A technique capable of measuring the currents flowing through single channels was devised in 1976 by Erwin Neher and Bert Sakmann at the Max Planck Institute in Goettingen. This remarkable approach, called patch clamping (Box A), revolutionized the study of membrane currents. In particular, the patch clamp method provided the means to test directly Hodgkin and Huxley's proposals about the characteristics of ion channels.

Currents flowing through Na^+ channels are best examined in experimental circumstances that prevent the flow of current through other types of channels that are present in the membrane (e.g., K^+ channels). Under such conditions, depolarizing a patch of membrane from a squid giant axon causes tiny inward currents to flow, but only occasionally (Figure 4.1). The size of these currents is minuscule—approximately l–2 pA (i.e., 10^{-12} ampere), which is orders of magnitude smaller than the Na^+ currents measured by voltage clamping the entire axon. The currents flowing through single channels are called **microscopic currents** to distinguish them from the **macroscopic currents** flowing through a large number of channels distributed over a much more extensive region of surface membrane. Although microscopic currents are certainly small, a current of 1 pA nonetheless reflects the flow of thousands of ions per millisecond. Thus, as predicted, a single channel can let many ions pass through the membrane in a very short time.

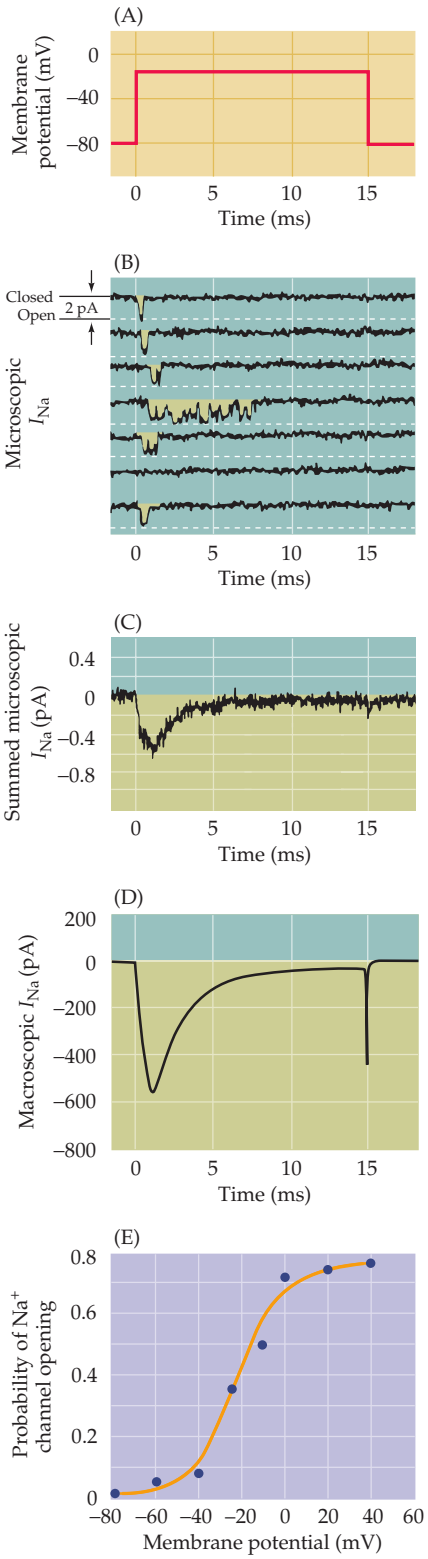


Figure 4.1 Patch clamp measurements of ionic currents flowing through single Na^+ channels in a squid giant axon. In these experiments, Cs^+ was applied to the axon to block voltage-gated K^+ channels. Depolarizing voltage pulses (A) applied to a patch of membrane containing a single Na^+ channel result in brief currents (B, downward deflections) in the seven successive recordings of membrane current (I_{Na}). (C) The sum of many such current records shows that most channels open in the initial 1–2 ms following depolarization of the membrane, after which the probability of channel openings diminishes because of channel inactivation. (D) A macroscopic current measured from another axon shows the close correlation between the time courses of microscopic and macroscopic Na^+ currents. (E) The probability of an Na^+ channel opening depends on the membrane potential, increasing as the membrane is depolarized. (B,C after Bezanilla and Correa, 1995; D after Vandenburg and Bezanilla, 1991; E after Correa and Bezanilla, 1994.)

Several observations further proved that the microscopic currents in Figure 4.1B are due to the opening of single, voltage-activated Na^+ channels. First, the currents are carried by Na^+; thus, they are directed inward when the membrane potential is more negative than E_{Na}, reverse their polarity at E_{Na}, are outward at more positive potentials, and are reduced in size when the Na^+ concentration of the external medium is decreased. This behavior exactly parallels that of the macroscopic Na^+ currents described in Chapter 3. Second, the channels have a time course of opening, closing, and inactivating that matches the kinetics of macroscopic Na^+ currents. This correspondence is difficult to appreciate in the measurement of microscopic currents flowing through a single open channel, because individual channels open and close in a stochastic (random) manner, as can be seen by examining the individual traces in Figure 4.1B. However, repeated depolarization of the membrane potential causes each Na^+ channel to open and close many times. When the current responses to a large number of such stimuli are averaged together, the collective response has a time course that looks much like the macroscopic Na^+ current (Figure 4.1C). In particular, the channels open mostly at the beginning of a prolonged depolarization, showing that they subsequently inactivate, as predicted from the macroscopic Na^+ current (compare Figures 4.1C and 4.1D). Third, both the opening and closing of the channels are voltage-dependent; thus, the channels are closed at –80 mV but open when the membrane potential is depolarized. In fact, the probability that any given channel will be open varies with membrane potential (Figure 4.1E), again as predicted from the macroscopic Na^+ conductance (see Figure 3.7). Finally, tetrodotoxin, which blocks the macroscopic Na^+ current (see Box C), also blocks microscopic Na^+ currents. Taken together, these results show that the macroscopic Na^+ current measured by Hodgkin and Huxley does indeed arise from the aggregate effect of many thousands of microscopic Na^+ currents, each representing the opening of a single voltage-sensitive Na^+ channel.

Patch clamp experiments have also revealed the properties of the channels responsible for the macroscopic K^+ currents associated with action potentials. When the membrane potential is depolarized (Figure 4.2A), microscopic outward currents (Figure 4.2B) can be observed under conditions that block Na^+ channels. The microscopic outward currents exhibit all the features expected for currents flowing through action-potential-related K^+ channels. Thus, the microscopic currents (Figure 4.2C), like their macroscopic counterparts (Figure 4.2D), fail to inactivate during brief depolarizations. Moreover, these single-channel currents are sensitive to ionic manipu-

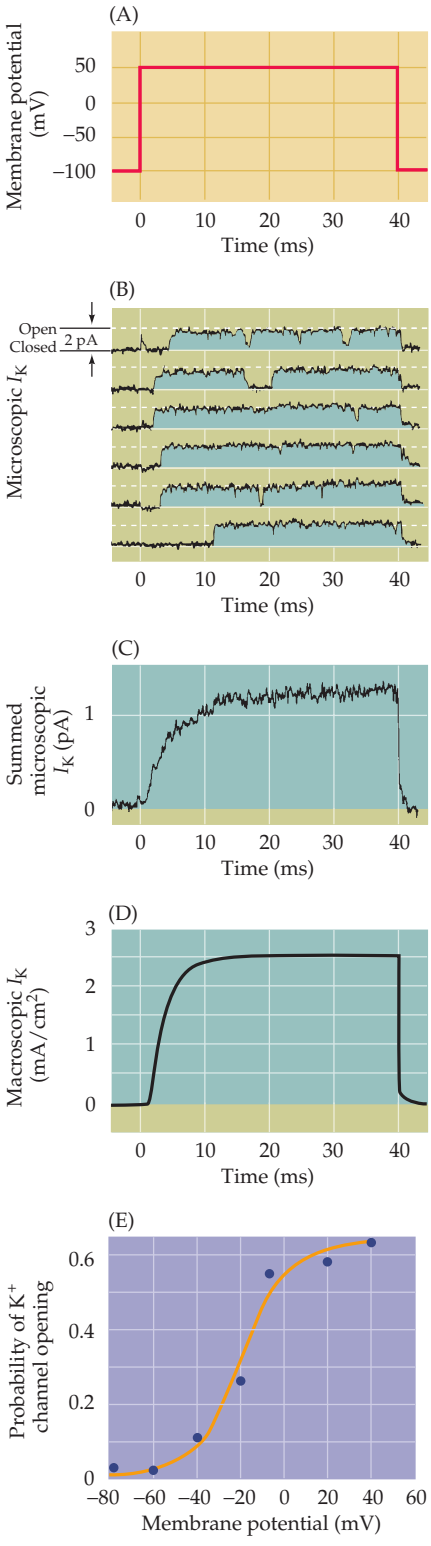


Figure 4.2 Patch clamp measurements of ionic currents flowing through single K^+ channels in a squid giant axon. In these experiments, tetrodotoxin was applied to the axon to block voltage-gated Na^+ channels. Depolarizing voltage pulses (A) applied to a patch of membrane containing a single K^+ channel results in brief currents (B, upward deflections) whenever the channel opens. (C) The sum of such current records shows that most channels open with a delay, but remain open for the duration of the depolarization. (D) A macroscopic current measured from another axon shows the correlation between the time courses of microscopic and macroscopic K^+ currents. (E) The probability of a K^+ channel opening depends on the membrane potential, increasing as the membrane is depolarized. (B and C after Augustine and Bezanilla, in Hille 1992; D after Augustine and Bezanilla, 1990; E after Perozo et al., 1991.)

lations and drugs that affect the macroscopic K^+ currents and, like the macroscopic K^+ currents, are voltage-dependent (Figure 4.2E). This and other evidence shows that macroscopic K^+ currents associated with action potentials arise from the opening of many voltage-sensitive K^+ channels.

In summary, patch clamping has allowed direct observation of microscopic ionic currents flowing through single ion channels, confirming that voltage sensitive Na^+ and K^+ channels are responsible for the macroscopic conductances and currents that underlie the action potential. Measurements of the behavior of single ion channels has also provided some insight into the molecular attributes of these channels. For example, single channel studies show that the membrane of the squid axon contains at least two types of channels—one selectively permeable to Na^+ and a second selectively permeable to K^+. Both channel types are **voltage-gated**, meaning that their opening is influenced by membrane potential (Figure 4.3). For each channel, depolarization increases the probability of channel opening, whereas hyperpolarization closes them (see Figures 4.1E and 4.2E). Thus, both channel types must have a **voltage sensor** that detects the potential across the membrane (Figure 4.3). However, these channels differ in important respects. In addition to their different ion selectivities, depolarization also inactivates the Na^+ channel but not the K^+ channel, causing Na^+ channels to pass into a nonconducting state. The Na^+ channel must therefore have an additional molecular mechanism responsible for **inactivation**. And, as expected from the macroscopic behavior of the Na^+ and K^+ currents described in Chapter 3, the kinetic properties of the gating of the two channels differs. This information about the physiology of single channels set the stage for subsequent studies of the molecular diversity of ion channels in various cell types, and of their detailed functional characteristics.

The Diversity of Ion Channels

Molecular genetic studies, in conjunction with the patch clamp method and other techniques, have led to many additional advances in understanding ion channels. Genes encoding Na^+ and K^+ channels, as well as many other channel types, have now been identified and cloned. A surprising fact that has emerged from these molecular studies is the diversity of genes that code for ion channels. Well over 100 ion channel genes have now been discovered, a number that could not have been anticipated from early studies of ion channel function. To understand the functional significance of this multitude of ion channel genes, the channels can be selectively expressed in well-

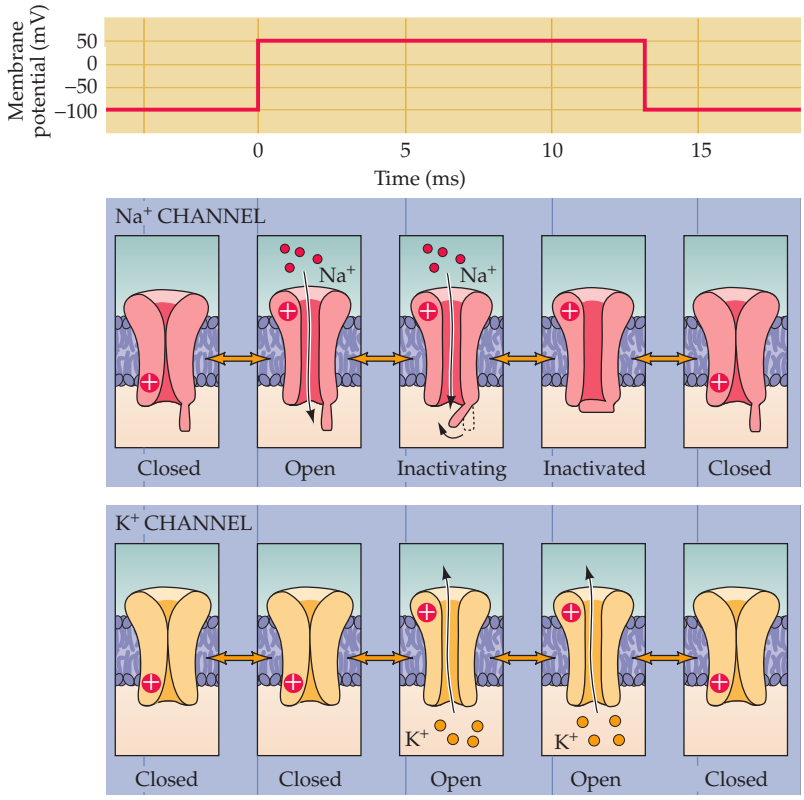


Figure 4.3 Functional states of voltage-gated Na^+ and K^+ channels. The gates of both channels are closed when the membrane potential is hyperpolarized. When the potential is depolarized, voltage sensors (indicated by +) allow the channel gates to open—first the Na^+ channels and then the K^+ channels. Na^+ channels also inactivate during prolonged depolarization, whereas many types of K^+ channels do not.

defined experimental systems, such as in cultured cells or frog oocytes (Box B), and then studied with patch clamping and other physiological techniques. Such studies have found many voltage-gated channels that respond to membrane potential in much the same way as the Na^+ and K^+ channels that underlie the action potential. Other channels, however, are gated by chemical signals that bind to extracellular or intracellular domains on these proteins and are insensitive to membrane voltage. Still others are sensitive to mechanical displacement, or to changes in temperature.

Further magnifying this diversity of ion channels are a number of mechanisms that can produce functionally different types of ion channels from a single gene. Ion channel genes contain a large number of coding regions that can be spliced together in different ways, giving rise to channel proteins that can have dramatically different functional properties. RNAs encoding ion channels also can be edited, modifying their base composition after transcription from the gene. For example, editing the RNA encoding of some receptors for the neurotransmitter glutamate (Chapter 6) changes a single amino acid within the receptor, which in turn gives rise to channels that differ in their selectivity for cations and in their conductance. Channel proteins can also undergo posttranslational modifications, such as phosphorylation by protein kinases (see Chapter 7), which can further change their functional characteristics. Thus, although the basic electrical signals of the nervous system are relatively stereotyped, the proteins responsible for generating these signals are remarkably diverse, conferring specialized signaling properties to many of the neuronal cell types that populate the nervous system. These channels also are involved in a broad range of neurological diseases.

Box B

Expression of Ion Channels in *Xenopus* Oocytes

Bridging the gap between the sequence of an ion channel gene and understanding channel function is a challenge. To meet this challenge, it is essential to have an experimental system in which the gene product can be expressed efficiently, and in which the function of the resulting channel can be studied with methods such as the patch clamp technique. Ideally, the vehicle for expression should be readily available, have few endogenous channels, and be large enough to permit mRNA and DNA to be microinjected with ease. Oocytes (immature eggs) from the clawed African frog, *Xenopus laevis* (Figure A), fulfill all these demands. These huge cells (approximately 1 mm in diameter; Figure B) are easily harvested from the female *Xenopus*. Work performed in the 1970s by John Gurdon, a developmental biologist, showed that injection of exogenous mRNA into frog oocytes causes them to synthesize foreign protein in prodigious quantities. In the early 1980s, Ricardo Miledi, Eric Barnard, and other neurobiologists demonstrated that *Xenopus* oocytes could express exogenous ion channels, and that physiological methods could be used to study the ionic currents generated by the newly-synthesized channels (Figure C).

As a result of these pioneering studies, heterologous expression experiments have now become a standard way of studying ion channels. The approach has been especially valuable in deciphering the relationship between channel structure and function. In such experiments, defined mutations (often affecting a single nucleotide) are made in the part of the channel gene that encodes a structure of interest; the resulting channel proteins are then expressed in oocytes to assess the functional consequences of the mutation.

The ability to combine molecular and physiological methods in a single cell system has made *Xenopus* oocytes a powerful experimental tool. Indeed, this system has been as valuable to contemporary studies of voltage-gated ion channels as the squid axon was to such studies in the 1950s and 1960s.

References

Gundersen, C. B., R. Miledi and I. Parker (1984) Slowly inactivating potassium channels induced in *Xenopus* oocytes by messenger ribonucleic acid from *Torpedo* brain. J. Physiol. (Lond.) 353: 231–248.

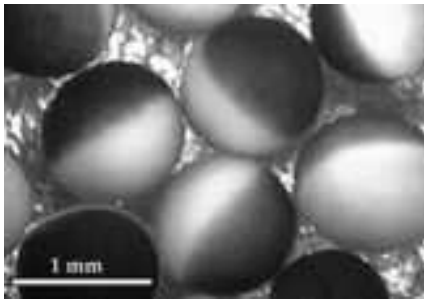
Gurdon, J. B., C. D. Lane, H. R. Woodland and G. Marbaix (1971) Use of frog eggs and oocytes for the study of messenger RNA and its translation in living cells. Nature 233: 177–182.

Stühmer, W. (1998) Electrophysiological recordings from *Xenopus* oocytes. Meth. Enzym. 293: 280–300.

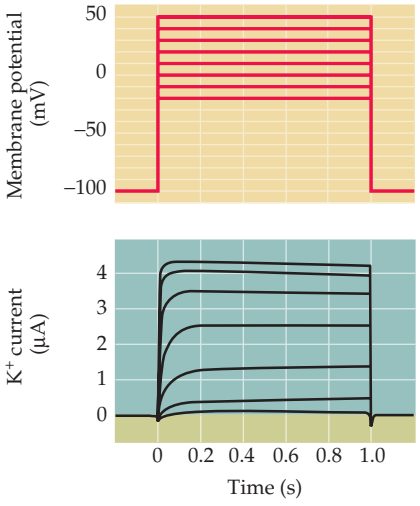
Sumikawa, K., M. Houghton, J. S. Emtage, B. M. Richards and E. A. Barnard (1981) Active multi-subunit ACh receptor assembled by translation of heterologous mRNA in *Xenopus* oocytes. Nature 292: 862– 864.

(A)

(B)



(C)



(A) The clawed African frog, *Xenopus laevis*. (B) Several oocytes from *Xenopus* highlighting the dark coloration of the original pole and the lighter coloration of the vegetal pole. (Courtesy of P. Reinhart.) (C) Results of a voltage clamp experiment showing K^+ currents produced following injection of K^+ channel mRNA into an oocyte. (After Gundersen et al., 1984.)

Voltage-Gated Ion Channels

Voltage-gated ion channels that are selectively permeable to each of the major physiological ions—Na^+, K^+, Ca^{2+}, and Cl^-—have now been discovered (Figure 4.4 A–D). Indeed, many different genes have been discovered for each type of voltage-gated ion channel. An example is the identification of 10 human Na^+ channel genes. This finding was unexpected because Na^+ channels from many different cell types have similar functional properties, consistent with their origin from a single gene. It is now clear, however, that all of these Na^+ channel genes (called SCN genes) produce proteins that differ in their structure, function, and distribution in specific tissues. For instance, in addition to the rapidly inactivating Na^+ channels discovered by Hodgkin and Huxley in squid axon, a voltage-sensitive Na^+ channel that does *not* inactivate has been identified in mammalian axons. As might be expected, this channel gives rise to action potentials of long duration and is a target of local anesthetics such as benzocaine and lidocaine.

Other electrical responses in neurons entail the activation of voltage-gated Ca^{2+} channels (Figure 4.4B). In some neurons, voltage-gated Ca^{2+} channels give rise to action potentials in much the same way as voltage-sensitive Na^+ channels. In other neurons, Ca^{2+} channels control the shape of action potentials generated primarily by Na^+ conductance changes. More generally, by affecting intracellular Ca^{2+} concentrations, the activity of Ca^{2+} channels regulates an enormous range of biochemical processes within cells (see Chapter 7). Perhaps the most important of the processes regulated by voltage-sensitive Ca^{2+} channels is the release of neurotransmitters at synapses (see Chapter 5). Given these crucial functions, it is perhaps not surprising that 16 different Ca^{2+} channel genes (called CACNA genes) have been identified. Like Na^+ channels, Ca^{2+} channels differ in their activation and inactivation properties, allowing subtle variations in both electrical and chemical signaling processes mediated by Ca^{2+}. As a result, drugs that block voltage-gated Ca^{2+} channels are especially valuable in treating a variety of conditions ranging from heart disease to anxiety disorders.

By far the largest and most diverse class of voltage-gated ion channels are the K^+ channels (Figure 4.4C). Nearly 100 K^+ channel genes are now known, and these fall into several distinct groups that differ substantially in their activation, gating, and inactivation properties. Some take minutes to inactivate, as in the case of squid axon K^+ channels studied by Hodgkin and Huxley (Figure 4.5A). Others inactivate within milliseconds, as is typical of most voltage-gated Na^+ channels (Figure 4.5B). These properties influence the

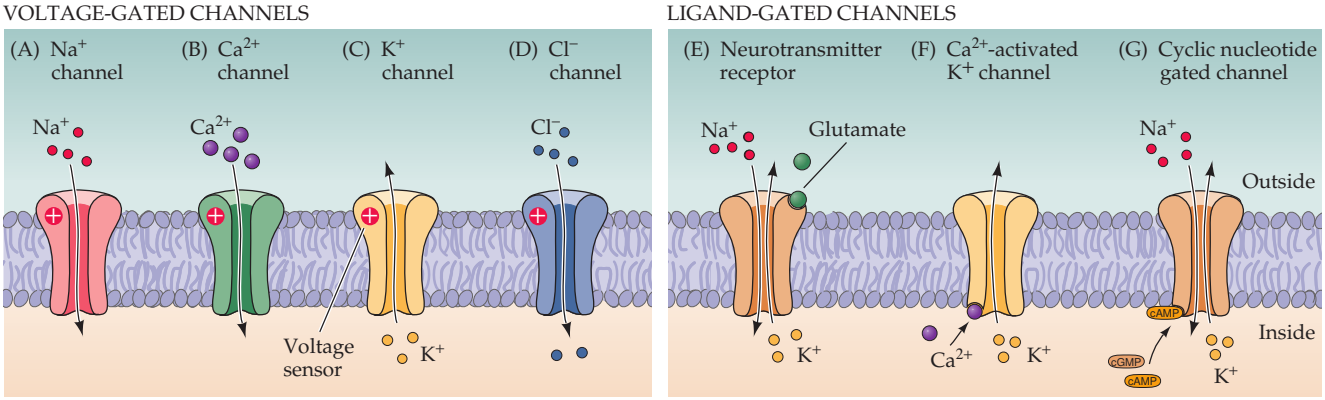


Figure 4.4 Types of voltage-gated ion channels. Examples of voltage-gated channels include those selectively permeable to Na^+ (A), Ca^{2+} (B), K^+ (C), and Cl^- (D). Ligand-gated ion channels include those activated by the extracellular presence of neurotransmitters, such as glutamate (E). Other ligand-gated channels are activated by intracellular second messengers, such as Ca^{2+} (F) or the cyclic nucleotides, cAMP and cGMP (G).

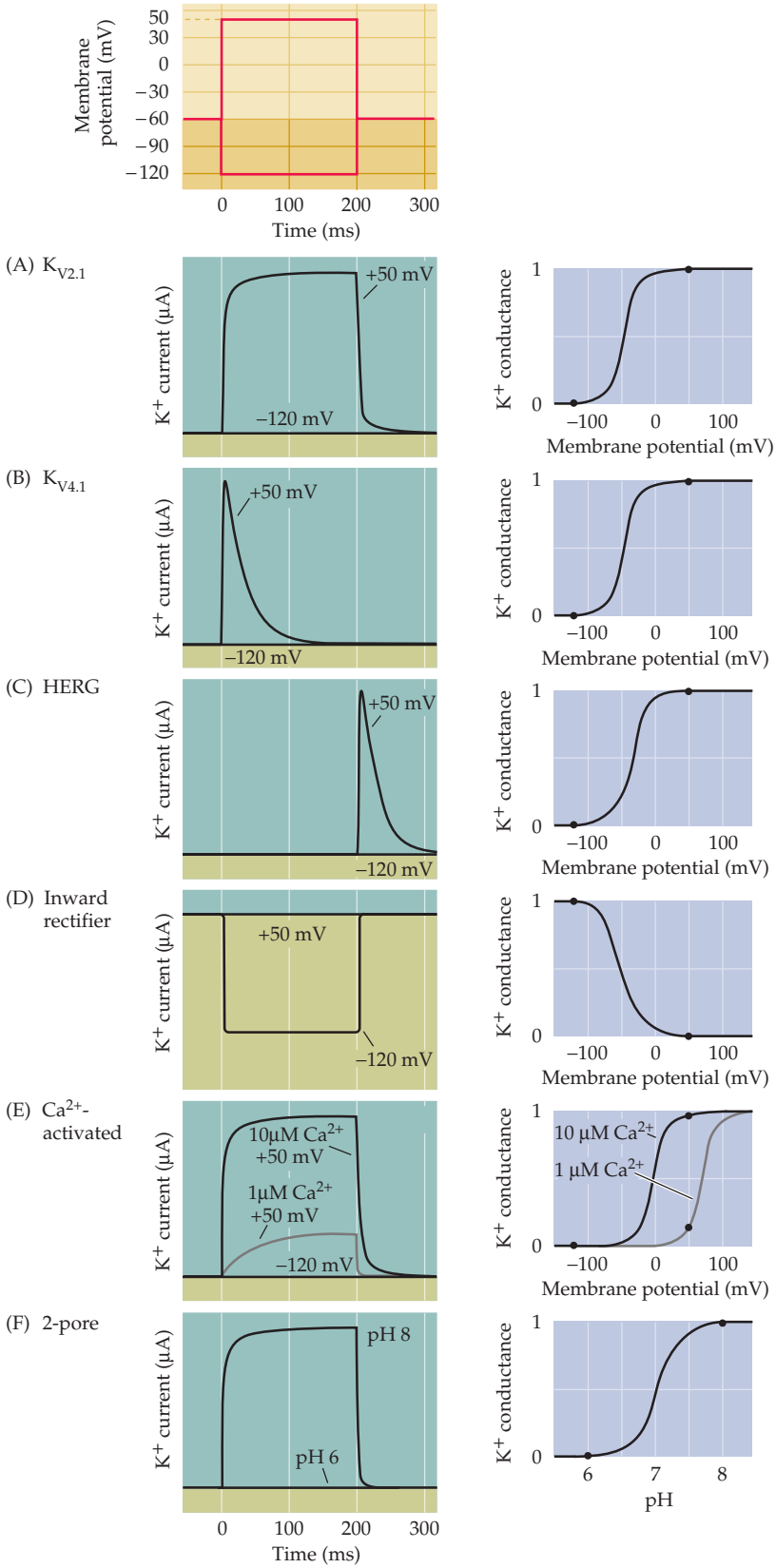


Figure 4.5 Diverse properties of K^+ channels. Different types of K^+ channels were expressed in *Xenopus* oocytes (see Box B), and the voltage clamp method was used to change the membrane potential (top) and measure the resulting currents flowing through each type of channel. These K^+ channels vary markedly in their gating properties, as evident in their currents (left) and conductances (right). (A) $K_{V2.1}$ channels show little inactivation and are closely related to the delayed rectifier K^+ channels involved in action potential repolarization. (B) $K_{V4.1}$ channels inactivate during a depolarization. (C) HERG channels inactivate so rapidly that current flows only when inactivation is rapidly removed at the end of a depolarization. (D) Inward rectifying K^+ channels allow more K^+ current to flow at hyperpolarized potentials than at depolarized potentials. (E) Ca^{2+}-activated K^+ channels open in response to intracellular Ca^{2+} ions and, in some cases, membrane depolarization. (F) K^+ channels with two pores usually respond to chemical signals, such as pH, rather than changes in membrane potential.

duration and rate of action potential firing, with important consequences for axonal conduction and synaptic transmission. Perhaps the most important function of K^+ channels is the role they play in generating the resting membrane potential (see Chapter 2). At least two families of K^+ channels that are open at substantially negative membrane voltage levels contribute to setting the resting membrane potential (Figure 4.5D).

Finally, several types of voltage-gated Cl^- channel have been identified (see Figure 4.4D). These channels are present in every type of neuron, where they control excitability, contribute to the resting membrane potential, and help regulate cell volume.

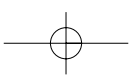
Ligand-Gated Ion Channels

Many types of ion channels respond to chemical signals (ligands) rather than to changes in the membrane potential (Figure 4.4E–G). The most important of these **ligand-gated ion channels** in the nervous system is the class activated by binding neurotransmitters (Figure 4.4E). These channels are essential for synaptic transmission and other forms of cell-cell signaling phenomena discussed in Chapters 5–7. Whereas the voltage-gated ion channels underlying the action potential typically allow only one type of ion to permeate, channels activated by extracellular ligands are usually less selective, allowing two or more types of ions to pass through the channel pore.

Other ligand-gated channels are sensitive to chemical signals arising within the cytoplasm of neurons (see Chapter 7), and can be selective for specific ions such as K^+ or Cl^-, or permeable to all physiological cations. Such channels are distinguised by ligand-binding domains on their *intracellular* surfaces that interact with second messengers such as Ca^{2+}, the cyclic nucleotides cAMP and cGMP, or protons. Examples of channels that respond to intracellular cues include Ca^{2+}-activated K^+ channels (Figure 4.4.F), the cyclic nucleotide gated cation channel (Figure 4.4G), or acid-sensing ion channels (ASICs). The main function of these channels is to convert intracellular chemical signals into electrical information. This process is particularly important in sensory transduction, where channels gated by cyclic nucleotides convert odors and light, for example, into electrical signals. Although many of these ligand-gated ion channels are located in the cell surface membrane, others are in membranes of intracellular organelles such as mitochondria or the endoplasmic reticulum . Some of these latter channels are selectively permeable to Ca^{2+} and regulate the release of Ca^{2+} from the lumen of the endoplasmic reticulum into the cytoplasm, where this second messenger can then trigger a spectrum of cellular responses such as described in Chapter 7.

Stretch- and Heat-Activated Channels

Still other ion channels respond to heat or membrane deformation. Heat-activated ion channels, such as some members of the transient receptor potential (TRP) gene family, contribute to the sensations of pain and temperature and help mediate inflammation (see Chapter 9). These channels are often specialized to detect specific temperature ranges, and some are even activated by cold. Other ion channels respond to mechanical distortion of the plasma membrane and are the basis of stretch receptors and neuromuscular stretch reflexes (see Chapters 8, 15 and 16). A specialized form of these channels enables hearing by allowing auditory hair cells to respond to sound waves (see Chapter 12).



In summary, this tremendous variety of ion channels allows neurons to generate electrical signals in response to changes in membrane potential, synaptic input, intracellular second messengers, light, odors, heat, sound, touch, and many other stimuli.

The Molecular Structure of Ion Channels

Understanding the physical structure of ion channels is obviously the key to sorting out how they actually work. Until recently, most information about channel structure was derived indirectly from studies of the amino acid composition and physiological properties of these proteins. For example, a great deal has been learned by exploring the functions of particular amino acids within the proteins using **mutagenesis** and the expression of such channels in *Xenopus* oocytes (see Box B). Such studies have discovered a general transmembrane architecture common to all the major ion channel families. Thus, these molecules are all integral membrane proteins that span the plasma membrane repeatedly. Na^+ (and Ca^{2+}) channel proteins, consist of repeating motifs of 6 membrane-spanning regions that are repeated 4 times, for a total of 24 transmembrane regions (Figure 4.6A,B). Na^+ (or Ca^{2+}) channels can be produced by just one of these proteins, although other accessory proteins, called β subunits, can regulate the function of these channels. K^+ channel proteins typically span the membrane six times (Figure 4.6C),

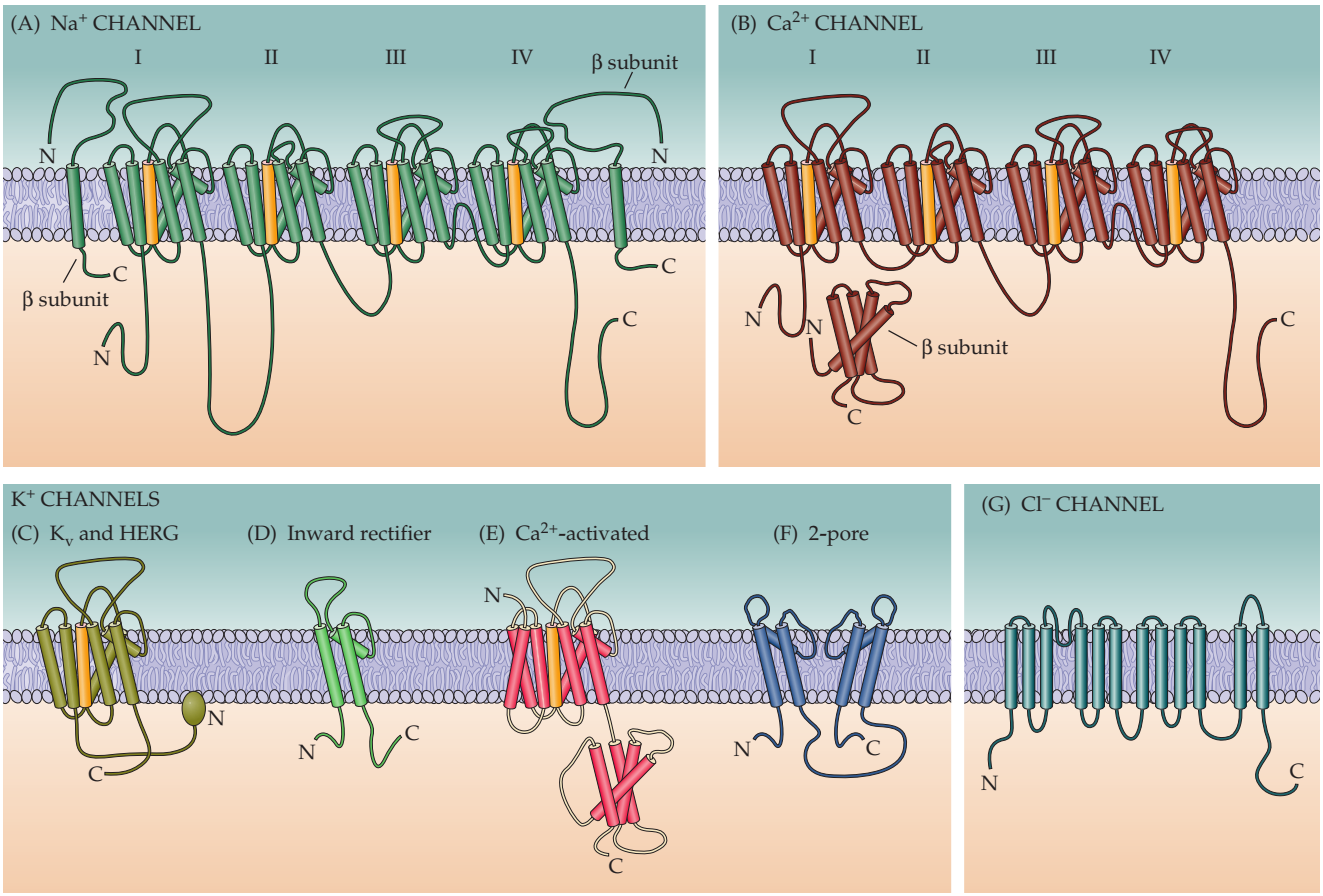


Figure 4.6 Topology of the principal subunits of voltage-gated Na^+, Ca^{2+}, K^+, and Cl^- channels. Repeating motifs of Na^+ (A) and Ca^{2+} (B) channels are labeled I, II, III, and IV; (C–F) K^+ channels are more diverse. In all cases, four subunits combine to form a functional channel. (G) Chloride channels are structurally distinct from all other voltage-gated channels.

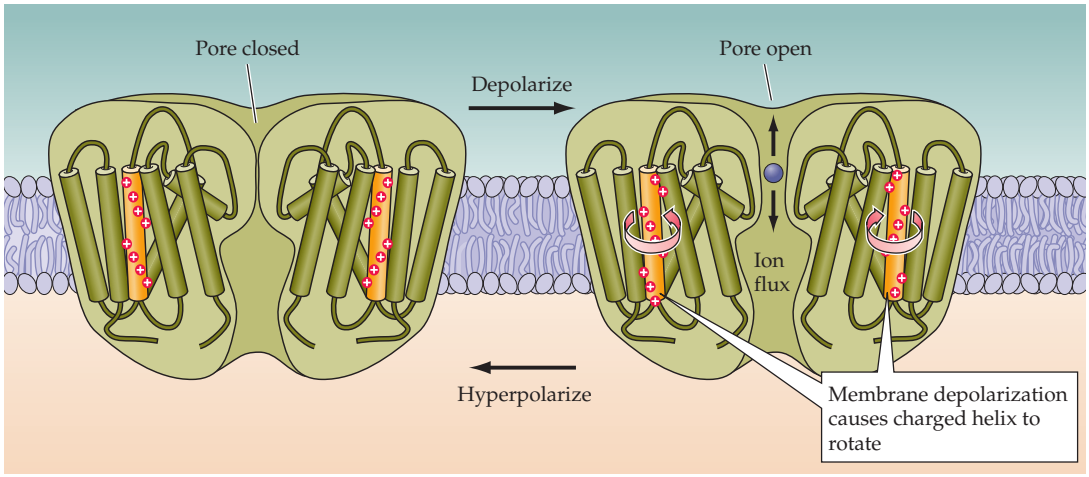


Figure 4.7 A charged voltage sensor permits voltage-dependent gating of ion channels. The process of voltage activation may involve the rotation of a positively charged transmembrane domain. This movement causes a change in the conformation of the pore loop, enabling the channel to conduct specific ions.

though there are some K^+ channels, such as a bacterial channel and some mammalian channels, that span the membrane only twice (Figure 4.6D), and others that span the membrane four times (Figure 4.6F) or seven times (Figure 4.6E). Each of these K^+ channel proteins serves as a channel subunit, with 4 of these subunits typically aggregating to form a single functional ion channel.

Other imaginative mutagenesis experiments have provided information about how these proteins function. Two membrane-spanning domains of all ion channels appear to form a central **pore** through which ions can diffuse, and one of these domains contains a protein loop that confers an ability to selectivity allow certain ions to diffuse through the channel pore (Figure 4.7). As might be expected, the amino acid composition of the pore loop differs among channels that conduct different ions. These distinct structural features of channel proteins also provide unique binding sites for drugs and for various neurotoxins known to block specific subclasses of ion channels (Box C). Furthermore, many voltage gated ion channels contain a distinct type of transmembrane helix containing a number of positively charged amino acids along one face of the helix (Figures 4.6 and 4.7). This structure evidently serves as a sensor that detects changes in the electrical potential across the membrane. Membrane depolarization influences the charged amino acids such that the helix undergoes a conformational change, which in turn allows the channel pore to open. One suggestion is that the helix rotates to cause the pore to open (Figure 4.7). Other types of mutagenesis experiments have demonstrated that one end of certain K^+ channels plays a key role in channel inactivation. This intracellular structure (labeled "N" in Figure 4.6C) can plug the channel pore during prolonged depolarization.

More recently, very direct information about the structural underpinnings of ion channel function has come from **X-ray crystallography** studies of bacterial K^+ channels (Figure 4.8). This molecule was chosen for analysis because the large quantity of channel protein needed for crystallography could be obtained by growing large numbers of bacteria expressing this molecule. The results of such studies showed that the channel is formed by subunits that each cross the plasma membrane twice; between these two membrane-spanning structures is a loop that inserts into the plasma membrane (Figure 4.8A). Four of these subunits are assembled together to form a chan-

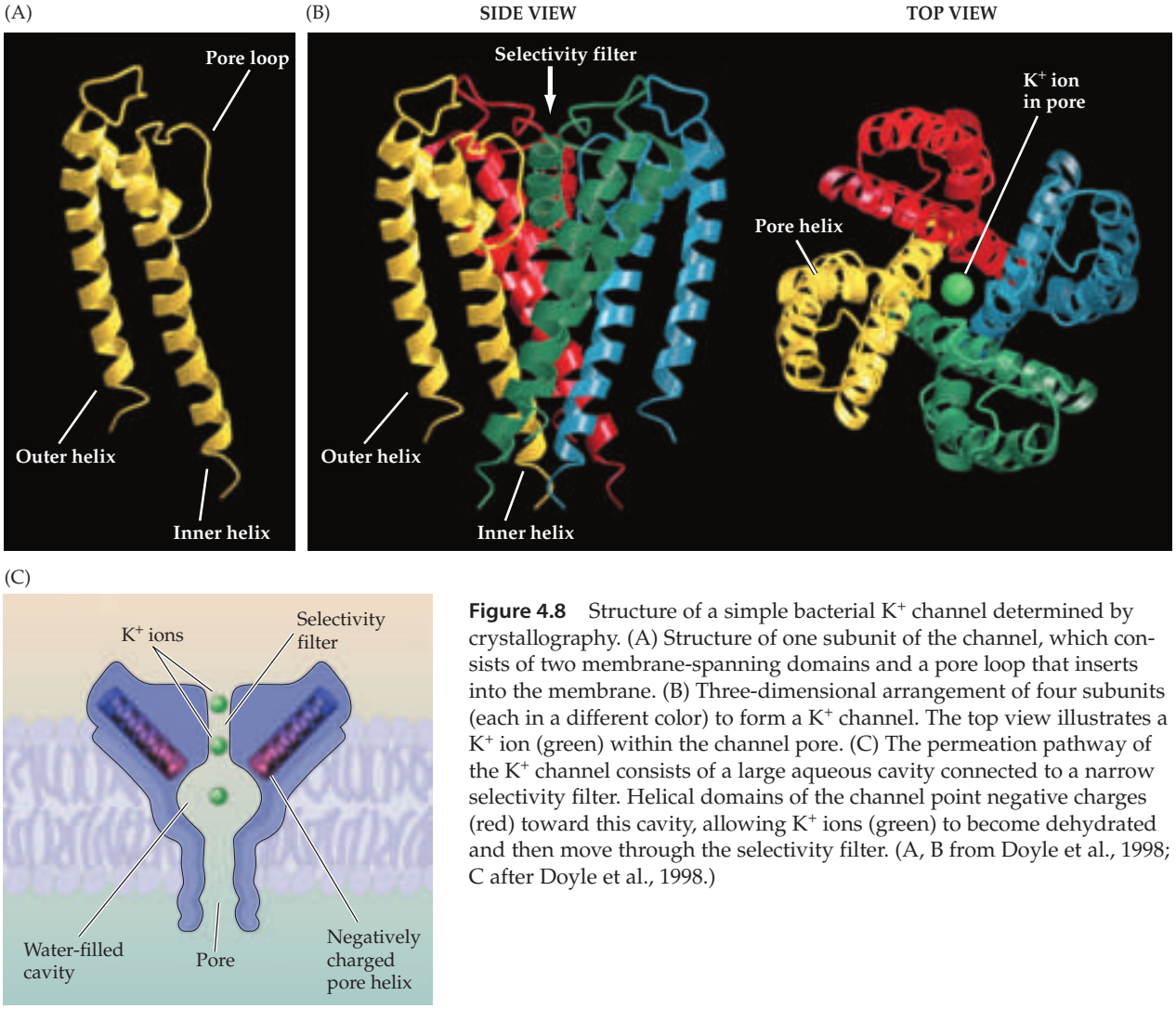


Figure 4.8 Structure of a simple bacterial K^+ channel determined by crystallography. (A) Structure of one subunit of the channel, which consists of two membrane-spanning domains and a pore loop that inserts into the membrane. (B) Three-dimensional arrangement of four subunits (each in a different color) to form a K^+ channel. The top view illustrates a K^+ ion (green) within the channel pore. (C) The permeation pathway of the K^+ channel consists of a large aqueous cavity connected to a narrow selectivity filter. Helical domains of the channel point negative charges (red) toward this cavity, allowing K^+ ions (green) to become dehydrated and then move through the selectivity filter. (A, B from Doyle et al., 1998; C after Doyle et al., 1998.)

nel (Figure 4.8B). In the center of the assembled channel is a narrow opening through the protein that allows K^+ to flow across the membrane. This opening is the channel pore and is formed by the protein loop, as well as by the membrane-spanning domains. The structure of the pore is well suited for conducting K^+ ions (Figure 4.8C). The narrowest part is near the outside mouth of the channel and is so constricted that only a non-hydrated K^+ ion can fit through the bottleneck. Larger cations, such as Cs^+, cannot traverse this region of the pore, and smaller cations such as Na^+ cannot enter the pore because the "walls" of the pore are too far apart to stabilize a dehydrated Na^+ ion. This part of the channel complex is responsible for the selective permeability to K^+ and is therefore called the **selectivity filter**. The sequence of amino acids making up part of this selectivity filter is often referred to as the K^+ channel "signature sequence". Deeper within the channel is a water-filled cavity that connects to the interior of the cell. This cavity evidently collects K^+ from the cytoplasm and, utilizing negative charges from the protein,

Box C

Toxins That Poison Ion Channels

Given the importance of Na^+ and K^+ channels for neuronal excitation, it is not surprising that a number of organisms have evolved channel-specific toxins as mechanisms for self-defense or for capturing prey. A rich collection of natural toxins selectively target the ion channels of neurons and other cells. These toxins are valuable not only for survival, but for studying the function of cellular ion channels. The best-known channel toxin is *tetrodotoxin*, which is produced by certain puffer fish and other animals. Tetrodotoxin produces a potent and specific obstruction of the Na^+ channels responsible for action potential generation, thereby paralyzing the animals unfortunate enough to ingest it. *Saxitoxin*, a chemical homologue of tetrodotoxin produced by dinoflagellates, has a similar action on Na^+ channels. The potentially lethal effects of eating shellfish that have ingested these "red tide" dinoflagellates are due to the potent neuronal actions of saxitoxin.

Scorpions paralyze their prey by injecting a potent mix of peptide toxins that also affect ion channels. Among these are the *α-toxins*, which slow the inactivation of Na^+ channels (Figure A1); exposure of neurons to these toxins prolongs the action potential (Figure A2), thereby scrambling information flow within the nervous system of the soon-to-be-devoured victim. Other peptides in scorpion venom, called *β-toxins*, shift the voltage dependence of Na^+ channel activation (Figure B). These toxins cause Na^+ channels to open at potentials much more negative than normal, disrupting action potential generation. Some alkaloid toxins combine these actions, both removing inactivation *and* shifting activation of Na^+ channels. One such toxin is *batrachotoxin*, produced by a species of frog; some tribes of South American Indians use this poison on their arrow tips. A number of plants produce similar toxins, including *aconitine*, from buttercups; *veratridine*, from lilies; and a number of insecticidal toxins produced by plants such as chrysanthemums and rhododendrons.

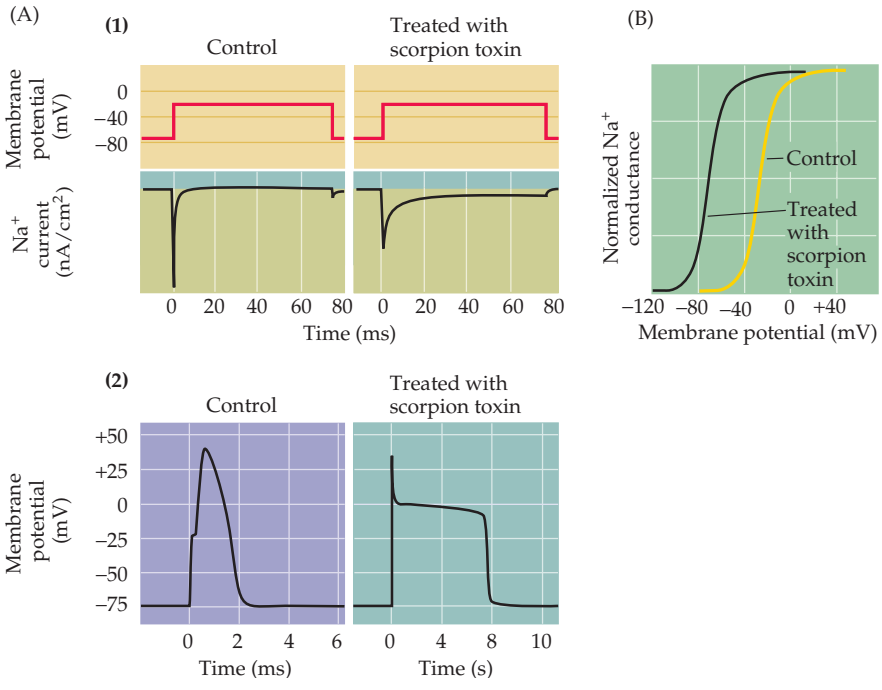
Potassium channels have also been targeted by toxin-producing organisms. Peptide toxins affecting K^+ channels include *dendrotoxin*, from wasps; *apamin*, from bees; and *charybdotoxin*, yet another toxin produced by scorpions. All of these toxins block K^+ channels as their primary action; no toxin is known to affect the activation or inactivation of these channels, although such agents may simply be awaiting discovery.

References

CAHALAN, M. (1975) Modification of sodium channel gating in frog myelinated nerve fibers by *Centruroides sculpturatus* scorpion venom. J. Physiol. (Lond.) 244: 511–534.

NARAHASHI, T. (2000) Neuroreceptors and ion channels as the basis for drug action: Present and future. J. Pharmacol. Exptl. Therapeutics 294: 1–26.

SCHMIDT, O. AND H. SCHMIDT (1972) Influence of calcium ions on the ionic currents of nodes of Ranvier treated with scorpion venom. Pflügers Arch. 333: 51–61.



(A) Effects of toxin treatment on frog axons. (1) α-Toxin from the scorpion *Leiurus quinquestriatus* prolongs Na^+ currents recorded with the voltage clamp method. (2) As a result of the increased Na^+ current, α-toxin greatly prolongs the duration of the axonal action potential. Note the change in timescale after treating with toxin. (B) Treatment of a frog axon with β-toxin from another scorpion, *Centruroides sculpturatus*, shifts the activation of Na^+ channels, so that Na^+ conductance begins to increase at potentials much more negative than usual. (A after Schmidt and Schmidt, 1972; B after Cahalan, 1975.)

allows K^+ ions to become dehydrated so they can enter the selectivity filter. These "naked" ions are then able to move through four K^+ binding sites within the selectivity filter to eventually reach the extracellular space (recall that the normal concentration gradient drives K^+ out of cells). On average, two K^+ ions reside within the selectivity filter at any moment, with electrostatic repulsion between the two ions helping to speed their transit through the selectivity filter, thereby permitting rapid ion flux through the channel.

Crystallographic studies have also determined the structure of the **voltage sensor** in another type of bacterial K^+ channel. Such studies indicate that the sensor is at the interface between proteins and lipid on the cytoplasmic surface of the channel, leading to the suggestion that the sensor is a paddle-like structure that moves through the membrane to gate the opening of the channel pore (Figure 4.9A), rather than being a rotating helix buried within the ion channel protein (as in Figure 4.7). Crystallographic work has also revealed the molecular basis of the rapid transitions between the closed and the open state of the channel during channel gating. By comparing data from K^+ channels crystallized in what is believed to be closed and open conformations (Figure 4.9B), it appears that channels gate by a conformational change in one of the transmembrane helices lining the channel pore. Producing a "kink" in one of these helices increases the opening from the central water-filled pore to the intracellular space, thereby permitting ion fluxes.

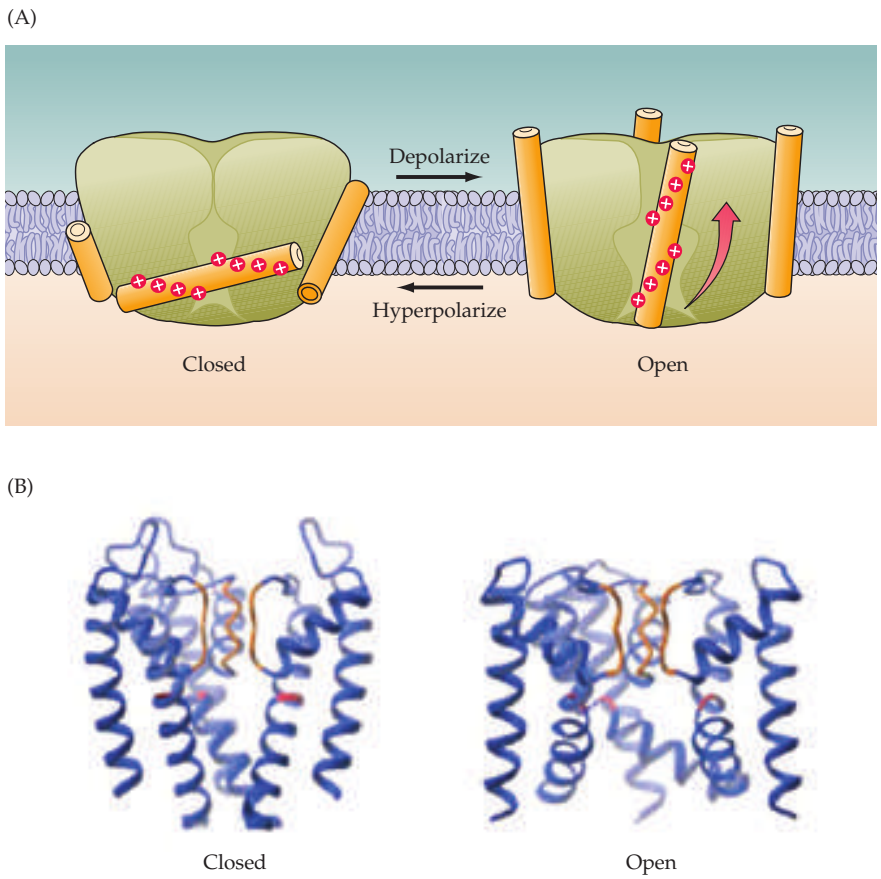


Figure 4.9 Structural features of K^+ channel gating. (A) Voltage sensing may involve paddle-like structures of the channel. These paddles reside within the lipid bilayer of the plasma membrane and may respond to changes in membrane potential by moving through the membrane. The gating charges that sense membrane potential are indicated by red "plus" signs. (B) Structure of K^+ channels in closed (left) and open (right) conformations. Three of the four channel subunits are shown. Opening of the pore of the channel involves kinking of a transmembrane domain at the point indicated in red, which then dilates the pore. (A after Jiang et al., 2003; B after MacKinnon, 2003).

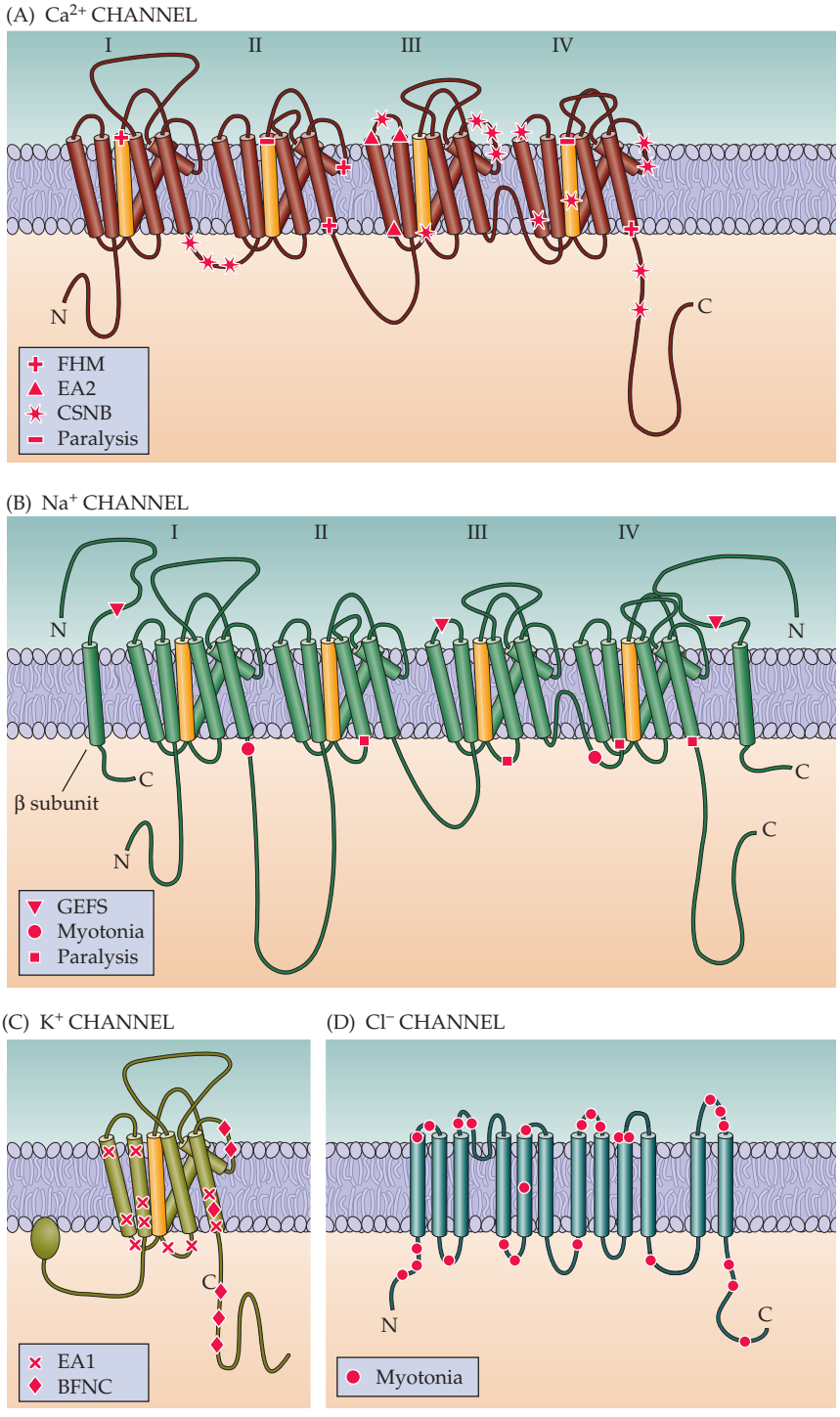
Box D

Diseases Caused by Altered Ion Channels

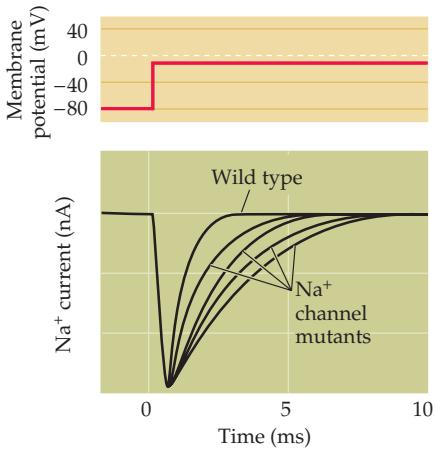
Several genetic diseases, collectively called *channelopathies*, result from small but critical alterations in ion channel genes. The best-characterized of these diseases are those that affect skeletal muscle cells. In these disorders, alterations in ion channel proteins produce either myotonia (muscle stiffness due to excessive electrical excitability) or paralysis (due to insufficient muscle excitability). Other disorders arise from ion channel defects in heart, kidney, and the inner ear.

Channelopathies associated with ion channels localized in brain are much more difficult to study. Nonetheless, voltage-gated Ca^{2+} channels have recently been implicated in a range of neurological diseases. These include episodic ataxia, spinocerebellar degeneration, night blindness, and migraine headaches. *Familial hemiplegic migraine* (FHM) is characterized by migraine attacks that typically last one to three days. During such episodes, patients experience severe headaches and vomiting. Several mutations in a human Ca^{2+} channel have been identified in families with FHM, each having different clinical symptoms. For example, a mutation in the pore-forming region of the channel produces hemiplegic migraine with progressive cerebellar ataxia, whereas other mutations cause only the usual FHM symptoms. How these altered Ca^{2+} channel properties lead to migraine attacks is not known.

Episodic ataxia type 2 (EA2) is a neurological disorder in which affected individuals suffer recurrent attacks of abnormal limb movements and severe ataxia. These problems are sometimes accompa-



Genetic mutations in (A) Ca^{2+} channels, (B) Na^{+} channels, (C) K^{+} channels, and (D) Cl^{-} channels that result in diseases. Red regions indicate the sites of these mutations; the red circles indicate mutations. (After Lehmann-Horn and Jurkat-Kott, 1999.)



Mutations in Na^+ channels slow the rate of inactivation of Na^+ currents. (After Barchi, 1995.)

nied by vertigo, nausea, and headache. Usually, attacks are precipitated by emotional stress, exercise, or alcohol and last for a few hours. The mutations in EA2 cause Ca^{2+} channels to be truncated at various sites, which may cause the clinical manifestations of the disease by preventing the normal assembly of Ca^{2+} channels in the membrane.

X-linked *congenital stationary night blindness* (CSNB) is a recessive retinal disorder that causes night blindness, decreased visual acuity, myopia, nystagmus, and strabismus. Complete CSNB causes retinal rod photoreceptors to be nonfunctional. Incomplete CSNB causes subnormal (but measurable) functioning of both rod and cone photoreceptors. Like EA2, the incomplete type of CSNB is caused by mutations producing truncated Ca^{2+} channels. Abnormal retinal function may arise from decreased Ca^{2+} currents and neurotransmitter release from photoreceptors (see Chapter 11).

A defect in brain Na^+ channels causes *generalized epilepsy with febrile seizures* (GEFS) that begins in infancy and usually continues through early puberty. This defect has been mapped to two mutations: one on chromosome 2 that encodes an α subunit for a voltage-gated Na^+ channel, and the other on chromosome 19 that encodes a Na^+ channel β subunit. These mutations cause a slowing of Na^+ channel inactivation (see figure above), which may explain the neuronal hyperexcitability underlying GEFS.

Another type of seizure, *benign familial neonatal convulsion* (BFNC), is due to K^+ channel mutations. This disease is characterized by frequent brief seizures commencing within the first week of life and disappearing spontaneously within a few months. The mutation has been mapped to at least two voltage-gated K^+ channel genes. A reduction in K^+ current flow through the mutated channels probably accounts for the hyperexcitability associated with this defect. A related disease, episodic ataxia type 1 (EA1), has been linked to a defect in another type of voltage-gated K^+ channel. EA1 is characterized by brief episodes of ataxia. Mutant channels inhibit the function of other, non-mutant K^+ channels and may produce clinical symptoms by impairing action potential repolarization. Mutations in the K^+ channels of cardiac muscle are responsible for the irregular heartbeat of patients with long Q-T syndrome. Numerous genetic disorders affect the voltage-gated channels of skeletal muscle and are responsible for a host of muscle diseases that either cause muscle weakness (*paralysis*) or muscle contraction (*myotonia*).

References

Barchi, R. L. (1995) Molecular pathology of the skeletal muscle sodium channel. Ann. Rev. Physiol. 57: 355–385.

Berkovic, S. F. and I. E. Scheffer (1997) Epilepsies with single gene inheritance. Brain Develop. 19 :13–28.

Cooper, E. C. and L. Y. Jan (1999) Ion channel genes and human neurological disease: Recent progress, prospects, and challenges. Proc. Natl. Acad. Sci. USA 96: 4759–4766.

Davies, N. P. and M. G. Hanna (1999) Neurological channelopathies: Diagnosis and therapy in the new millennium. Ann. Med. 31: 406–420.

Jen, J. (1999) Calcium channelopathies in the central nervous system. Curr. Op. Neurobiol. 9: 274–280.

Lehmann-Horn, F. and K. Jurkat-Rott (1999) Voltage-gated ion channels and hereditary disease. Physiol. Rev. 79: 1317–1372.

Ophoff, R. A., G. M. Terwindt, R. R. Frants and M. D. Ferrari (1998) P/Q-type Ca^{2+} channel defects in migraine, ataxia and epilepsy. Trends Pharm. Sci. 19: 121–127.

In short, ion channels are integral membrane proteins with characteristic features that allow them to assemble into multimolecular aggregates. Collectively, these structures allow channels to conduct ions, sense the transmembrane potential, to inactivate, and to bind to various neurotoxins. A combination of physiological, molecular biological and crystallographic studies has begun to provide a detailed physical picture of K^+ channels. This work has now provided considerable insight into how ions are conducted from one side of the plasma membrane to the other, how a channel can be selectively permeable to a single type of ion, how they are able to sense changes in membrane voltage, and how they gate the opening of their pores. It is likely that other types of ion channels will be similar in their functional architecture. Finally, this sort of work has illuminated how mutations in ion channel genes can lead to a variety of neurological disorders (Box D).

Active Transporters Create and Maintain Ion Gradients

Up to this point, the discussion of the molecular basis of electrical signaling has taken for granted the fact that nerve cells maintain ion concentration gradients across their surface membranes. However, none of the ions of physiological importance (Na^+, K^+, Cl^-, and Ca^{2+}) are in electrochemical equilibrium. Because channels produce electrical effects by allowing one or more of these ions to diffuse down their electrochemical gradients, there would be a gradual dissipation of these concentration gradients unless nerve cells could restore ions displaced during the current flow that occurs as a result of both neural signaling and the continual ionic leakage that occurs at rest. The work of generating and maintaining ionic concentration gradients for particular ions is carried out by a group of plasma membrane proteins known as **active transporters**.

Active transporters carry out this task by forming complexes with the ions that they are translocating. The process of ion binding and unbinding for transport typically requires several milliseconds. As a result, ion translocation by active transporters is much slower than ion movement through channels: Recall that ion channels can conduct thousands of ions across a membrane each millisecond. In short, active transporters gradually store energy in the form of ion concentration gradients, whereas the opening of ion channels rapidly dissipates this stored energy during relatively brief electrical signaling events.

Several types of active transporter have now been identified (Figure 4.10). Although the specific jobs of these transporters differ, all must translocate ions against their electrochemical gradients. Moving ions uphill requires the consumption of energy, and neuronal transporters fall into two classes based on their energy sources. Some transporters acquire energy directly from the hydrolysis of ATP and are called **ATPase pumps** (Figure 4.10, left)**.** The most prominent example of an ATPase pump is the **Na^+ pump** (or, more properly, the Na^+/K^+ ATPase pump), which is responsible for maintaining transmembrane concentration gradients for both Na^+ and K^+ (Figure 4.10A). Another is the Ca^{2+} pump, which provides one of the main mechanisms for removing Ca^{2+} from cells (Figure 4.10B). The second class of active transporter does not use ATP directly, but depends instead on the electrochemical gradients of other ions as an energy source. This type of transporter carries one or more ions *up* its electrochemical gradient while simultaneously taking another ion (most often Na^+) *down* its gradient. Because at least two species of ions are

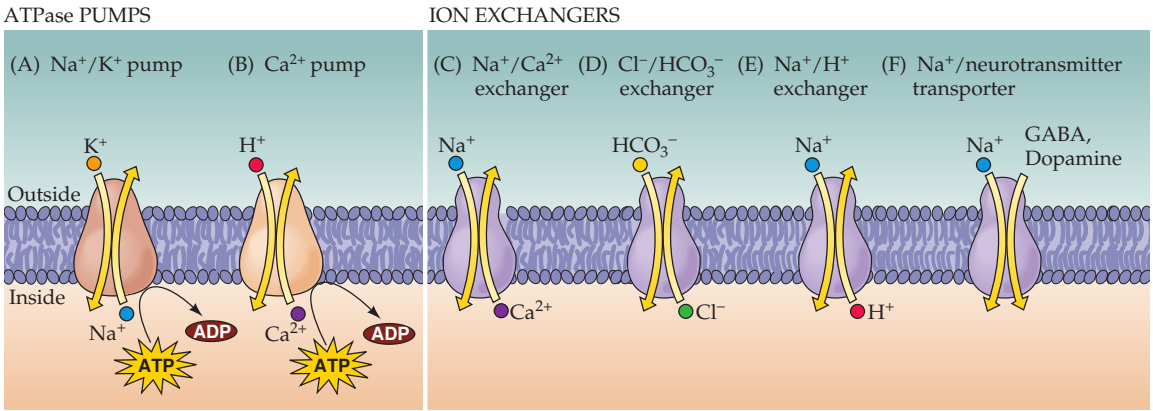


Figure 4.10 Examples of ion transporters found in cell membranes. (A,B) Some transporters are powered by the hydrolysis of ATP (ATPase pumps), whereas others (C–F) use the electrochemical gradients of co-transported ions as a source of energy (ion exchangers).

involved in such transactions, these transporters are usually called **ion exchangers** (Figure 4.10, right). An example of such a transporter is the Na^+/Ca^{2+} exchanger, which shares with the Ca^{2+} pump the important job of keeping intracellular Ca^{2+} concentrations low (Figure 4.10C). Another exchanger in this category regulates both intracellular Cl^- concentration and pH by swapping intracellular Cl^- for another extracellular anion, bicarbonate (Figure 4.10D). Other ion exchangers, such as the Na^+/H^+ exchanger (Figure 4.10E), also regulate intracellular pH, in this case by acting directly on the concentration of H^+. Yet other ion exchangers are involved in transporting neurotransmitters into synaptic terminals (Figure 4.10F), as described in Chapter 6. Although the electrochemical gradient of Na^+ (or other counter ions) is the proximate source of energy for ion exchangers, these gradients ultimately depend on the hydrolysis of ATP by ATPase pumps, such as the Na^+/K^+ ATPase pump.

Functional Properties of the Na^+/K^+ Pump

Of these various transporters, the best understood is the Na^+/K^+ pump. The activity of this pump is estimated to account for 20–40% of the brain's energy consumption, indicating its importance for brain function. The Na^+ pump was first discovered in neurons in the 1950s, when Richard Keynes at Cambridge University used radioactive Na^+ to demonstrate the energy-dependent efflux of Na^+ from squid giant axons. Keynes and his collaborators found that this efflux ceased when the supply of ATP in the axon was interrupted by treatment with metabolic poisons (Figure 4.11A, point 4). Other conditions that lower intracellular ATP also prevent Na^+ efflux. These experiments showed that removing intracellular Na^+ requires cellular metabolism. Further studies with radioactive K^+ demonstrated that Na^+ efflux is associated with simultaneous, ATP-dependent influx of K^+. These opposing fluxes of Na^+ and K^+ are operationally inseparable: Removal of external K^+ greatly reduces Na^+ efflux (Figure 4.11, point 2), and vice versa. These energy-dependent movements of Na^+ and K^+ implicated an ATP-hydrolyzing Na^+/K^+ pump in the generation of the transmembrane gradients of both Na^+ and K^+. The exact mechanism responsible for these fluxes of Na^+ and K^+ is still not entirely clear, but the pump is thought to alternately shuttle these ions across the membranes in a cycle fueled by the transfer of a phosphate group from ATP to the pump protein (Figure 4.11B).

Additional quantitative studies of the movements of Na^+ and K^+ indicate that the two ions are not pumped at identical rates: The K^+ influx is only about two-thirds the Na^+ efflux. Thus, the pump apparently transports two K^+ into the cell for every three Na^+ that are removed (see Figure 4.11B). This stoichiometry causes a net loss of one positively charged ion from inside of the cell during each round of pumping, meaning that the pump generates an electrical current that can hyperpolarize the membrane potential. For this reason, the Na^+/K^+ pump is said to be **electrogenic**. Because pumps act much more slowly than ion channels, the current produced by the Na^+/K^+ pump is quite small. For example, in the squid axon, the net current generated by the pump is less than 1% of the current flowing through voltage-gated Na^+ channels and affects the resting membrane potential by only a millivolt or less.

Although the electrical current generated by the activity of the Na^+/K^+ pump is small, under special circumstances the pump can significantly influence the membrane potential. For instance, prolonged stimulation of

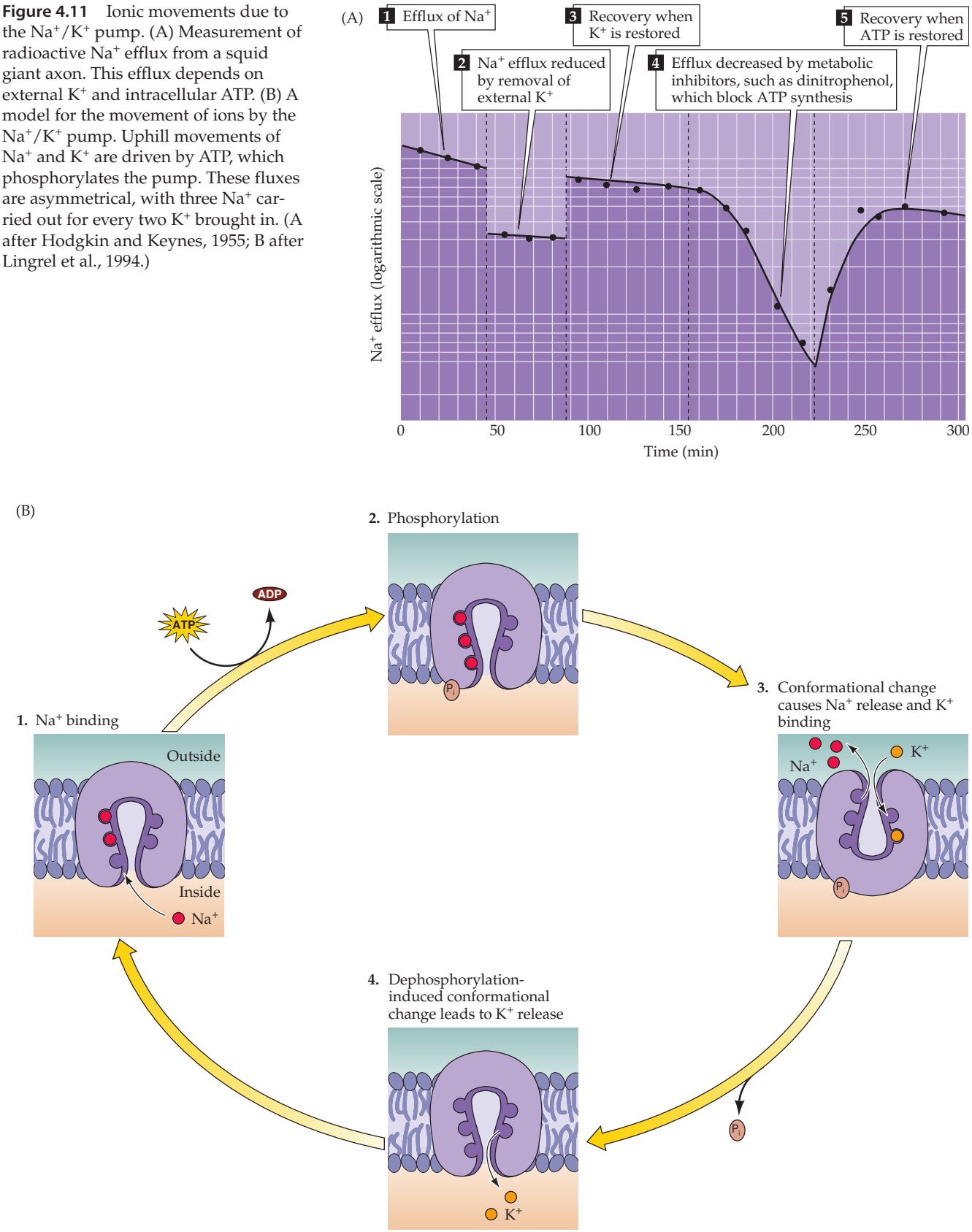


Figure 4.11 Ionic movements due to the Na^+/K^+ pump. (A) Measurement of radioactive Na^+ efflux from a squid giant axon. This efflux depends on external K^+ and intracellular ATP. (B) A model for the movement of ions by the Na^+/K^+ pump. Uphill movements of Na^+ and K^+ are driven by ATP, which phosphorylates the pump. These fluxes are asymmetrical, with three Na^+ carried out for every two K^+ brought in. (A after Hodgkin and Keynes, 1955; B after Lingrel et al., 1994.)

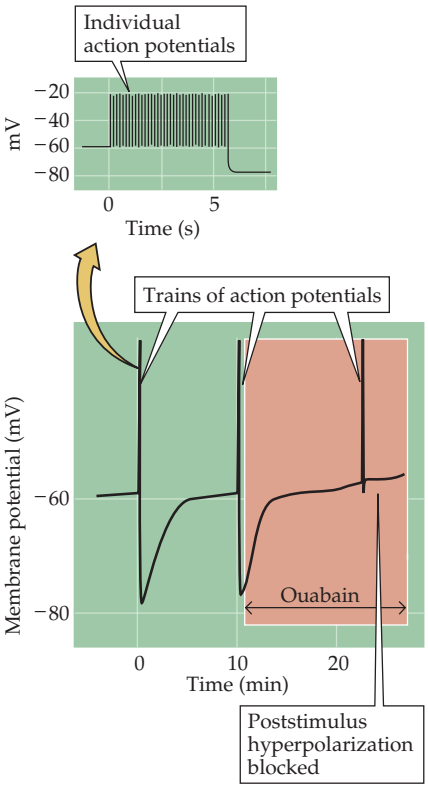


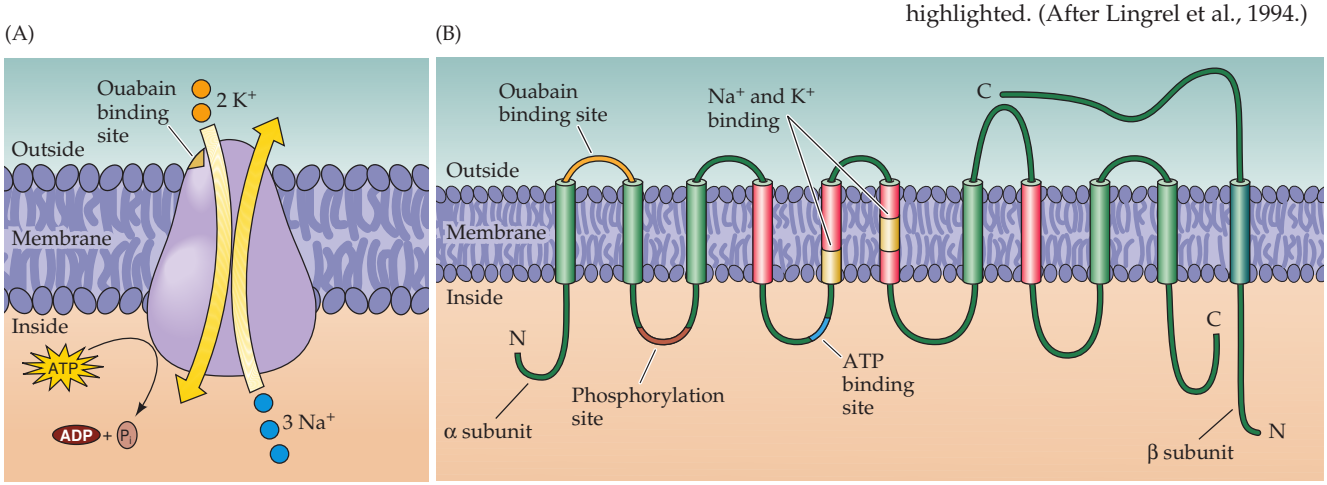
Figure 4.12 The electrogenic transport of ions by the Na^+/K^+ pump can influence membrane potential. Measurements of the membrane potential of a small unmyelinated axon show that a train of action potentials is followed by a long-lasting hyperpolarization. This hyperpolarization is blocked by ouabain, indicating that it results from the activity of the Na^+/K^+ pump. (After Rang and Ritchie, 1968.)

small unmyelinated axons produces a substantial hyperpolarization (Figure 4.12). During the period of stimulation, Na^+ enters through voltage-gated channels and accumulates within the axons. As the pump removes this extra Na^+, the resulting current generates a long-lasting hyperpolarization. Support for this interpretation comes from the observation that conditions that block the Na^+/K^+ pump—for example, treatment with ouabain, a plant glycoside that specifically inhibits the pump—prevent the hyperpolarization. The electrical contribution of the Na^+/K^+ pump is particularly significant in these small-diameter axons because their large surface-to-volume ratio causes intracellular Na^+ concentration to rise to higher levels than it would in other cells. Nonetheless, it is important to emphasize that, in most circumstances, the Na^+/K^+ pump plays no part in generating the action potential and has very little *direct* effect on the resting potential.

The Molecular Structure of the Na^+/K^+ Pump

These observations imply that the Na^+ and K^+ pump must exhibit several molecular properties: (1) It must bind both Na^+ and K^+; (2) it must possess sites that bind ATP and receive a phosphate group from this ATP; and (3) it must bind ouabain, the toxin that blocks this pump (Figure 4.13A). A variety of studies have now identified the aspects of the protein that account for these properties of the Na^+/K^+ pump. This pump is a large, integral membrane protein made up of at least two subunits, called α and β. The primary sequence shows that the α subunit spans the membrane 10 times, with most of the molecule found on the cytoplasmic side, whereas the β subunit spans the membrane once and is predominantly extracellular. Although a detailed account of the functional domains of the Na^+/K^+ pump is not yet available, some parts of the amino acid sequence have identified functions (Figure 4.13B). One intracellular domain of the protein is required for ATP binding

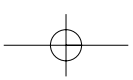
Figure 4.13 Molecular structure of the Na^+/K^+ pump. (A) General features of the pump. (B) The molecule spans the membrane 10 times. Amino acid residues thought to be important for binding of ATP, K^+, and ouabain are highlighted. (After Lingrel et al., 1994.)



and hydrolysis, and the amino acid phosphorylated by ATP has been identified. Another extracellular domain may represent the binding site for ouabain. However, the sites involved in the most critical function of the pump—the movement of Na^+ and K^+—have not yet been defined. Nonetheless, altering certain membrane-spanning domains (red in Figure 4.13B) impairs ion translocation; moreover, kinetic studies indicate that both ions bind to the pump at the same site. Because these ions move across the membrane, it is likely that this site traverses the plasma membrane; it is also likely that the site has a negative charge, since both Na^+ and K^+ are positively charged. The observation that removing negatively charged residues in a membrane-spanning domain of the protein (pale yellow in Figure 4.13B) greatly reduces Na^+ and K^+ binding provides at least a hint about the ion-translocating domain of the transporter molecule.

Summary

Ion transporters and channels have complementary functions. The primary purpose of transporters is to generate transmembrane concentration gradients, which are then exploited by ion channels to generate electrical signals. Ion channels are responsible for the voltage-dependent conductances of nerve cell membranes. The channels underlying the action potential are integral membrane proteins that open or close ion-selective pores in response to the membrane potential, allowing specific ions to diffuse across the membrane. The flow of ions through single open channels can be detected as tiny electrical currents, and the synchronous opening of many such channels generates the macroscopic currents that produce action potentials. Molecular studies show that such voltage-gated channels have highly conserved structures that are responsible for features such as ion permeation and voltage sensing, as well as the features that specify ion selectivity and toxin sensitivity. Other types of channels are sensitive to chemical signals, such as neurotransmitters or second messengers, or to heat or membrane deformation. A large number of ion channel genes create channels with a correspondingly wide range of functional characteristics, thus allowing different types of neurons to have a remarkable spectrum of electrical properties. Ion transporter proteins are quite different in both structure and function. The energy needed for ion movement against a concentration gradient (e.g., in maintaining the resting potential) is provided either by the hydrolysis of ATP or by the electrochemical gradient of co-transported ions. The Na^+/K^+ pump produces and maintains the transmembrane gradients of Na^+ and K^+, while other transporters are responsible for the electrochemical gradients for other physiologically important ions, such as Cl^-, Ca^{2+}, and H^+. Together, transporters and channels provide a reasonably comprehensive molecular explanation for the ability of neurons to generate electrical signals.



Additional Reading

Reviews

ARMSTRONG, C. M. AND B. HILLE (1998) Voltage-gated ion channels and electrical excitability. Neuron 20: 371–380.

BEZANILLA, F. AND A. M. CORREA (1995) Single-channel properties and gating of Na^+ and K^+ channels in the squid giant axon. In *Cephalopod Neurobiology*, N. J. Abbott, R. Williamson and L. Maddock (eds.). New York: Oxford University Press, pp. 131–151.

CATTERALL, W. A. (1988) Structure and function of voltage-sensitive ion channels. Science 242: 50–61.

ISOM, L. L., K. S. DE JONGH AND W. A. CATTERALL (1994) Auxiliary subunits of voltage-gated ion channels. Neuron 12: 1183–1194.

JAN, L. Y. AND Y. N. JAN (1997) Voltage-gated and inwardly rectifying potassium channels. J. Physiol. 505: 267–282.

JENTSCH, T. J., T. FRIEDRICH, A. SCHRIEVER AND H. YAMADA (1999) The CLC chloride channel family. Pflügers Archiv. 437: 783–795.

KAPLAN, J. H. (2002) Biochemistry of Na,K-ATPase. Annu. Rev. Biochem. 71: 511–535.

KRISHTAL, O. (2003). The ASICs: Signaling molecules? Modulators? Trends Neurosci, 26: 477–483.

LINGREL, J. B., J. VAN HUYSSE, W. O'BRIEN, E. JEWELL-MOTZ, R. ASKEW AND P. SCHULTHEIS (1994) Structure-function studies of the Na, K-ATPase. Kidney Internat. 45: S32–S39.

MACKINNON, R. (2003) Potassium channels. FEBS Lett. 555: 62–65.

NEHER, E. (1992) Nobel lecture: Ion channels for communication between and within cells. Neuron 8: 605–612.

PATAPOUTIAN, A., A. M. PEIER, G. M. STORY AND V. VISWANATH (2003). ThermoTRP channels and beyond: Mechanisms of temperature sensation. Nat. Rev. Neurosci. 4: 529–539.

SEEBURG, P. H. (2002). A-to-I editing: New and old sites, functions and speculations. Neuron 35: 17–20.

SKOU, J. C. (1988) Overview: The Na,K pump. Meth. Enzymol. 156: 1–25.

Important Original Papers

ANTZ, C. AND 7 OTHERS (1997) NMR structure of inactivation gates from mammalian voltage-dependent potassium channels. Nature 385: 272–275.

BEZANILLA, F., E. PEROZO, D. M. PAPAZIAN AND E. STEFANI (1991) Molecular basis of gating charge immobilization in Shaker potassium channels. Science 254: 679–683.

BOULTER, J. AND 6 OTHERS (1990) Molecular cloning and functional expression of glutamate receptor subunit genes. Science 249: 1033–1037.

CATERINA, M. J., M. A. SCHUMACHER, M. TOMINAGA, T. A. ROSEN, J. D. LEVINE AND D. JULIUS (1997) The capsaicin receptor: A heat-activated ion channel in the pain pathway. Nature 389: 816–824.

CHA, A., G. E. SNYDER, P. R. SELVIN AND F. BEZANILLA (1999) Atomic scale movement of the voltage-sensing region in a potassium channel measured via spectroscopy. Nature 402: 809–813.

DOYLE, D. A. AND 7 OTHERS (1998) The structure of the potassium channel: Molecular basis of K^+ conduction and selectivity. Science 280: 69–77.

FAHLKE, C., H. T. YU, C. L. BECK, T. H. RHODES AND A. L. GEORGE JR. (1997) Pore-forming segments in voltage-gated chloride channels. Nature 390: 529–532.

HO, K. AND 6 OTHERS (1993) Cloning and expression of an inwardly rectifying ATP-regulated potassium channel. Nature 362: 31–38.

HODGKIN, A. L. AND R. D. KEYNES (1955) Active transport of cations in giant axons from *Sepia* and *Loligo*. J. Physiol. 128: 28–60.

HOSHI, T., W. N. ZAGOTTA AND R. W. ALDRICH (1990) Biophysical and molecular mechanisms of *Shaker* potassium channel inactivation. Science 250: 533–538.

JIANG, Y. AND 6 OTHERS (2003) X-ray structure of a voltage-dependent K^+ channel. Nature 423: 33–41.

LLANO, I., C. K. WEBB AND F. BEZANILLA (1988) Potassium conductance of squid giant axon. Single-channel studies. J. Gen. Physiol. 92: 179–196.

MIKAMI, A. AND 7 OTHERS (1989) Primary structure and functional expression of the cardiac dihydropyridine-sensitive calcium channel. Nature 340: 230–233.

NODA, M. AND 6 OTHERS (1986) Expression of functional sodium channels from cloned cDNA. Nature 322: 826–828.

NOWYCKY, M. C., A. P. FOX AND R. W. TSIEN (1985) Three types of neuronal calcium channel with different calcium agonist sensitivity. Nature 316: 440–443.

PAPAZIAN, D. M., T. L. SCHWARZ, B. L. TEMPEL, Y. N. JAN AND L. Y. JAN (1987) Cloning of genomic and complementary DNA from *Shaker*, a putative potassium channel gene from *Drosophila*. Science 237: 749–753.

RANG, H. P. AND J. M. RITCHIE (1968) On the electrogenic sodium pump in mammalian non-myelinated nerve fibres and its activation by various external cations. J. Physiol. 196: 183–221.

SIGWORTH, F. J. AND E. NEHER (1980) Single Na^+ channel currents observed in cultured rat muscle cells. Nature 287: 447–449.

THOMAS, R. C. (1969) Membrane current and intracellular sodium changes in a snail neurone during extrusion of injected sodium. J. Physiol. 201: 495–514.

TOYOSHIMA, C., M. NAKASAKO, H. NOMURA AND H. OGAWA (2000) Crystal structure of the calcium pump of sarcoplasmic reticulum at 2.6 Å resolution. Nature 405: 647–655.

VANDERBERG, C. A. AND F. BEZANILLA (1991) A sodium channel model based on single channel, macroscopic ionic, and gating currents in the squid giant axon. Biophys. J. 60: 1511–1533.

WALDMANN, R., G. CHAMPIGNY, F. BASSILANA, C. HEURTEAUX AND M. LAZDUNSKI (1997) A proton-gated cation channel involved in acid-sensing. Nature 386: 173–177.

WEI, A. M., A. COVARRUBIAS, A. BUTLER, K. BAKER, M. PAK AND L. SALKOFF (1990) K^+ current diversity is produced by an extended gene family conserved in *Drosophila* and mouse. Science 248: 599–603.

YANG, N., A. L. GEORGE JR. AND R. HORN (1996) Molecular basis of charge movement in voltage-gated sodium channels. Neuron 16: 113–22.

Books

AIDLEY, D. J. AND P. R. STANFIELD (1996) *Ion Channels: Molecules in Action*. Cambridge: Cambridge University Press.

ASHCROFT, F. M. (2000) *Ion Channels and Disease.* Boston: Academic Press.

HILLE, B. (2001) *Ion Channels of Excitable Membranes*, 3rd Ed. Sunderland, MA: Sinauer Associates.

JUNGE, D. (1992) *Nerve and Muscle Excitation*, 3rd Ed. Sunderland, MA: Sinauer Associates.

NICHOLLS, D. G. (1994) *Proteins, Transmitters and Synapses*. Oxford: Blackwell Scientific

SIEGEL, G. J., B. W. AGRANOFF, R. W. ALBERS, S. K. FISHER AND M. D. UHLER (1999) *Basic Neurochemistry*. Philadelphia: Lippincott-Raven.