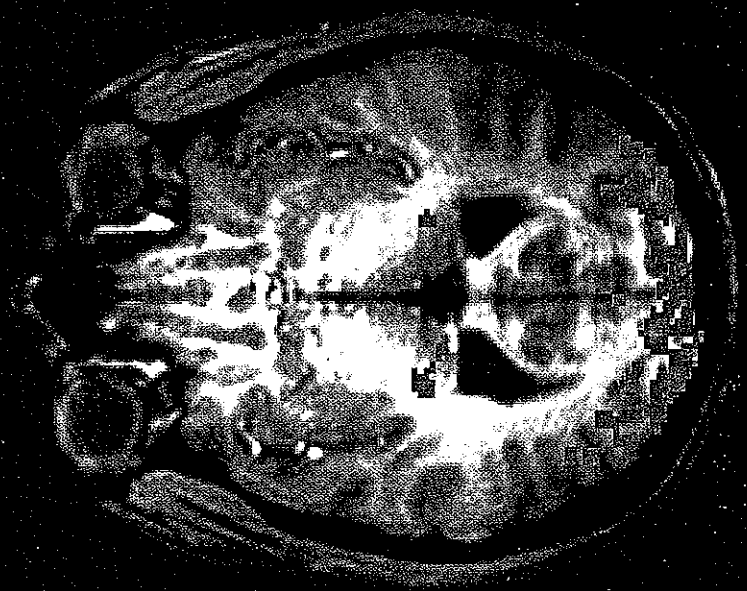
FROM NEURON TO BRAIN

FOURTH EDITION



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5 Ionic Basis of the Resting Potential

AT REST, A NEURON HAS A STEADY ELECTRICAL POTENTIAL across its plasma membrane, the inside being negative with respect to the outside. In relation to the extracellular fluid, the neuron has a high intracellular potassium concentration and low intracellular concentrations of sodium and chloride, so that potassium tends to diffuse out of the cell and sodium and chloride tend to diffuse in. The tendency for potassium and chloride to diffuse down their concentration gradients is opposed by the membrane potential. In a model cell permeable only to potassium and chloride, the concentration gradients and the membrane potential can be balanced exactly so that there is no net flux of either ion across the membrane. The membrane potential is then equal to the equilibrium potential for both potassium and chloride.

In the model cell, changing the extracellular potassium concentration changes the potassium equilibrium potential, and hence the membrane potential. In contrast, changing the extracellular chloride concentration eventually leads to a change in intracellular chloride. As a result the chloride equilibrium potential and the membrane potential are unchanged.

Real cells are also permeable to sodium. At rest, sodium ions constantly move into the cell, reducing the internal negativity of the membrane. As a result, potassium, being no longer in equilibrium, leaks out. If there were no compensation, these fluxes would lead to changes in the internal concentrations of sodium and potassium. However, the concentrations are maintained by the sodium–potassium exchange pump, which transports sodium out and potassium in across the cell membrane in a ratio of 3 sodium to 2 potassium. The resting membrane potential depends on the potassium equilibrium potential, the sodium equilibrium potential, the relative permeabilities of the cell membrane to the two ions, and the pump ratio. At the resting potential, the passive fluxes of sodium and potassium are exactly matched by the rates at which they are transported in the opposite direction. Because the sodium–potassium exchange pump transports more positive ions outward than inward across the membrane, it makes a direct contribution of several millivolts to the membrane potential.

The chloride equilibrium potential may be positive or negative with respect to the resting membrane potential, depending on chloride transport processes. Although the chloride distribution plays little role in determining the resting membrane potential, a substantial chloride permeability is important in some cells for electrical stability.

Electrical signals are generated in nerve cells and muscle fibers primarily by changes in permeability of the cell membrane to ions such as sodium and potassium. Increases in permeability allow ions to move inward or outward across the cell membrane down their electrochemical gradients. As we discussed in Chapter 2, permeability increases are due to activation of ion channels. Ions moving through the open channels change the charge on the cell membrane, and hence change the membrane potential. In order to understand how signals are generated, it is necessary to understand the nature of the standing ionic gradients across the cell membrane, and how these influence the resting membrane potential.

A MODEL CELL

It is useful to begin with the model cell shown in Figure 5.1. This cell contains potassium, sodium, chloride, and a large anion species, and it is bathed in a solution of sodium and potassium chloride. Other ions present in real cells, such as calcium or magnesium, are ignored for the moment, as their direct contributions to the resting membrane potential are negligible. The extracellular and intracellular ion concentrations in the model cell are similar to those found in frogs. In birds and mammals, ion concentrations are somewhat higher; in marine invertebrates such as the squid, very much higher (see Table 5.1). The model cell membrane is permeable to potassium and chloride, but not to sodium or to the internal anion. There are three major requirements for such a cell to remain in a stable condition:

1. The intracellular and extracellular solutions must each be electrically neutral. For example, a solution of chloride ions alone cannot exist; their charges must be balanced by an equal number of positive charges on cations such as sodium or potassium (otherwise electrical repulsion would literally blow the solution apart).
2. The cell must be in osmotic balance. If not, water will enter or leave the cell, causing it to swell or shrink, until osmotic balance is achieved. Osmotic balance is achieved when the total concentration of solute particles inside the cell is equal to that on the outside.
3. There must be no net movement of any particular ion into or out of the cell.

Ionic Equilibrium

How are the concentrations of the permeant ions maintained in the model cell, and what electrical potential is developed across the cell membrane? Figure 5.1 shows that the two ions are distributed in reverse ratio: Potassium is more concentrated on the inside of the cell, chloride on the outside. Imagine first that the membrane is permeable only to potassium; the question that arises immediately is why potassium ions do not diffuse out of the cell until the concentrations on either side of the cell membrane are equal. The answer is that they cannot because as they diffuse outward, positive charges accumulate on

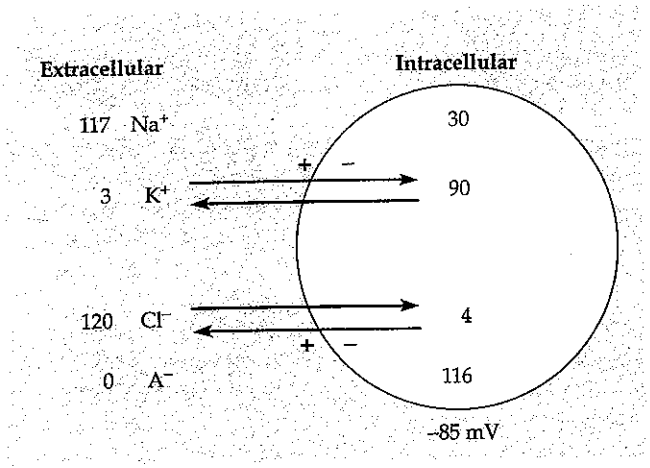


FIGURE 5.1 Ion Distributions in a Model Cell. The cell membrane is impermeable to Na^+ and to the internal anion (A^-), and permeable to K^+ and Cl^-. The concentration gradient for K^+ tends to drive it out of the cell (black arrow); the potential gradient tends to attract K^+ into the cell (orange arrow). In a cell at rest the two forces are exactly in balance. Concentration and electrical gradients for Cl^- are in the reverse directions. Ion concentrations are expressed in millimolar (m*M*).

the outer surface of the membrane and an excess of negative charges is left on the inner surface. As a result, a difference in potential develops across the membrane, the inside being negative with respect to the outside. The electrical gradient slows the efflux of positively charged potassium ions, and when the potential becomes sufficiently large, further net efflux of potassium is stopped. This is the potassium equilibrium potential (E_K). At E_K the effects of the concentration gradient and the potential gradient on ion flux through the membrane balance one another exactly. Individual potassium ions still enter and leave the cell, but no *net* movement occurs. The potassium ion is in equilibrium.

The conditions for potassium to be in equilibrium across the cell membrane are the same as those described in Chapter 2 for maintaining zero net flux through an individual channel in a membrane patch. There, a concentration gradient was balanced by a potential applied to the patch pipette. The important difference here is that the ion flux itself produces the required transmembrane potential. In other words, equilibrium in the model cell is automatic and inevitable. Recall from Chapter 2 that the potassium equilibrium potential is given by the Nernst equation:

$$E_K = \frac{RT}{zF} \ln \frac{[K]_o}{[K]_i} = 58 \log \frac{[K]_o}{[K]_i}$$

where $[K]_o$ and $[K]_i$ are the external and internal potassium ion concentrations. For the cell shown in Figure 5.1, E_K is 58 log (1/30) = −85 mV.

Suppose now that, in addition to potassium channels, the membrane has chloride channels. Because for an anion $z = -1$, the equilibrium potential for chloride is:

$$E_{Cl} = -58 \log \frac{[Cl]_o}{[Cl]_i}$$

or (from the properties of logarithmic ratios):

$$E_{Cl} = 58 \log \frac{[Cl]_i}{[Cl]_o}$$

In our model cell, the chloride concentration ratio is again 1:30, and E_{Cl} is also −85 mV. As with potassium, the membrane potential of −85 mV balances exactly the tendency for chloride to move down its concentration gradient, in this case *into* the cell.

In summary, the tendency for potassium ions to leave the cell and for chloride ions to diffuse inward are both opposed by the membrane potential. Because the concentration ratios for the two ions are of exactly the same magnitude (1:30), their equilibrium potentials are exactly the same. Since potassium and chloride are the only two ions that can move across the membrane and both are in equilibrium at −85 mV, the model cell can exist indefinitely with no net gain or loss of ions.

Electrical Neutrality

The charge separation across the membrane of our model cell, produced by outward movement of potassium and inward movement of chloride, means that there is an excess of anions inside the cell and of cations outside. This appears to violate the principle of electrical neutrality that we started with, but in fact does not. Potassium ions diffusing outward collect as excess cations against the outer membrane surface, leaving excess anions closely attracted to the inner surface. Both the potassium ions and the counterions they leave behind are, in effect, removed from the intracellular bulk solution, leaving it neutral. Similarly, chloride ions diffusing inward add to the collection of excess anions on the inner surface and leave counterions in the outer charged layer, so that the extracellular solution remains neutral as well. The outer layer of cations and inner layer of anions, of equal and opposite charge, are not in free solution, but are held to the membrane surface by mutual attraction. Thus, the membrane acts as a capacitor, separating and storing charge.

This does not mean that any given anion or cation is locked in position against the membrane. Ions in the charged layer interchange freely with those in the bulk solution.

The point is that although the identities of the ions in the layer are constantly changing, their total number remains constant, and the bulk solution remains neutral.

Another question we might ask about charge separation is whether the number of ions accumulated in the charged layer represents a significant fraction of the total number of ions in the cell. The answer is that it does not. If we consider our cell to have a radius of 25 μm, then at a concentration of 120 m*M* there are roughly 4×10^{12} cations and an equal number of anions in the cytoplasm. At a membrane potential of −85 mV, the amount of charge separated by the membrane is about 5×10^{11} univalent ions/cm^2 (Chapter 7). Our cell has a surface area of about 8×10^{-5} cm^2, so there are approximately 4×10^7 negative ions collected at the inner surface of the membrane, or 1/100,000 the number in free solution. Thus, the movements of potassium and chloride ions required to establish the membrane potential have no significant effect on intracellular ion concentrations.

The Effect of Extracellular Potassium and Chloride on Membrane Potential

In neurons, and in many other cells, the resting membrane potential is sensitive to changes in extracellular potassium concentration but is relatively unaffected by changes in extracellular chloride. To understand how this comes about it is useful to consider the consequences of such changes in the model cell. We will assume throughout this discussion that the volume of the extracellular fluid is infinitely large. Thus, movements of ions and water into or out of the cell have no significant effect on extracellular concentrations. Figure 5.2A shows the changes in intracellular composition and membrane potential that result from increasing extracellular potassium from 3 to 6 m*M*. This is done by replacing 3 m*M* NaCl with 3 m*M* KCl, thereby keeping the osmolarity unchanged, with a total

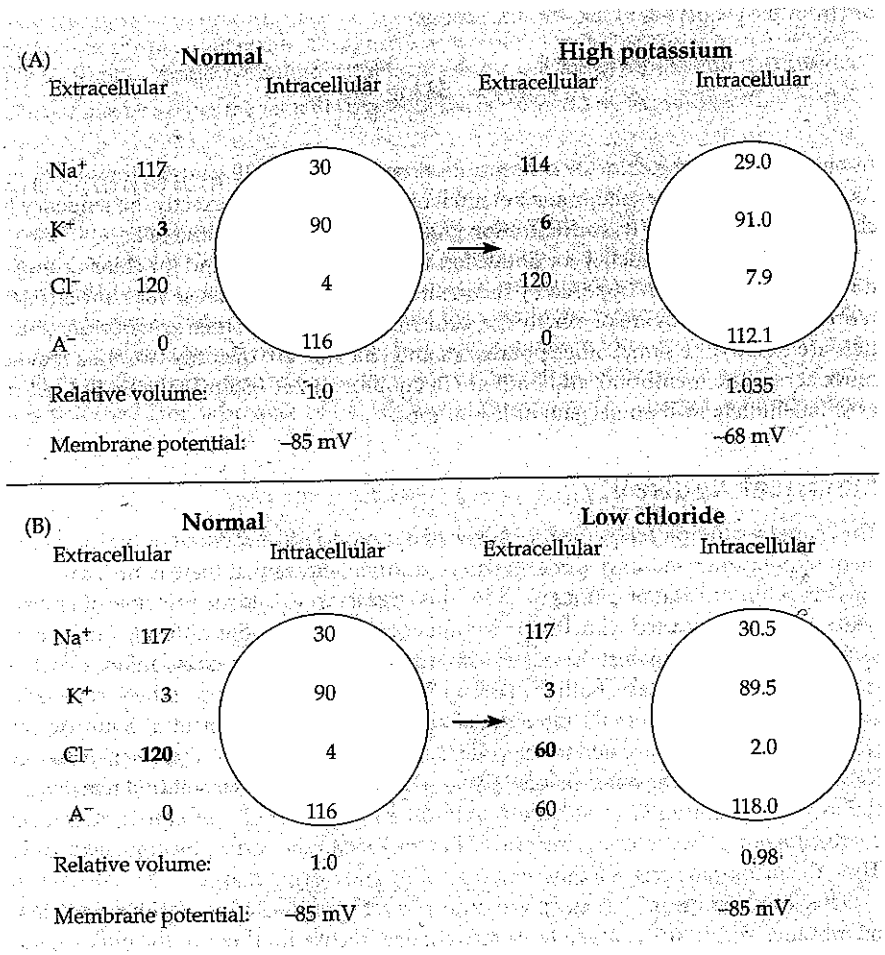


FIGURE 5.2 Effects of Changing Extracellular Ion Composition on intracellular ion concentrations and on membrane potential. (A) Extracellular K^+ concentration is doubled and, to keep osmolarity constant, Na^+ concentration is reduced. (B) Half the extracellular Cl^- is replaced by an impermeant anion, A^-. Ion concentrations are in millimolar (m*M*), and extracellular volumes are assumed to be very large with respect to cell volumes, so fluxes into and out of the cell do not change extracellular concentrations.

solute concentration of 240 m*M*. The increase in extracellular potassium reduces the concentration gradient for outward potassium movement, while initially leaving the electrical gradient unchanged. As a result there will be a net inward movement of potassium ions. As positive charges accumulate on its inner surface, the membrane is depolarized. This, in turn, means that chloride ions are no longer in equilibrium, and they move into the cell as well. Potassium and chloride entry continues until a new equilibrium is established, with both ions at a new concentration ratio consistent with the new membrane potential, in this example –68 mV.

Potassium and chloride entry is accompanied by the entry of water to maintain osmotic balance, resulting in a slight increase in cell volume. When the new equilibrium is reached, intracellular potassium has increased in concentration from 90 to 91 m*M*, intracellular chloride has increased in concentration from 4 to 7.9 m*M*, and the cell volume has increased by 3.5%.

At first glance it seems that more chloride than potassium has entered the cell, but think what the concentrations would be if the cell did *not* increase in volume: The concentrations of both ions would be greater than the indicated values by 3.5%. Thus, the intracellular chloride concentration would be about 8.2 m*M* (instead of 7.9 m*M*), and intracellular potassium would be about 94.2 m*M*, both 4.2 m*M* higher than in the original solution. In other words, we can think first of potassium and chloride entering in equal quantities (except for the trivial difference required to change the charge on the membrane), and then of water following to achieve the final concentrations shown in the figure.

Similar considerations apply to changes in extracellular chloride concentration, but with a marked difference: When the new steady state is finally reached, the membrane potential is essentially unchanged. The consequences of a 50% reduction in extracellular chloride concentration are shown in Figure 5.2B, in which 60 m*M* of chloride in the solution bathing the cell is replaced by an impermeant anion. Chloride leaves the cell, depolarizing the membrane toward the new chloride equilibrium potential (–68 mV). Potassium, being no longer in equilibrium, leaves as well. As in the previous example, potassium and chloride leave the cell in equal quantities (accompanied by water). Because the intracellular concentration of potassium is high, the fractional change in concentration produced by the efflux is relatively small. However, the efflux of chloride causes a sizable fractional change in the intracellular chloride concentration, and hence in the chloride equilibrium potential. As chloride continues to leave the cell, the equilibrium potential returns toward its original value. The process continues until the chloride and potassium equilibrium potentials are again equal and the membrane potential is restored.

MEMBRANE POTENTIALS IN SQUID AXONS

The idea that the resting membrane potential is the result of an unequal distribution of potassium ions between the extracellular and intracellular fluids was first proposed by Bernstein[1] in 1902. He could not test this hypothesis directly, however, because there was no satisfactory way of measuring membrane potential. It is now possible to measure membrane potential accurately, and to see whether changes in external and internal potassium concentrations produce the potential changes predicted by the Nernst relation.

The first such experiments were done on giant axons that innervate the mantle of the squid. The axons are up to 1 mm in diameter,[2] and their large size permits the insertion of recording electrodes into their cytoplasm to measure transmembrane potential directly (Figure 5.3A). Further, they are remarkably resilient and continue to function even when their axoplasm has been squeezed out with a rubber roller and replaced with an internal perfusate (Figure 5.3B and C). Thus, their internal, as well as external, ion composition can be controlled. A. L. Hodgkin, who together with A. F. Huxley initiated many experiments on squid axon (for which they later received the Nobel prize), has said,[3]

> It is arguable that the introduction of the squid giant nerve fiber by J. Z. Young in 1936 did more for axonology than any other single advance during the last forty years. Indeed a distinguished neurophysiologist remarked recently at a congress dinner (not, I thought, with the utmost tact), "It's the squid that really ought to be given the Nobel Prize."

[1]Bernstein, J. 1902. *Pflügers Arch.* 92: 521–562.

[2]Young, J. Z. 1936. *Q. J. Microsc. Sci.* 78: 367–386.

[3]Hodgkin, A. L. 1973. *Proc. R. Soc. Lond. B* 183: 1–19.

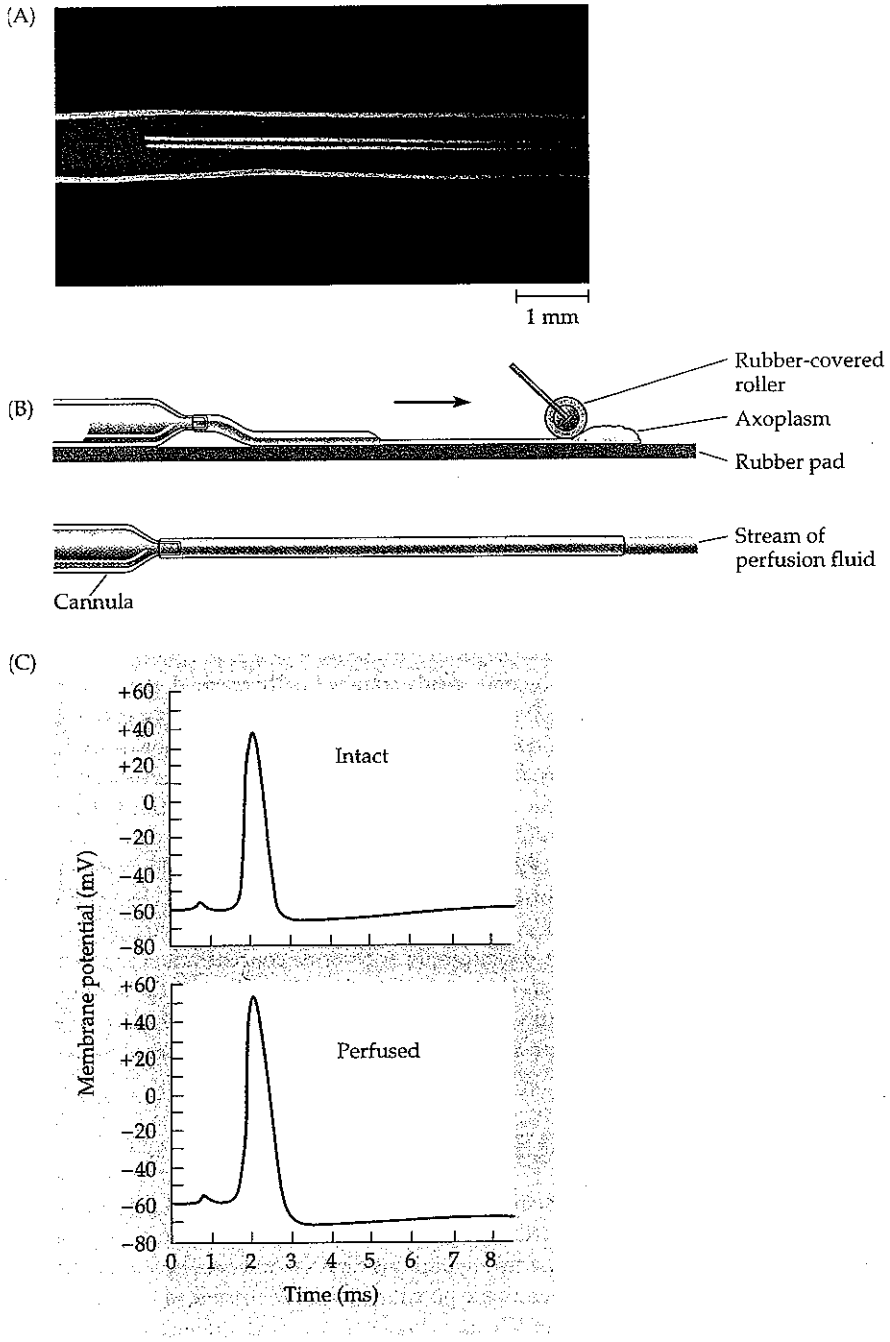


FIGURE 5.3 Recording from a Squid Axon. (A) Isolated giant axon of the squid, with axial recording electrode inside. (B) Extrusion of axoplasm from the axon, which is then cannulated and perfused internally. (C) Comparison of records before (intact) and after perfusion shows that the resting and action potentials are unaffected by removal of the axoplasm. (A from Hodgkin and Keynes, 1956; B and C after Baker, Hodgkin, and Shaw, 1962.)

The concentrations of some of the major ions in squid blood and in the axoplasm of the squid nerves are given in Table 5.1 (several ions, such as magnesium and internal anions, are omitted). Experiments on isolated axons are usually done in seawater, with the ratio of intracellular to extracellular potassium concentrations 40:1. If the membrane potential (V_m) were equal to the potassium equilibrium potential, it would be −93 mV. In fact, the measured membrane potential is considerably less negative (about −65 to −70 mV). On the other hand, the membrane potential is more negative than the chloride equilibrium potential, which is about −55 mV.

Bernstein's hypothesis was tested by measuring the resting membrane potential and comparing it with the potassium equilibrium potential at various extracellular potassium concentrations. (As with our model cell, such changes would be expected to produce no significant change in internal potassium concentration.) From the Nernst equation

Table 5.1
Concentrations of ions inside and outside freshly isolated axons of squid

Ion	Concentration (m*M*)		
	Axoplasm	Blood	Seawater
Potassium	400	20	10
Sodium	50	440	460
Chloride	60	560	540
Calcium	0.1 μ*M*[a]	10	10

Source: After Hodgkin, 1964.
[a] Ionized intracellular calcium from Baker, Hodgkin, and Ridgeway, 1971.

(Chapter 2), changing the concentration ratio by a factor of 10 should change the membrane potential by 58 mV at room temperature. The results of such an experiment on squid axon, in which the external potassium concentration was changed, are shown in Figure 5.4. The external concentration is plotted on a logarithmic scale on the abscissa and the membrane potential on the ordinate. The expected slope of 58 mV per 10-fold change in extracellular potassium concentration is realized only at relatively high concentrations (straight line), with the slope becoming less and less as external potassium is reduced. This result indicates that the potassium ion distribution is not the only factor contributing to the membrane potential.

The Effect of Sodium Permeability

From the experiments on squid axon we can conclude that the hypothesis made by Bernstein in 1902 is almost correct: The membrane potential is strongly but not exclusively dependent on the potassium concentration ratio. How do we account for the deviation from the Nernst relation shown in Figure 5.4? Simply by abandoning the notion that the membrane is impermeable to sodium. A real cell membrane has, in fact, a permeability to sodium that ranges between 1 and 10% of its permeability to potassium.

To consider the effect of sodium permeability, we begin with our model cell and, for the moment, ignore any movement of chloride ions. The membrane potential is at the potassium equilibrium potential, so there is no net movement of potassium across the membrane. If we now make the cell permeable to sodium, both the concentration gradi-

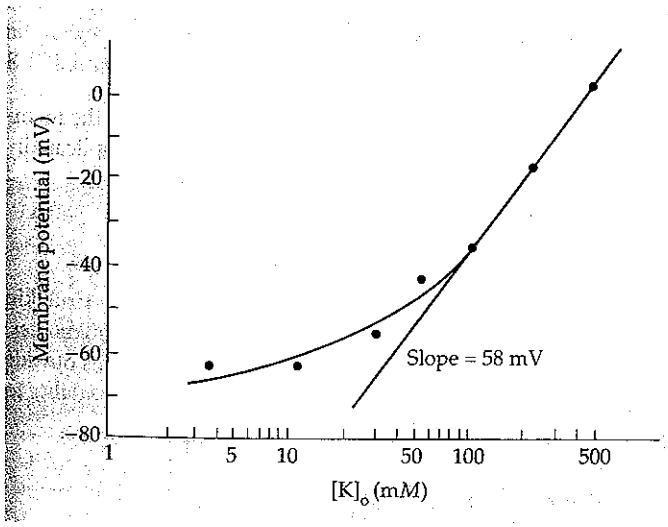


FIGURE 5.4 Membrane Potential versus External Potassium Concentration in squid axon, plotted on a semilogarithmic scale. The straight line is drawn with a slope of 58 mV per 10-fold change in extracellular potassium concentration, according to the Nernst equation. Because the membrane is also permeable to sodium, the points deviate from the straight line, especially at low potassium concentrations. (After Hodgkin and Keynes, 1955.)

ent and the membrane potential tend to drive sodium into the cell. As sodium ions enter, the accumulation of positive charge depolarizes the membrane. As a result, potassium is no longer in equilibrium and potassium ions leave the cell. As the depolarization progresses, the driving force for sodium influx decreases and that for potassium efflux increases. The process continues until the influx of sodium is exactly balanced by the efflux of potassium. At that point there is no further charge accumulation, and the membrane potential remains constant. In summary, the membrane potential lies between the potassium and sodium equilibrium potentials, and is the potential at which the sodium and potassium currents are exactly equal and opposite.

Chloride ions participate in the process as well, but as we have already seen, there is ultimately an adjustment in intracellular chloride concentration in the model cell so that the chloride equilibrium potential matches the new membrane potential. As the cation fluxes gradually reach a balance, the intracellular chloride concentration increases until there is no net chloride flux across the membrane.

The Constant Field Equation

To determine the exact membrane potential in our model cell we have to consider the individual ion currents across the membrane. The inward sodium current (i_{Na}) depends on the driving force for sodium ($V_m - E_{Na}$) (Chapter 2), and on the membrane conductance for sodium (g_{Na}). The conductance depends on the average number of sodium channels that are open in the resting membrane: The more open channels, the greater the conductance. So the sodium current is:

$$i_{Na} = g_{Na}(V_m - E_{Na})$$

This is also true for potassium and chloride:

$$i_K = g_K(V_m - E_K)$$

$$i_{Cl} = g_{Cl}(V_m - E_{Cl})$$

If we assume that chloride is in equilibrium, so that $i_{Cl} = 0$, then for the membrane potential to remain constant, the potassium and sodium currents must be equal and opposite:

$$g_K(V_m - E_K) = -g_{Na}(V_m - E_{Na})$$

It is useful to examine this relation in more detail. Suppose g_K is much larger than g_{Na}. Then, if the currents are to be equal, the driving force for potassium efflux must be much smaller than that for sodium entry. In other words, the membrane potential must be much closer to E_K than to E_{Na}. Conversely, if g_{Na} is relatively large, the membrane potential will be closer to E_{Na}.

By rearranging the equation we arrive at an expression for the membrane potential:

$$V_m = \frac{g_K E_K + g_{Na} E_{Na}}{g_K + g_{Na}}$$

If, for some reason, chloride is not at equilibrium, then chloride currents across the membrane must be considered as well, and the equation becomes slightly more complicated:

$$V_m = \frac{g_K E_K + g_{Na} E_{Na} + g_{Cl} E_{Cl}}{g_K + g_{Na} + g_{Cl}}$$

These ideas were developed originally by Goldman,[4] and independently by Hodgkin and Katz.[5] However, instead of considering equilibrium potentials and conductances, they derived an equation for membrane potential in terms of ion concentrations outside the cell ($[K]_o$, $[Na]_o$, $[Cl]_o$) and inside the cell ($[K]_i$, etc.), and membrane *permeability* to each ion (p_K, p_{Na}, and p_{Cl}):

$$V_m = 58 \log \frac{p_K[K]_o + p_{Na}[Na]_o + p_{Cl}[Cl]_i}{p_K[K]_i + p_{Na}[Na]_i + p_{Cl}[Cl]_o}$$

[4]Goldman, D. E. 1943. *J. Gen. Physiol.* 27: 37–60.

[5]Hodgkin, A. L., and Katz, B. 1949. *J. Physiol.* 108: 37–77.

As before, if chloride is in equilibrium, the chloride terms disappear. This equation is sometimes called the GHK equation after its originators and is known also as the **constant field equation** because one of the assumptions made in arriving at the expression was that the voltage gradient (or "field") across the membrane is uniform. It is entirely analogous to the conductance equation and makes the same predictions: When the permeability to potassium is very high relative to the sodium and chloride permeabilities, the sodium and chloride terms become negligible and the membrane potential approaches the equilibrium potential for potassium: $V_m = 58 \log ([K]_o/[K]_i)$. Increasing sodium permeability causes the membrane potential to move toward the sodium equilibrium potential.

The constant field equation provides us with a useful general principle to remember. The membrane potential depends on the relative conductances (or permeabilities) of the membrane to the major ions, and on the equilibrium potentials for those ions. In real cells the resting permeabilities to potassium and chloride are relatively high, so the resting membrane potential is close to the potassium and chloride equilibrium potentials. When sodium permeability is increased, as during an action potential (Chapter 6) or an excitatory postsynaptic potential (Chapter 9), the membrane potential moves toward the sodium equilibrium potential.

The Resting Membrane Potential

As useful as the constant field equation is, it does not provide us with an accurate description of the resting membrane potential. This is because the requirement for zero net current across the membrane is not, in itself, adequate: Our third requirement for the cell to remain in a stable condition—namely, that *each* individual ionic current must be zero—is not satisfied. As a result, the cell will gradually fill up with sodium and chloride and lose potassium. In real cells, intracellular sodium and potassium concentrations are kept constant by sodium–potassium ATPase (Chapter 4). To counteract the constant influx of sodium and the efflux of potassium, the pump transports a matching amount of each ion in the opposite direction (Figure 5.5). Thus, metabolic energy is used to maintain the cell in a **steady state.**

In order to have a more complete and accurate description of the resting membrane potential, we must consider both the passive ion fluxes and the activity of the pump. Again, we first consider the currents carried by passive fluxes of sodium and potassium across the membrane:

$$i_{Na} = g_{Na}(V_m - E_{Na})$$

$$i_K = g_K(V_m - E_K)$$

We no longer assume that the sodium and potassium currents are equal and opposite, but if we know how they are related we can, as before, obtain an equation for the membrane potential in terms of the sodium and potassium equilibrium potentials and their relative conductances. This is where the pump comes in. Because it keeps intracellular sodium and potassium concentrations constant by transporting the ions in the ratio of 3 Na to 2K (Chapter 4), it follows that the passive ion fluxes must be in the same ratio: $i_{Na}/i_K =$

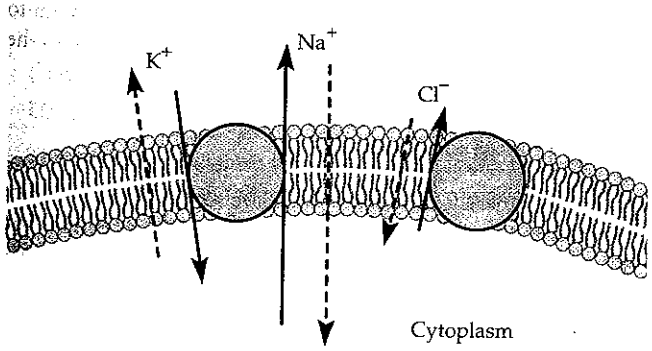


FIGURE 5.5 Passive Ion Fluxes and Pumps in a Steady State. Net passive ion movements across the membrane are indicated by dashed arrows, transport systems by solid arrows and circles. Lengths of arrows indicate the relative magnitudes of net ion movements. Total flux is zero for each ion. For example, the net inward leak of Na^+ is equal to the rate of outward transport. Na:K transport is coupled with a ratio of 3:2. In any particular cell, Cl^- transport may be outward (as shown) or inward.

3/2. So we can write

$$\frac{i_{Na}}{i_K} = \frac{g_{Na}(V_m - E_{Na})}{g_K(V_m - E_K)} = -1.5$$

The ratio is negative because the sodium and potassium currents are flowing in opposite directions. By rearranging, we get

$$V_m = \frac{1.5g_K E_K + g_{Na}E_{Na}}{1.5g_K + g_{Na}}$$

This equation is similar to the expression derived previously for the model cell, and it makes the same kinds of predictions: The membrane potential depends on the relative magnitudes of g_K and g_{Na}. The difference is that the potassium term is multiplied by a factor of 1.5. Because of this factor, the membrane potential is closer to E_K than would otherwise be the case. Thus, the driving force for sodium entry is increased, and that for potassium efflux is reduced. As a result the Na/K passive fluxes are in a ratio of 3:2 rather than 1:1.

In summary, the real cell differs from the model cell in that the resting membrane potential is the potential at which the passive influx of sodium is 1.5 times the passive efflux of potassium, rather than the potential at which the two fluxes are equal and opposite. The passive inward and outward currents are determined by the equilibrium potentials and conductances for the two ions; the required ratio of 3:2 is determined by the transport characteristics of the pump.

The problem of finding an expression for the resting membrane potential of real cells, taking into account the transport activity, was first considered by Mullins and Noda,[6] who used intracellular microelectrodes to study the effects of ionic changes on membrane potential in muscle. Like Goldman and Hodgkin and Katz, they derived an expression for membrane potential in terms of permeabilities and concentrations. The result is equivalent to the equation we have just derived using conductances and equilibrium potentials:

$$V_m = 58 \log \frac{rp_K[K]_o + p_{Na}[Na]_o}{rp_K[K]_i + p_{Na}[Na]_i}$$

where r is the absolute value of the transport ratio (3:2). The equation provides an accurate description of the resting membrane potential, provided all the other permeant ions (e.g., chloride) are in a steady state.

Chloride Distribution

How do these considerations apply to chloride? As for all other ions, there must be no net chloride current across the resting membrane. As already discussed (see Figure 5.2B), chloride is able to reach equilibrium simply by an appropriate adjustment in internal concentration, without affecting the steady-state membrane potential. In many cells, however, there are transport systems for chloride as well (Chapter 4). In squid axon and in muscle, chloride is transported actively into the cells; in many nerve cells active transport is outward (see Figure 5.5). The effect of inward transport is to add an increment to the equilibrium concentration such that there is an outward leak of chloride equal to the rate of transport in the opposite direction.[7] Outward transport has the reverse effect.

An Electrical Model of the Membrane

For those attuned to electrical diagrams, these considerations are summarized in Figure 5.6. E_{Na}, E_K, and E_{Cl} are represented by batteries, and the conductance pathways for sodium, potassium, and chloride by resistors. Passive ion currents through the resistors are equal and opposite to the corresponding currents generated by the pumps, so that the net current across the membrane for each ion is zero.

[6] Mullins, L. J., and Noda, K. 1963. *J. Gen. Physiol.* 47: 117–132.

[7] Matthews, G., and Wickelgren, W. O. 1979. *J. Physiol.* 293: 393–414, Appendix (by A. R. Martin).

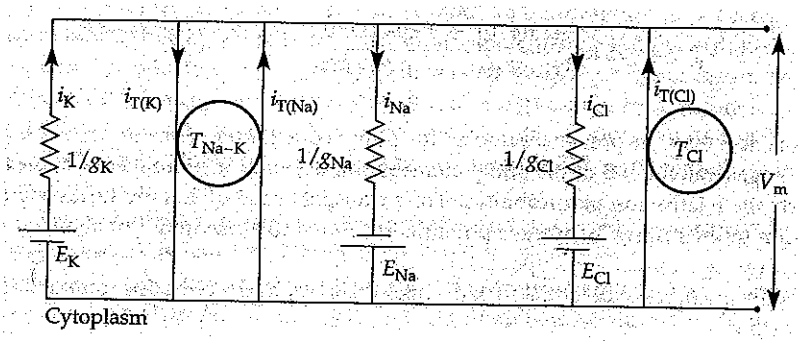


FIGURE 5.6 Electrical Model of the Steady-State Cell Membrane shown in Figure 5.5. E_K, E_{Na}, and E_{Cl} are the Nernst potentials for the individual ions. The individual ion conductances are represented by resistors (having a resistance of $1/g$ for each ion). The individual ion currents (i_K, i_{Na}, and i_{Cl}) are equal and opposite to the currents ($i_{T(K)}$, $i_{T(Na)}$, and $i_{T(Cl)}$) supplied by the sodium–potassium exchange pump (T_{Na-K}), and the chloride pump (T_{Cl}), so the net flux of each ion across the membrane is zero. The resulting membrane potential is V_m.

Predicted Values of Membrane Potential

How do these considerations explain the relation between potassium concentration and membrane potential shown in Figure 5.4? The answer becomes evident if we use real numbers in the equations. In squid axon, the permeability constants for sodium and potassium are roughly in the ratio 0.04:1.0.[5] We can use these relative values, together with the ion concentrations given in Table 5.1, to calculate the resting membrane potential in seawater:

$$V_m = 58 \log \frac{(1.5)(10) + (0.04)(460)}{(1.5)(400) + (0.04)(50)} = -73\,\text{mV}$$

Now we can see quantitatively why, when extracellular potassium is altered, the membrane potential fails to follow the Nernst potential for potassium, as shown in Figure 5.4. If, in the numerator of the equation, we look at the magnitude of the term involving extracellular potassium concentration ($1.5 \times 10 = 15$) and the term that involves extracellular sodium concentration ($0.04 \times 460 = 18.4$), we see that potassium contributes only about 45% to the total. Because of this, doubling the external potassium concentration does not double the numerator (as would happen in the Nernst equation), and as a consequence, the effect on the membrane potential of changing the extracellular potassium concentration is less than would be expected if potassium were the only permeant ion. When the external potassium concentration is raised to a high enough level (100 m*M* in Figure 5.4), the potassium term becomes sufficiently dominant for the relation to approach the theoretical limit of 58 mV per 10-fold change in concentration. This effect is enhanced by a factor discussed in Chapter 3: Many potassium channels are voltage-activated and open when the membrane is depolarized by increasing extracellular potassium. Because of the increased permeability to potassium, the relative contribution of sodium to the membrane potential is further reduced.

In general, nerve cells have resting potentials on the order of −70 mV. In some cells, such as vertebrate skeletal muscle,[8] the resting potential can be −90 mV or larger, reflecting a low ratio of sodium permeability to potassium permeability. Glial cells in particular have a very low permeability to sodium, so that their resting potentials are nearly identical to the potassium equilibrium potential (Chapter 8). Other cells, such as leech ganglion cells[9] and receptors in the retina,[10] have relatively high membrane permeabilities to sodium and resting membrane potentials as small as −40 mV.

Contribution of the Sodium–Potassium Pump to the Membrane Potential

The sodium–potassium transport system is **electrogenic** because each cycle of the pump results in the net outward transfer of one positive ion, thereby contributing to the excess negative charge on the inner face of the membrane. How large is this contribution? An easy way to find out is to calculate what the membrane potential would be if the pump were *not* electrogenic, or, in other words, if $r = 1$. Repeating the previous calculation with this condition yields the following:

[8]Fatt, P., and Katz, B. 1951. *J. Physiol.* 115: 320–370.

[9]Nicholls, J. G., and Baylor, D. A. 1968. *J. Neurophysiol.* 31: 740–756.

[10]Baylor, D. A., and Fuortes, M. G. F. 1970. *J. Physiol.* 207: 77–92.

$$V_m = 58 \log \frac{(1.0)(10) + (0.04)(460)}{(1.0)(400) + (0.04)(50)} = -67 \text{ mV}$$

This is 6 mV less than the previous value, so the pump contributes −6 mV to the resting potential. In general, the size of the pump contribution depends on a number of factors, particularly the relative ion permeabilities. For a transport ratio of 3:2, the steady-state contribution to the resting membrane potential is limited to a maximum of about −11 mV.[11] If the transport process is stopped, the electrogenic contribution disappears immediately, and the membrane potential then declines gradually as the cell gains sodium and loses potassium.

Ion Channels Associated with the Resting Potential

The resting permeabilities of membranes to sodium, potassium, and chloride have been determined in many nerve cells. It is a curious fact, however, that none of the channels underlying these resting permeabilities have been precisely identified in any specific cell. Candidates for potassium channels active in the resting membrane vary from one cell to the next. Among these are channels activated by intracellular cations: sodium-activated and calcium-activated potassium channels. In addition, many nerve cells have "M" potassium channels that are open at rest and closed by intracellular messengers (Chapter 16). Although it is unlikely that a large fraction of voltage-activated potassium channels ("delayed rectifier" and "A" channels) are open at rest, only 0.1 to 1% of the total number would be required to account for a substantial fraction of the resting conductance.[12]

The specific sources of the resting sodium permeability of nerve cells are also uncertain. A part can be attributed to the movement of sodium through potassium channels, most of which have a sodium/potassium permeability ratio that ranges between 1% and 3%.[13] In addition, both inward sodium and outward potassium fluxes may occur through cation channels that show little selectivity for potassium over sodium.[14,15] An additional sodium influx is through sodium-dependent secondary active transport systems (Chapter 4). Finally, tetrodotoxin has been shown to block a small fraction of the resting sodium conductance, indicating a contribution by voltage-activated sodium channels.[9]

Chloride channels of the CLC family (Chapter 3) are widely distributed in nerve and muscle. The presence of chloride channels is important in that they serve to stabilize the membrane potential (see the next section). The channels also interact with chloride transport systems to determine intracellular chloride concentrations.[16,17] When CLC channel expression is low—for example, in embryonic neurons in the hippocampus—E_{Cl} is positive with respect to the resting membrane potential because of inward transport and accumulation of chloride in the cytoplasm. In adult neurons, the expression of CLC channels increases the chloride conductance of the membrane so that excess accumulation cannot occur, and E_{Cl} becomes equal to the membrane potential. In central nervous system neurons, chloride channels can account for as much as 10% of the resting membrane conductance.[18]

CHANGES IN MEMBRANE POTENTIAL

It is important to keep in mind that the discussion of resting membrane potential is always in reference to *steady-state* conditions. For example, we have said that changing the extracellular chloride concentration has little effect on membrane potential because the intracellular chloride concentration accommodates to the change. This is true in the long run, but the intracellular adjustment takes time, and while it is occurring there will indeed be a transient effect.

The steady-state potential is the baseline upon which all changes in membrane potential are superimposed. How are such changes in potential produced? In general, transient changes, such as those that mediate signaling between cells in the nervous system, are the result of transient changes in membrane permeability. As we already know from

[11]Martin, A. R., and Levinson, S. R. 1985. *Muscle Nerve* 8: 354–362.

[12]Edwards, C. 1982. *Neuroscience* 7: 1335–1366.

[13]Hille, B. 1992. *Ionic Channels of Excitable Membranes*, 2nd Ed. Sinauer Associates, Sunderland, MA, p. 352.

[14]Yellen, G. 1982. *Nature* 296: 357–359.

[15]Chua, M., and Betz, W. J. 1991. *Biophys. J.* 59: 1251–1260.

[16]Staley, K., et al. 1996. *Neuron* 17: 543–551.

[17]Mladinić, M., et al. 1999. *Proc. R. Soc. Lond. B* 266: 1207–1213.

[18]Gold, M. R., and Martin, A. R. 1983. *J. Physiol.* 342: 99–117.

the constant field equation, an increase in sodium permeability (or a decrease in potassium permeability) will move the membrane potential toward the sodium equilibrium potential, producing depolarization. Conversely, an increase in potassium permeability will produce hyperpolarization. Another ion of importance in signaling is calcium. Intracellular calcium concentration is very low (Chapter 4), and in most cells E_{Ca} is greater than +150 mV. Thus, an increase in calcium permeability results in calcium influx and depolarization.

The role of chloride permeability in the control of membrane potential is of particular interest. As we have noted, chloride makes little contribution to the resting membrane potential. Instead, intracellular chloride concentration adjusts to the potential and is modified by whatever chloride transport mechanisms are operating in the cell membrane. The effect of a transient increase in chloride permeability can be either hyperpolarizing or depolarizing, depending on whether the chloride equilibrium potential is negative or positive with respect to the resting potential. This, in turn, depends on whether intracellular chloride is depleted or concentrated by the transport system. In either case, the change in potential is usually relatively small. Even so, the increased chloride permeability can be important for the regulation of signaling because it tends to hold the membrane potential near the chloride equilibrium potential and thus attenuates changes in potential that are produced by other influences.

This stabilization of the membrane potential is important for controlling the excitability of many cells, such as skeletal muscle fibers, that have a relatively high chloride permeability at rest. In such cells a transient influx of positive ions causes less depolarization than would otherwise be the case because it is countered by an influx of chloride through already open channels. Chloride channel mutations that reduce chloride conductance are responsible for several muscle diseases. The diseased muscles are hyperexcitable (myotonic) because of a loss of the normal stabilizing influence of a high chloride conductance.[19,20]

[19]Barchi, R. L. 1997. *Neurobiol. Dis.* 4: 254–264.

[20]Cannon, S. C. 1996. *Trends Neurosci.* 19: 3–10.

SUMMARY

■ Nerve cells have a high intracellular concentration of potassium and low intracellular concentrations of sodium and chloride, so that potassium tends to diffuse out of the cell, and sodium and chloride tend to diffuse in. The tendency for potassium and chloride to diffuse down their concentration gradients is opposed by the electrical potential across the cell membrane.

■ In a model cell permeable only to potassium and chloride, the concentration gradients can be balanced exactly by the membrane potential, so that there is no net flux of either ion across the membrane. The membrane potential is then equal to the equilibrium potential for both potassium and chloride.

■ Changing the extracellular potassium concentration changes the potassium equilibrium potential, and hence the membrane potential. Changing the extracellular chloride concentration, on the other hand, leads ultimately to a change in intracellular chloride, so that the chloride equilibrium potential and the membrane potential differ from their original values only transiently.

■ The plasma membranes of real cells are permeable to sodium, as well as to potassium and chloride. As a result, there is a constant passive influx of sodium into the cell, and an efflux of potassium. These fluxes are balanced exactly by active transport of the ions in the opposite directions, in the ratio of 3 sodium to 2 potassium. Under these circumstances, the membrane potential depends on the sodium equilibrium potential, the potassium equilibrium potential, the relative conductance of the membrane to the two ions, and the pump ratio.

■ Because the sodium–potassium exchange pump transports more positive ions outward than inward across the membrane, it makes a direct contribution of several millivolts to the membrane potential.

■ The chloride equilibrium potential may be positive or negative with respect to the resting membrane potential, depending on chloride transport processes. Although the chloride distribution plays little role in determining the resting membrane potential, a high chloride permeability is important for electrical stability.

SUGGESTED READING

Hodgkin, A. L., and Katz, B. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol.* 108: 37–77. (The constant field equation is derived in Appendix A of this paper.)

Junge, D. 1992. *Nerve and Muscle Excitation*, 3rd Ed. Sinauer Associates, Sunderland, MA, Chapters 1–3.

Mullins, L. J., and Noda, K. 1963. The influence of sodium-free solutions on membrane potential of frog muscle fibers. *J. Gen. Physiol.* 47: 117–132.

6 Ionic Basis of the Action Potential

THE IONIC MECHANISMS RESPONSIBLE FOR GENERATING action potentials have been described quantitatively by using the voltage clamp method to measure membrane currents. From such measurements it is possible to determine which components of the currents are carried by different ion species, and to deduce the magnitude and time course of the underlying changes in ionic conductances. Such experiments have shown that depolarization increases sodium conductance and, more slowly, potassium conductance. The activation of sodium conductance is transient, being followed by inactivation. The increase in potassium conductance persists for as long as the depolarizing pulse is maintained. The dependence of sodium and potassium conductances on membrane potential and their sequential timing account quantitatively for the amplitude and time course of the action potential, as well as for other phenomena, such as threshold and refractory period.

Patch clamp experiments have been used to examine the behavior of individual sodium and potassium channels associated with the action potential. The behavior of the channels is consistent with previous voltage clamp experiments on whole cells: Depolarization increases the probability that sodium and potassium channels will open. For both ion channels the increase in this probability follows the same time course as that of the corresponding voltage clamp currents. For example, sodium channels open most frequently near the beginning of a depolarizing pulse and openings then become less frequent as inactivation develops.

Other cation channels can be involved in action potential generation. In some cells, voltage-activated calcium channels are responsible for the rising phase of the action potential, and repolarization can involve activation of a variety of potassium channel types.

A. L. Hodgkin, 1949

A. F. Huxley, 1974

SODIUM AND POTASSIUM CURRENTS

In Chapter 5 we showed that the resting potential is determined mainly by the potassium concentration ratio (as postulated by Bernstein in 1902), but it is influenced as well by the concentration ratio of sodium and, to a lesser extent, by that of chloride. At the same time that Bernstein proposed his hypothesis about the nature of the resting potential, Overton[1] made the important discovery that sodium ions are necessary for nerve and muscle cells to produce action potentials, and he suggested (somewhat hesitantly) that the action potential might come about by sodium entering the cell. Further clarification of this idea came with experiments on the squid axon.

In 1939, Hodgkin and Huxley[2] showed that at the peak of the action potential there was an overshoot during which the membrane potential became transiently positive on the inside. This suggested that sodium was indeed involved, because sodium entry across the membrane would continue beyond zero membrane potential until the sodium equilibrium potential (E_{Na}) was reached. Ten years later, Hodgkin and Katz[3] showed that reducing external sodium concentration, and hence E_{Na}, produced corresponding reductions in the overshoot (Figure 6.1). They concluded that the action potential was the result of a large, transient increase in the sodium permeability of the membrane. We now know that this permeability increase is due to the opening of a large number of voltage-activated sodium channels.

What about the falling phase of the action potential? One might expect that the membrane potential would return to the resting level if the sodium channels simply closed. Indeed, this is one factor involved. If nothing else occurred, however, the return in most cells would be much slower than that observed experimentally. This is because the overall resting permeability of the membrane is relatively small, and consequently the loss of the accumulated positive charge through resting potassium and chloride channels would take several, or even tens of, milliseconds. The return to normal is very rapid because of a second large increase in membrane permeability—this time due to the opening of voltage-activated potassium channels. The membrane potential, having raced toward E_{Na}, now returns with almost equal rapidity toward E_K. The increase in potassium permeability can last for several milliseconds, so that in many cells the membrane is actually *hyperpolarized* beyond its normal resting potential for a time (see Figure 6.1).

To summarize, the action potential is the result of a sudden, large increase in sodium permeability of the membrane. The resulting inrush of sodium and accumulation of positive charge on the inner surface of the membrane drives the potential toward E_{Na}. Repolarization is accomplished by a subsequent large increase in potassium permeability, and loss of the accumulated positive charge, carried now by the efflux of potassium ions as the membrane returns toward E_K. Explanation of the mechanisms underlying generation of the action potential leads directly to understanding impulse propagation, discussed in Chapter 7.

[1]Overton, E. 1902. *Pflügers Arch.* 92: 346–386.

[2]Hodgkin, A. L., and Huxley, A. F. 1939. *Nature* 144: 710–711.

[3]Hodgkin, A. L., and Katz, B. 1949. *J. Physiol.* 108: 37–77.

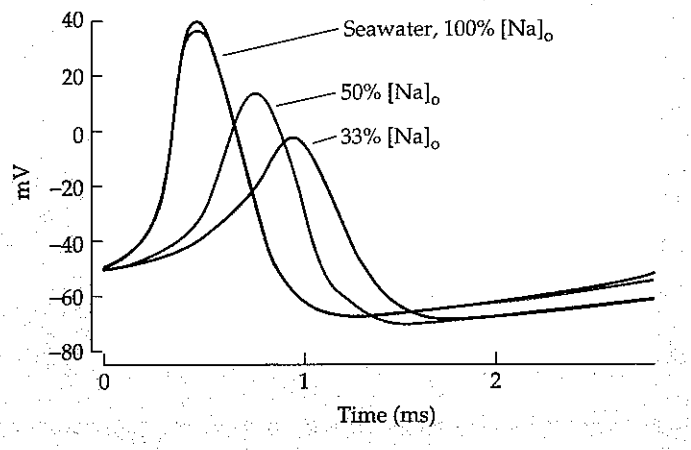


FIGURE 6.1 Role of Sodium in Action Potential Generation. Action potentials recorded from a squid axon bathed in seawater (blue), in solutions containing 50% (green) and 33% normal sodium (red), and then returned to seawater (orange). (After Hodgkin and Katz, 1949.)

How Many Ions Enter and Leave during an Action Potential?

If the interior of the nerve gains sodium during the rising phase of the action potential and loses potassium during the falling phase, then it follows that the sodium and potassium concentrations in the cytoplasm must change. The magnitude of the concentration change can be determined in two ways: by calculation and by direct measurement.

Calculation of the relation between membrane potential and charge separation by the membrane is discussed in detail in Chapter 7. At a membrane potential of −67 mV, about 4×10^{11} negative charges/cm^2 are collected on the inner surface of the membrane. At the peak of the action potential (+40 mV) these negative charges are replaced by 2.4×10^{11} positive charges, requiring an influx of 6.4×10^{11} sodium ions/cm^2. This is equivalent to about 10^{-12} mol/cm^2. Experimental measurements of radioactive sodium entering and radioactive potassium leaving the fiber during action potential activity[4] gave values in the range of 3×10^{-12} to 4×10^{-12} mol/cm^2.The values are higher than those calculated theoretically, largely because the calculation takes no account of the fact that the sodium and potassium fluxes overlap in time. Thus, the actual amount of sodium influx is greater than that required to charge the membrane to the peak of the action potential because potassium efflux (carrying charge in the opposite direction) begins before the peak is reached.

How does the sodium influx affect concentration? A 1 cm segment of squid axon, 1 mm in diameter, has a surface area of 0.31 cm^2, so that an influx of 3.5×10^{-12} mol/cm^2 into the segment amounts to about 10^{-12} mol of sodium. The same length of axon has a volume of 7.85×10^{-12} l, and contains (at 50 mmol/l) 4×10^{-7} mol of sodium, so that the influx changes the sodium concentration by only about 2.5 parts in a million. The same potassium efflux represents only about 3 parts in 10 million of the potassium content of the fiber.

Action potential activity in very small nerve processes can produce changes in intracellular ion concentrations that are more significant than those seen in large squid axons. For example, a nerve terminal 1 μm in diameter and 100 μm long has a surface area of 3×10^{-6} cm^2 and a volume of 8×10^{-14} l. During an action potential, an influx of 3.5×10^{-12} mol/cm^2, like that seen in squid axon, would result in about 10^{-17} mol of sodium accumulating in the terminal. At an intracellular concentration of 20 m*M*, the terminal contains about 1.5×10^{-15} mol, so a single impulse increases the intracellular sodium concentration by 0.7%. A rapid burst of 50 impulses would, in theory, increase the intracellular sodium concentration by 35%, with a corresponding reduction in intracellular potassium. Sodium influx accelerates the activity of the sodium–potassium exchange pump (Chapter 4) so that the concentrations are restored rapidly to their resting values.

Positive and Negative Feedback during Conductance Changes

The main feature underlying the ion currents associated with the action potential is that both the sodium and potassium conductances are voltage-dependent: The probability that the channels will open increases with depolarization. Depolarization increases the membrane conductance to sodium and, with a delay, to potassium as well. The effect on sodium conductance is **regenerative**: A small depolarization increases the number of open sodium channels; the resulting entry of sodium down its electrochemical gradient produces still more depolarization, opening more sodium channels, leading to still more rapid sodium entry, and so on (Figure 6.2A). This process of cumulative self-enhancement is known as **positive feedback**. In contrast, the voltage dependence of potassium conductance is self-limiting and involves **negative feedback** (Figure 6.2B). Depolarization increases the number of open potassium channels, resulting in the efflux of potassium down its electrochemical gradient. Rather than reinforcing the depolarization, the efflux leads to repolarization and return of the potassium conductance to its resting level.

Measuring Conductance

The ideas we have discussed so far were proposed by Hodgkin, Huxley, and Katz[5] and developed in detail by Hodgkin and Huxley,[6–9] who carried out and analyzed elegant electrophysiological experiments on the giant axon of the squid. They showed experimentally

[4]Keynes, R. D., and Lewis, P. R. 1951. *J. Physiol.* 114: 151–182.

[5]Hodgkin, A. L., Huxley, A. F., and Katz, B. 1952. *J. Physiol.* 116: 424–448.

[6]Hodgkin, A. L., and Huxley, A. F. 1952. *J. Physiol.* 116: 449–472.

[7]Hodgkin, A. L., and Huxley, A. F. 1952. *J. Physiol.* 116: 473–496.

[8]Hodgkin, A. L., and Huxley, A. F. 1952. *J. Physiol.* 116: 497–506.

[9]Hodgkin, A. L., and Huxley, A. F. 1952. *J. Physiol.* 117: 500–544.

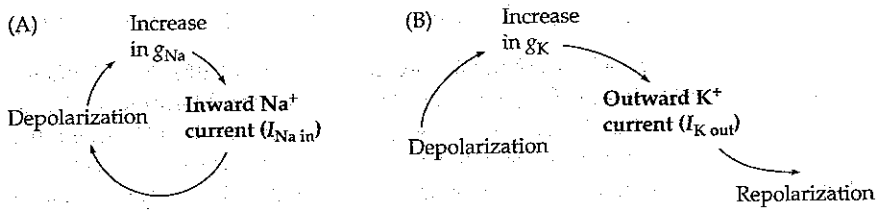


FIGURE 6.2 Effects of Increasing Conductances (g_{Na} and g_K) on membrane potential. (A) Sodium entry reinforces depolarization. (B) Potassium efflux leads to repolarization.

that changes in sodium and potassium conductances occurred, and that the changes were timed appropriately and were of the correct magnitude to account exactly for the magnitude and time course of the action potential.

What kinds of experiments were done to arrive at these conclusions? At first thought, it appears simple to obtain the appropriate measurements of conductance of the membrane to sodium (g_{Na}) or potassium (g_K). All that is needed is to measure the amount of current (I) flowing inward or outward across the membrane at various levels of potential (V_m), since

$$g_{Na} = \frac{I_{Na}}{(V_m - E_{Na})}$$

$$g_K = \frac{I_K}{(V_m - E_K)}$$

However, there are two problems to be solved before this approach becomes practical. The first is that current flowing across the membrane will change the membrane potential; this, in turn, will alter the membrane conductances. The solution was to devise a method for rapidly setting the membrane potential to any desired level and then holding it at that level while measuring the magnitude and time course of the membrane current. Because the voltage is fixed for the period of observation, the observed current will represent accurately the underlying changes in membrane conductance. The second problem is to separate the ionic components of the current so that their individual characteristics can be assessed. This has been accomplished in a number of ways, including the replacement of sodium with impermeant ions and, later, the use of selective toxins and poisons.

VOLTAGE CLAMP EXPERIMENTS

The voltage clamp was devised by Cole and his colleagues[10,11] and developed further by Hodgkin, Huxley, and Katz.[6] The experimental arrangement is described in Box 6.1. All we need to know to understand the experiments themselves is that the method permits us to set the membrane potential of the cell almost instantaneously at any level and hold it there ("clamp" it), while at the same time recording the current flowing across the membrane. Figure 6.3A shows an example of the currents that occur when the membrane potential is stepped suddenly from its resting value (in this example –65 mV) to a depolarized level (–9 mV). The current produced by the voltage step consists of three phases: (1) a brief outward surge lasting only a few microseconds, (2) an early inward current, and (3) a late outward current.

Capacitative and Leak Currents

The initial brief surge of current is the capacitative current, which occurs because the step from one potential to another alters the charge on the membrane capacitance. If the clamp amplifier is capable of delivering a large amount of current, then the membrane can be charged rapidly and this current will last only a very short time. Once the new potential is reached, there is no more capacitative current. In practice the surge of capacitative current lasts only about 20 μs and is followed by a small steady outward current.

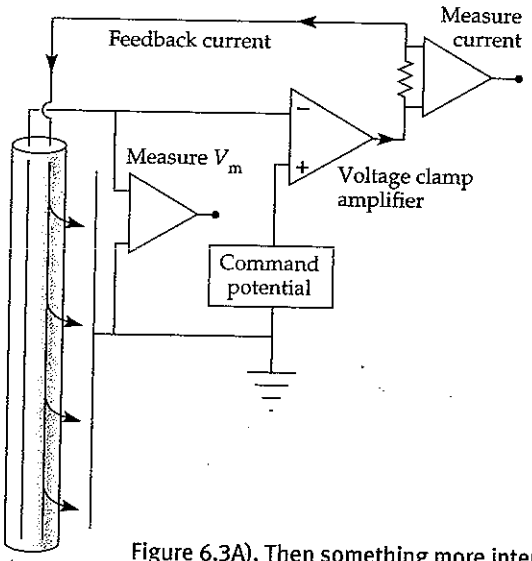
The steady outward current is through the resting membrane conductances and is known as leak current. Leak current is carried largely by potassium and chloride ions,

Box 6.1 The Voltage Clamp

The figure illustrates an experimental arrangement for voltage clamp experiments on squid axons. The axon is bathed in seawater, and into one end two fine silver wires are inserted longitudinally. One of the wires provides a measure of the potential inside the fiber with respect to that of the seawater (which is grounded) or, in other words, a measure of the membrane potential (V_m). It is also connected to one input of the voltage clamp amplifier. The other input is connected to a variable voltage source, which can be set by the person doing the experiment; the value to which it is set is thus known as the **command potential**. The voltage clamp amplifier delivers current from its output whenever there is a voltage difference between the inputs. The output current flows across the cell membrane between the second fine silver wire and the seawater (arrows); it is measured by the voltage drop across a small series resistor.

The circuit is arranged so that the output current tends to cancel any voltage difference between the two inputs, and it works as follows: Suppose that the resting potential of the fiber is –70 mV and the command potential is set to –70 mV as well. Because the voltages at the two inputs of the amplifier are equal, there will be no output current. If the command potential is stepped to, say, –65 mV, then because of the 5 mV difference between the inputs, the amplifier delivers positive current into the axon and across the cell membrane. The current produces a voltage drop across the membrane, driving V_m to –65 mV and removing the voltage difference between the two inputs. In this way the membrane potential is kept equal to the command potential. If the circuitry is properly designed, the change in V_m is achieved within a few microseconds.

Now suppose that the command potential is stepped from –70 to –15 mV. We would expect that the amplifier would deliver positive current to the axon to drive V_m to –15 mV. This is indeed what happens, but only transiently (see Figure 6.3A). Then something more interesting occurs. The depolarization to –15 mV produces an increase in sodium conductance, and there is a consequent flow of sodium ions *inward* across the membrane. In the absence of the clamp, this would tend to depolarize the membrane still further (toward the sodium equilibrium potential); with the clamp in place, however, the amplifier provides just the correct amount of negative current to hold the membrane potential constant. In other words, the current provided by the amplifier is exactly equal to the current flowing across the membrane. Here, then, is the great power of the voltage clamp: In addition to holding the membrane potential constant, it provides an exact measure of the membrane current required to do so. Voltage clamp measurements can now be made in small nerve cells by using the whole-cell method of patch clamp recording (Chapter 2).



varies linearly with voltage displacement from rest in either direction, and lasts throughout the duration of the voltage step. However, during most of the response it is obscured by much larger ion currents.

Currents Carried by Sodium and Potassium

Hodgkin and Huxley showed that the second and third phases of the current were due first to the entry of sodium and then to the exit of potassium across the cell membrane. In addition, they were able to deduce the relative size and time course of the separate currents. One convenient way was to abolish the sodium current by replacing most of the extracellular sodium by choline (an impermeant cation). With an appropriate reduction in extracellular sodium concentration, the sodium equilibrium potential could be made equal to the potential during the depolarizing step (–9 mV in Figure 6.3A). Consequently there was no net current through the activated sodium channels. This left only the potassium current, shown

[10]Marmont, G. 1940. *J. Cell. Comp. Physiol.* 34: 351–382.

[11]Cole, K. S. 1968. *Membranes, Ions and Impulses*. University of California Press, Berkeley.

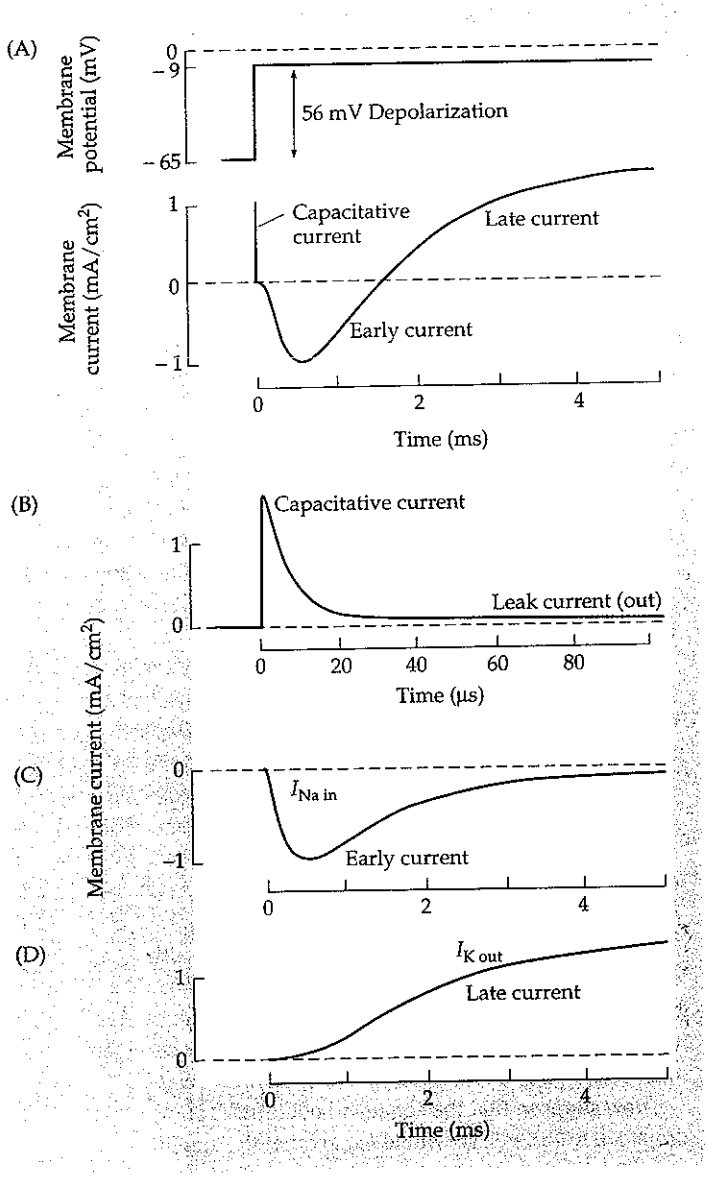


FIGURE 6.3 Membrane Currents produced by depolarization. (A) Currents measured by a voltage clamp during a 56 mV depolarization of a squid axon membrane. The currents (lower trace) consist of a brief positive capacitative current, an early transient phase of inward current, and a late, maintained outward current. These are shown separately in B, C, and D. The capacitative current (B) lasts for only a few microseconds (note the change in timescale). The small outward leak current is due to the movement of potassium and chloride. The early inward current (C) is due to sodium entry, the late outward current (D) to potassium movement out of the fiber.

in Figure 6.3D. Subtraction of the potassium current from the total ion current (Figure 6.3A) then revealed the magnitude and time course of the sodium current (Figure 6.3C).

Selective Poisons for Sodium and Potassium Channels

Since the original experiments of Hodgkin and Huxley, convenient pharmacological methods have been found for blocking sodium and potassium currents selectively. Tetrodotoxin (TTX) and its pharmacological companion saxitoxin (STX) have been particularly useful for blocking sodium channels (Chapter 3). TTX is a virulent poison, concentrated in the ovaries and other organs of puffer fish, whose potent effects have given rise to the Chinese proverb "To throw away life eat blowfish." Kao[12] has reviewed the fascinating history of TTX, beginning with the discovery of its effects by the Chinese emperor Shun Nung (2838–2698 B.C.), who personally tasted 365 drugs while compiling a pharmacopoeia and lived (for an amazingly long time) to tell the tale. STX is synthesized by marine dinoflagellates and concentrated by filter-feeding shellfish, such as the Alaskan butter clam *Saxidomus*. Its virulence competes with that of TTX: Ingestion of a single clam (cooked or not) can be fatal.

[12]Kao, C. T. 1966. *Pharmacol. Rev.* 18: 977–1049.

The great advantage of TTX for neurophysiological studies is that its action is highly specific. Working with squid axons, Moore, Narahashi, and colleagues showed that it blocks the voltage-activated sodium conductance selectively at concentrations of less than 1 μM.[13] When a TTX-poisoned axon is subjected to a depolarizing voltage step, no inward sodium current is seen, but only the outward potassium current (Figure 6.4A and B). The potassium current is unchanged in amplitude and time course by the poison. Application of TTX to the inside of the membrane by adding it to an internal perfusing solution has no effect. The actions of STX are indistinguishable from those of TTX. Both toxins appear to bind to the same site in the outer mouth of the channel through which sodium ions move, thereby physically blocking ion current through the channel.[14]

Just as TTX and STX block sodium channels selectively, a number of substances have been found that have similar effects on the voltage-activated potassium channels associated with the action potential. For example, in squid axons and in frog myelinated axons Armstrong, Hille, and others have shown that voltage-activated potassium currents are blocked by tetraethylammonium (TEA, in concentrations greater than 10 mM) (see Figure 6.4C).[15] In squid axon, TEA must be added to the internal solution, and exerts its action at the inner mouth of the potassium channel; in other preparations, such as the frog node of Ranvier, TEA is effective at an external site as well. Other compounds, such as 4-aminopyridine (4-AP) and 3,4-diaminopyridine (DAP), block potassium currents when applied in millimolar concentrations to either the inside or the outside of the membrane.

Dependence of Ion Currents on Membrane Potential

Having established that the early and late currents are due to sodium influx, followed by potassium efflux, Hodgkin and Huxley then determined how the magnitude and time course of the currents depend on membrane potential. Currents produced by various levels of depolarization from a holding potential of −65 mV are shown in Figure 6.5A. First, a step hyperpolarization to −85 mV (the bottom trace in Figure 6.5A) produces only a small inward current, as would be expected from the resting properties of the membrane. As already shown in Figure 6.3, moderate depolarizing steps each produce an early inward

[13]Narahashi, T., Moore, J. W., and Scott, W. R. 1964. *J. Gen. Physiol.* 47: 965–974.

[14]Hille, B. 1970. *Prog. Biophys. Mol. Biol.* 21: 1–32.

[15]Armstrong, C. M., and Hille, B. 1972. *J. Gen. Physiol.* 59: 388–400.

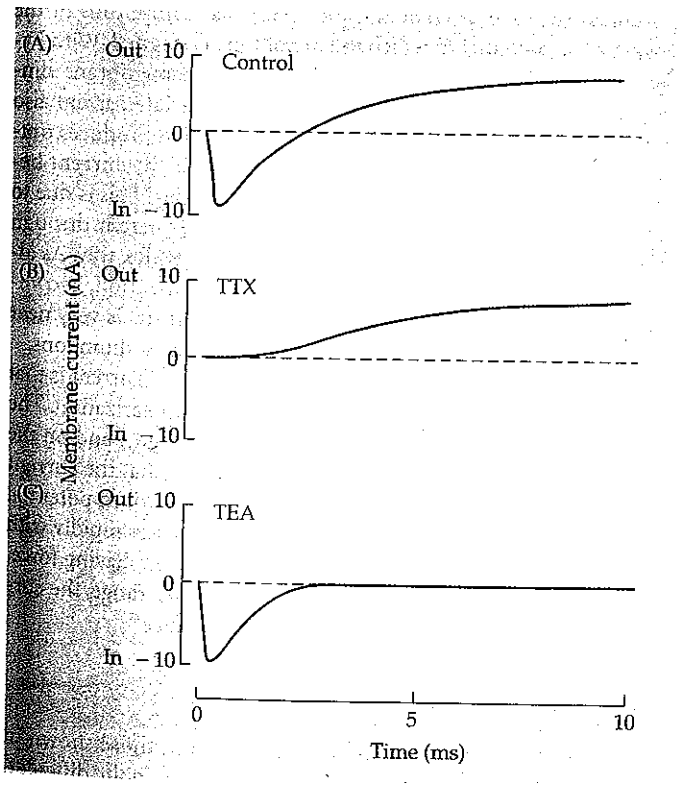


FIGURE 6.4 Pharmacological Separation of Membrane Currents into sodium and potassium components. Membrane currents were produced by clamping of the membrane potential to 0 mV in a frog myelinated nerve. (A) Control record in normal bathing solution. (B) The addition of 300 nM tetrodotoxin (TTX) causes the sodium current to disappear while the potassium current remains. (C) The addition of tetraethylammonium (TEA) blocks the potassium current, leaving the sodium current intact. (After Hille, 1970.)

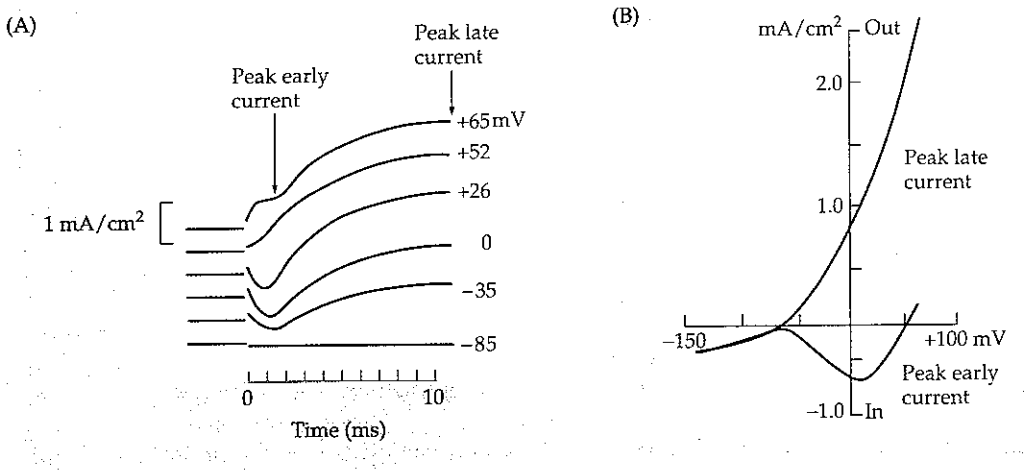


FIGURE 6.5 Dependence of Early and Late Currents on Potential. (A) Currents produced by voltage steps from a holding potential of −65 mV to a hyperpolarized level (−85 mV) and to successively increasing depolarized levels as indicated. The late potassium current increases as the depolarizing steps increase. The early sodium current first increases, then decreases with increasing depolarization; it is absent at +52 mV and reversed in sign at +65 mV. (B) Peak currents plotted against the potential to which the membrane is stepped. Late outward current increases rapidly with depolarization. Early inward current first increases in magnitude, then decreases, reversing to outward current at about +55 mV (the sodium equilibrium potential). (After Hodgkin, Huxley, and Katz, 1952.)

current followed by a sustained outward current. With greater depolarizations the early current becomes smaller, at about +52 mV it is absent, and as the depolarizing step is increased still further it reverses, becoming outward.

The current–voltage relations for the early and late currents are shown in Figure 6.5B, in which the peak amplitude of the early current and the steady-state amplitude of the late current are plotted against the potential to which the membrane is stepped. With hyperpolarizing steps there is no separation of early and late currents; the membrane simply responds as a passive resistor, with the expected inward current. The late current also behaves as one would expect of a resistor in the sense that depolarization produces outward current, but as the depolarization is increased the magnitude of the current becomes much greater than expected from the resting membrane properties. This is due to the voltage-activated potassium conductance, which allows additional current through the membrane. The early inward current behaves in a much more complex way. As already noted, it first increases and then decreases with increasing depolarization, becoming zero at about +52 mV and then reversing in sign. The reversal potential is very near the equilibrium potential for sodium, as expected for a current carried by sodium ions.

One point of interest in the current–voltage relation for the early inward current is that between about −50 and +10 mV the current increases with increasing depolarization. The magnitude of the sodium current depends on the sodium conductance (g_{Na}) and on the driving force for sodium entry ($V_m - E_{Na}$). One might expect, therefore, that the current will *decrease* as the membrane potential moves toward the sodium equilibrium potential and the driving force is reduced. However, the sodium conductance increases rapidly with depolarization (see Figure 6.7), and this increase outweighs the decrease in driving force. Thus, the sodium current—$i_{Na} = g_{Na}(V_m - E_{Na})$—increases. In this voltage range, the current–voltage relation is said to have a region of "negative slope conductance."

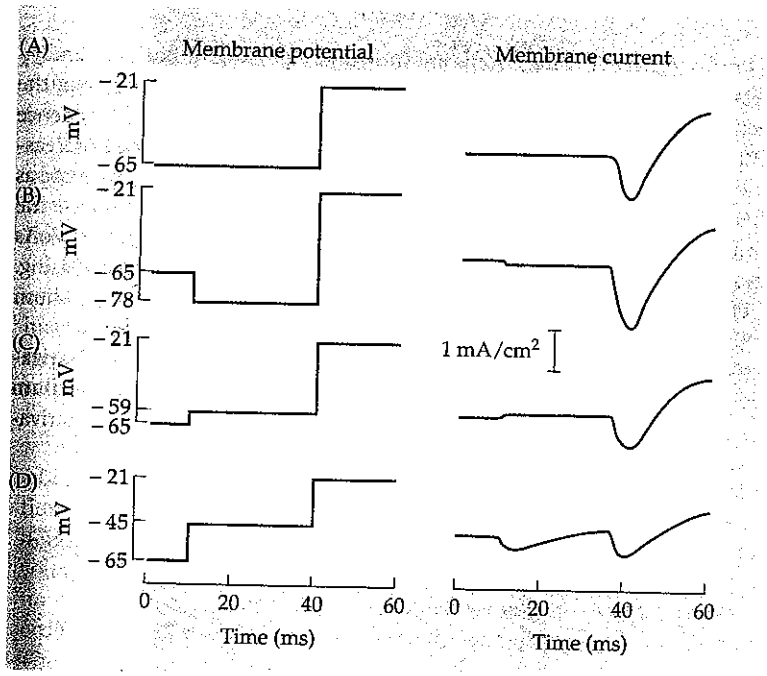
Inactivation of the Sodium Current

It is apparent from the experiments of Hodgkin and Huxley and from those shown in Figure 6.4 that the time courses of the sodium and potassium currents are quite different.

The potassium current is much delayed compared to the onset of the sodium current, but once developed it remains high throughout the duration of the step. The sodium current, on the other hand, rises much more rapidly but then decreases to zero, even though the membrane is still depolarized. This decline of the sodium current is called **inactivation.**

Hodgkin and Huxley studied the nature of the inactivation process in detail. In particular, they investigated the effect of hyperpolarizing and depolarizing prepulses on the peak amplitude of the sodium current produced by a subsequent depolarizing step. Records from such an experiment are shown in Figure 6.6. In Figure 6.6A the membrane is stepped from a holding potential of −65 to −21 mV, producing a peak sodium current of about 1 mA/cm^2. When the step is preceded by a hyperpolarizing prepulse of −13 mV, the peak sodium current is increased (Figure 6.6B). Depolarizing prepulses, on the other hand, cause a decrease in the sodium current (Figure 6.6C and D). The effects of hyperpolarizing and depolarizing prepulses are time-dependent; brief pulses of only a few milliseconds duration have little effect. In the experiment shown here, the prepulses are of sufficient duration (30 ms) for the effects to reach their maximum.

The results are shown quantitatively in Figure 6.6E, in which the peak sodium current is plotted against the potential during the prepulse. The peak current after a prepulse is expressed as a fraction of the control current. With a depolarizing prepulse to about −30



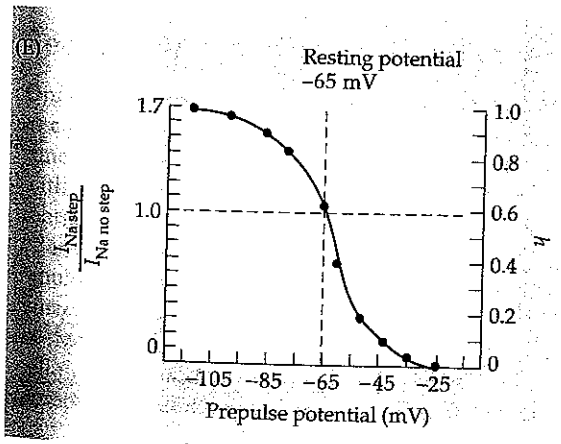


FIGURE 6.6 Effect of Membrane Potential on Sodium Currents. (A) A depolarizing step from −65 to −21 mV produces inward sodium current, followed by outward potassium current. (B) When the depolarizing step is preceded by a 30 ms hyperpolarizing step, the sodium current is increased. Prior depolarizing steps (C and D) reduce the size of the inward current. (E) The fractional increase or reduction of the sodium current as a function of membrane potential during the preceding conditioning step. The maximum current with a hyperpolarizing step to −105 mV is about 1.7 times larger than the control value. A depolarizing step to −25 mV reduces the subsequent response to zero. Full range of the sodium current is scaled from zero to unity by the *h* ordinate.

mV, the subsequent sodium current was reduced to zero; that is, inactivation was complete. Hyperpolarizing prepulses to −95 mV or beyond increased the sodium current by a maximum of about 70%. Hodgkin and Huxley represented this range of sodium currents from zero to their maximum value with a single parameter (h), varying between zero (complete inactivation) to 1 (no inactivation), as indicated on the right-hand ordinate of Figure 6.6E. In these experiments there was about 40% inactivation at the resting potential. Subsequent experiments have shown that all neurons show some degree of sodium channel inactivation at rest.

Sodium and Potassium Conductances as Functions of Potential

Having measured the magnitude and time course of sodium and potassium currents as a function of the membrane potential, V_m, and having determined the equilibrium potentials E_{Na} and E_K, Hodgkin and Huxley were then able to deduce the magnitude and time courses of the sodium and potassium conductance changes, using the relations noted earlier:

$$g_{Na} = \frac{I_{Na}}{(V_m - E_{Na})}$$

$$g_K = \frac{I_K}{(V_m - E_K)}$$

The results for five different voltage steps are shown in Figure 6.7A. Both g_{Na} and g_K increase progressively with increasing membrane depolarization. The time course of the sodium conductance is similar to that of the sodium current, but its voltage dependence is quite different (see Figure 6.5). The conductance increases progressively with increasing depolarization, whereas the current first increases and then decreases in magnitude as the voltage steps increase in amplitude. The current decreases progressively because the larger depolarizations come progressively closer to the sodium equilibrium potential. As a result, the inward current decreases, even though the sodium conductance is increasing. The relations between peak conductance and membrane potential are shown for sodium and potassium in Figure 6.7B. The curves are remarkably similar.

In summary, the results obtained by Hodgkin and Huxley indicated that depolarization of the nerve membrane leads to three distinct processes: (1) activation of a sodium conductance mechanism, (2) subsequent inactivation of the mechanism, and (3) activation of a potassium conductance mechanism.

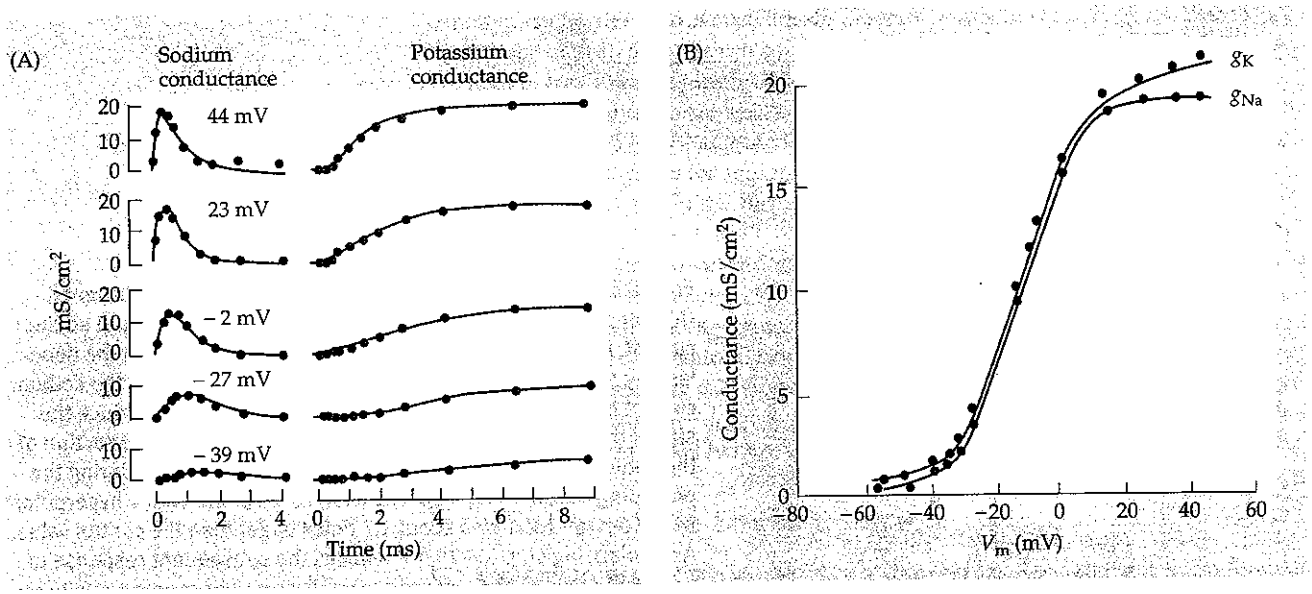


FIGURE 6.7 Sodium and Potassium Conductances. (A) Conductance changes produced by voltage steps from −65 mV to the indicated potentials. Peak sodium conductance and steady-state potassium conductance both increase with increasing depolarization. (B) Peak sodium conductance and steady-state potassium conductance plotted against the potential to which the membrane is stepped. Both increase steeply with depolarization between −20 and +10 mV. (After Hodgkin and Huxley, 1952b.)

Quantitative Description of Potassium and Sodium Conductances

After obtaining the experimental results, Hodgkin and Huxley proceeded to develop a mathematical description of the precise time courses of the sodium and potassium conductance changes produced by the depolarizing voltage steps. To deal first with the potassium conductance, one might imagine that the effect of a sudden change in membrane potential would be to provide a driving force for the movement of one or more charges in the voltage-activated potassium channel that would then lead to channel opening. If a single process were involved, the change in the overall potassium conductance might be expected to be governed by ordinary first-order kinetics; that is, its rise after the onset of the voltage step would be exponential.

Instead the onset of the potassium conductance change was found to be S-shaped, with a marked delay (see Figure 6.7A). Because of this delay, and because the potassium conductance increase occurred during depolarization but not hyperpolarization (i.e., it rectified), it was called the **delayed rectifier**. Hodgkin and Huxley were able to account for the S-shaped onset of the conductance by assuming that the opening of each potassium channel required the activation of four first-order processes—for example, the movement of four charged particles in the membrane. In other words, the S-shaped time course of activation could be fitted by the product of four exponential functions. The increase in potassium conductance for a given voltage step, then, was described by the relation

$$g_K = g_{K(max)} n^4$$

where $g_{K(max)}$ is the maximum conductance reached for the particular voltage step and n is a rising exponential function varying between zero and unity, given by $n = 1 - e^{-t/\tau_n}$.

The dependence of $g_{K(max)}$ on voltage is shown in Figure 6.7. The exponential time constant, τ_n, is also voltage-dependent: The increase in conductance becomes more rapid with larger depolarizing steps. At 10°C τ_n ranges from about 4 ms for small depolarizations to 1 ms for depolarization to zero.

The time course of the rise in sodium conductance, also S-shaped, was fitted by an exponential raised to the third power. In contrast, the fall in sodium conductance due to inactivation was consistent with a simple exponential decay process. For a given voltage step the overall time course of the sodium conductance change was the product of the activation and inactivation processes:

$$g_{Na} = g_{Na(max)} m^3 h$$

where $g_{Na(max)}$ is the maximum level to which g_{Na} would rise if there were no inactivation, and $m = 1 - e^{-t/\tau_m}$. The inactivation process is a falling, rather than a rising, exponential and is given by $h = e^{-t/\tau_h}$. As with the potassium conductance, $g_{Na(max)}$ is voltage-dependent, as are the activation and inactivation time constants. The activation time constant, τ_m, is much shorter than that for potassium, having a value at 10°C on the order of 0.6 ms near the resting potential, decreasing to about 0.2 ms at zero potential. The inactivation time constant, τ_h, on the other hand, is similar in magnitude to τ_n.

Reconstruction of the Action Potential

Once the empirical expressions were obtained for sodium and potassium conductances as a function of voltage and time, Hodgkin and Huxley were able to predict the entire time course of the action potential, and of the underlying conductance changes. Starting with a depolarizing step to just above threshold, they calculated what the subsequent potential changes would be at successive intervals of 0.01 ms. Thus, during the first 0.01 ms after the membrane had been depolarized to, say, −45 mV, they calculated how g_{Na} and g_K would change, what increments of I_{Na} and I_K would result, and then the effect of the net current on V_m. Knowing the change in V_m at the end of the first 0.01 ms, they then repeated the calculations for the next time increment, and so on all through the rising and falling phases of the action potential (a laborious exercise to undertake in the days before computers, or even electronic calculators, were available).

The calculations duplicated with remarkable accuracy the naturally occurring action potential in the squid axon. Calculated and observed action potentials produced by brief depolarizing pulses at three different stimulus strengths are compared in Figure 6.8A. To appreciate fully the magnitude of this accomplishment, it is necessary to keep in mind that the calculations used to duplicate the action potential were based on measurements of current that were made under completely artificial conditions with the membrane potential clamped first at one value, then at another.

The mechanisms of action potential generation are summarized in Figure 6.8B, which shows the calculated magnitude and time course of a propagated action potential in a squid axon, together with the calculated changes in sodium and potassium conductance.

Threshold and Refractory Period

In addition to describing the action potential, Hodgkin and Huxley were able to explain in terms of ionic conductance many other properties of excitable axons, such as **thresh-**

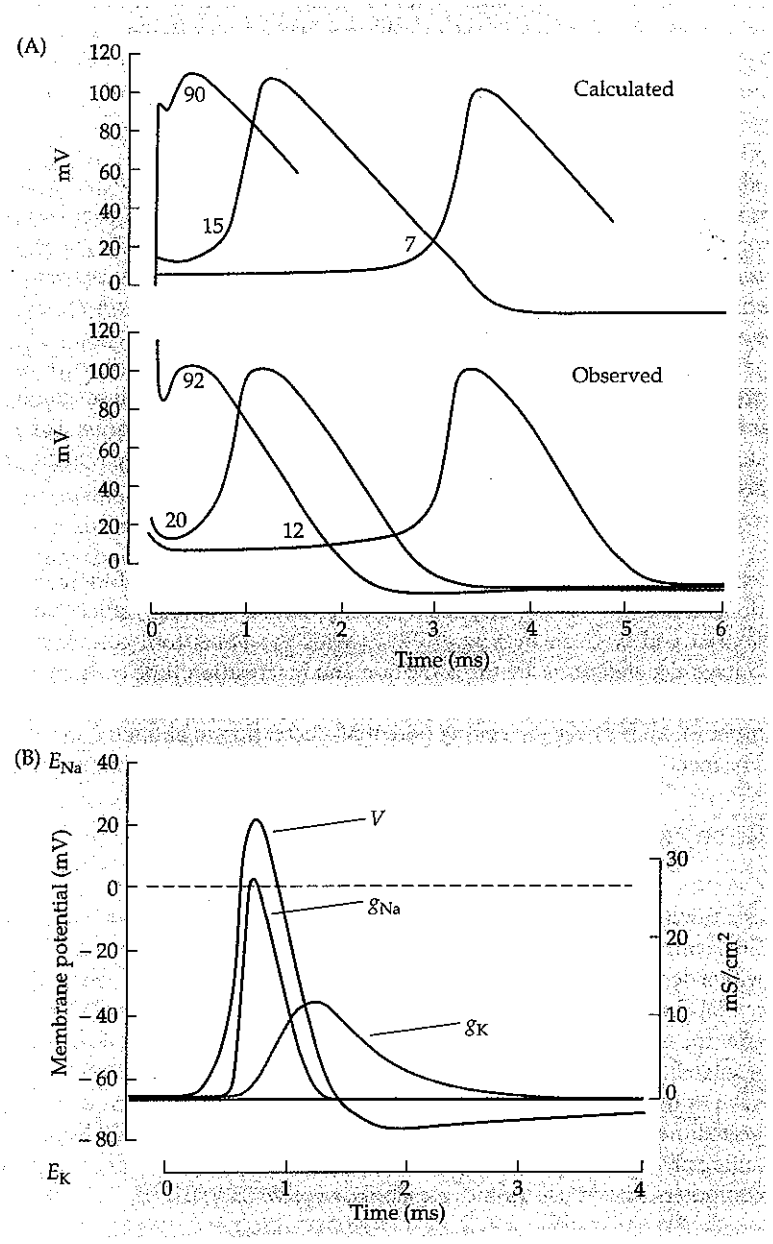


FIGURE 6.8 Reconstruction of the Action Potential. (A) Calculated action potentials produced by brief depolarizations of three different amplitudes (upper panel) are compared with those recorded under the same conditions (lower panel). (B) Relation between conductance changes (g_{Na} and g_K) and the action potential (V), calculated for a propagated action potential in a squid axon. (After Hodgkin and Huxley, 1952d.)

old and refractory period. Further, their findings have been found to be applicable to a wide variety of other excitable tissues.

How do the findings predict the threshold membrane potential at which the impulse takes off, especially when it might seem that a discontinuity like threshold would require a discontinuity in g_{Na} or g_K? The phenomenon can be understood if we imagine passing current through the membrane to depolarize it just to threshold, and then turning the current off. Because the membrane is depolarized, there will be an increase in outward current over that at rest (through potassium and leak channels). We will also have activated some sodium channels, increasing inward sodium current. At threshold the inward and outward currents are exactly equal and opposite, just as they are at rest. However, there is an important difference: The balance of currents is now unstable. If an extra sodium ion enters the cell, the depolarization is increased, g_{Na} increases, and more sodium enters. The outward current can no longer keep up with the sodium influx, and the regenerative process explodes. If, on the other hand, an extra potassium ion leaves the cell, the depolarization is decreased, g_{Na} decreases, sodium current decreases, and the excess outward current causes repolarization. As the membrane potential approaches its resting level, the potassium current decreases until it again equals the resting inward sodium current. Depolarization above threshold results in an increase in g_{Na} sufficient for inward sodium movement to swamp outward potassium movement immediately. Subthreshold depolarization fails to increase g_{Na} sufficiently to override the resting potassium conductance.

And how is the refractory period explained? Two changes develop during an action potential that make it impossible for the nerve fiber to produce a second action potential immediately: (1) Inactivation of sodium channels is maximal during the falling phase of the action potential and requires several more milliseconds to be removed. During this time few if any channels are available to contribute to an increase in g_{Na}. (2) Because of activation of potassium channels, g_K is very large during the falling phase of the action potential and decreases slowly back to its resting level. During this time a very large increase in g_{Na} is required to initiate any regenerative depolarization. These two factors result in an **absolute refractory period** lasting throughout the falling phase of the action potential during which no amount of externally applied depolarization can initiate a second regenerative response. This is followed by a **relative refractory period**, during which the threshold gradually returns to normal as sodium channels recover from inactivation and potassium channels close.

It was an extraordinary achievement for Hodgkin and Huxley to have provided rigorous quantitative explanations of such complex biophysical properties of membranes. Although subsequent observations on single channels have provided a new depth to our understanding of the underlying molecular mechanisms, by no stretch of the imagination would single-channel studies on their own, without the previous voltage clamp experiments and insights, have been able to account for how a nerve cell generates and conducts impulses. The older work has become enriched, rather than supplanted, by the new.

Gating Currents

Hodgkin and Huxley suggested that sodium channel activation was associated with the translocation of charged structures, or particles, within the membrane. Such charge movements would be expected to contribute to the capacitative current produced by a depolarizing voltage step. After a number of technical difficulties were resolved, such gating currents were finally seen.[16,17]

How is the gating current separated from the usual capacitative current expected with a step change in membrane potential (e.g., see Figure 6.3)? Briefly, currents associated merely with charging and discharging the membrane capacitance should be symmetrical. That is, they should be of the same magnitude for depolarizing steps as for hyperpolarizing steps. On the other hand, currents associated with sodium channel activation should appear upon depolarization of, say, 50 mV from a holding potential of −70 mV, but not upon hyperpolarization. In other words, if the channels are already closed, there should be no gating current upon further hyperpolarization. Similarly, gating currents associated with channel closing might be expected at the termination of a brief depolarizing pulse

[16]Armstrong, C. M., and Bezanilla, F. 1974. *J. Gen. Physiol.* 63: 533–552.

[17]Keynes, R. D., and Rojas, E. 1974. *J. Physiol.* 239: 393–434.

but not after a hyperpolarizing pulse. One experimental way of recording gating currents, then, is to sum the currents produced by two identical voltage steps of opposite polarity. The asymmetry due to gating currents is shown in parts a and b of Figure 6.9A. The current at the beginning of the depolarizing pulse is larger than that produced by the hyperpolarizing pulse because of the additional charge movement associated with gating of the sodium channel. When the two currents are added (part c of Figure 6.9A), the net result is the gating current (or "asymmetry current") alone.

An example of gating current in a squid axon, obtained by cancellation of the capacitative current, is shown in Figure 6.9B. Voltage-sensitive potassium currents were blocked with TEA. A step depolarization of perfused squid axon produced an outward gating current, followed by an inward sodium current. The sodium current was much smaller than usual because extracellular sodium concentration was reduced to 20% of normal. The gating current is shown alone in Figure 6.9C, after tetrodotoxin was added to the solution to eliminate the sodium current entirely (note the change in scale). The evidence that asymmetry currents observed in this way are, in fact, associated with sodium channel activation has been summarized by Armstrong.[18]

Activation and Inactivation of Single Channels

Patch clamp techniques have now provided detailed information about the way in which single sodium channels respond to depolarization. One experiment using this technique is illustrated in Figure 6.10. The records are from a cell-attached patch on a cultured rat muscle fiber.[19] To remove inactivation of sodium channels, a steady command potential was applied to the electrode, hyperpolarizing the patch membrane to −100 mV or so. On successive trials, a 40 mV depolarizing pulse was applied to the electrode for about 20 ms (part a of Figure 6.10B). In about one-third of the trials no sodium channels were activated. In the remainder, one or more single-channel currents appeared during the pulse, occurring most frequently near the onset of depolarization (part b of Figure 6.10B). The mean channel current was 1.6 pA. Assuming the sodium equilibrium potential to be +30 mV, the driving potential for sodium entry was about −90 mV; thus, the single-channel conductance was about 18 pS. This is comparable to sodium channel conductances mea-

[18]Armstrong, C. M. 1981. *Physiol. Rev.* 61: 644–683.

[19]Sigworth, F. J., and Neher, E. 1980. *Nature* 287: 447–449.

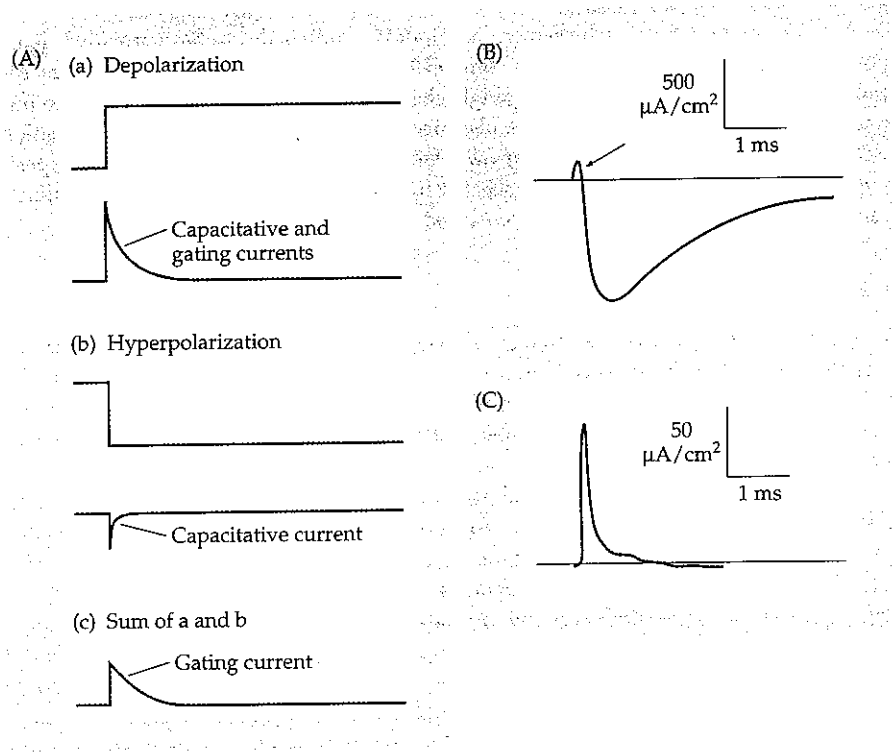


FIGURE 6.9 Sodium Channel Gating Current. (A) Method of separating gating current from capacitative current. A depolarizing pulse (a) produces capacitative current in the membrane, plus gating current. A hyperpolarizing pulse of the same amplitude (b) produces capacitative current only. When the responses to a hyperpolarizing and a depolarizing pulse are summed (c), capacitative currents cancel out and only gating current remains. (B) Record of current from a squid axon in response to a depolarizing pulse, after cancellation of capacitative current. Inward sodium current was reduced by lowering extracellular sodium to 20% of normal. The small outward current (arrow) preceding the inward current is the sodium channel gating current. (C) Response to depolarization from the same preparation after adding TTX to the bathing solution, recorded at higher amplification. Only the gating current remains. (B and C after Armstrong and Bezanilla, 1977.)

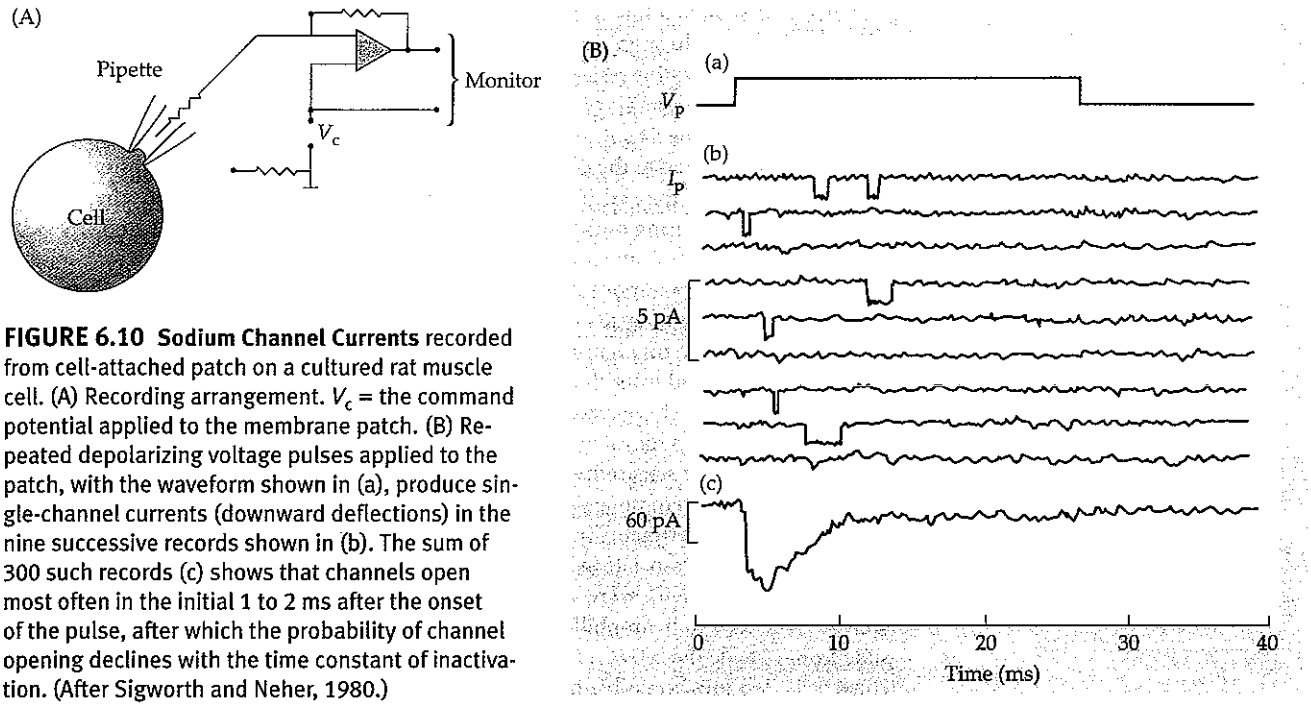


FIGURE 6.10 Sodium Channel Currents recorded from cell-attached patch on a cultured rat muscle cell. (A) Recording arrangement. V_c = the command potential applied to the membrane patch. (B) Repeated depolarizing voltage pulses applied to the patch, with the waveform shown in (a), produce single-channel currents (downward deflections) in the nine successive records shown in (b). The sum of 300 such records (c) shows that channels open most often in the initial 1 to 2 ms after the onset of the pulse, after which the probability of channel opening declines with the time constant of inactivation. (After Sigworth and Neher, 1980.)

sured in a variety of other cells. When 300 of the individual traces were summed (part c of Figure 6.10B), the result was an inward current that followed the same time course as that expected from the whole-cell sodium current.

A major point of interest in Figure 6.10 is that the mean channel open time (0.7 ms) is short relative to the overall time course of the summed current. Specifically, the time constant of decay of the summed current (about 4 ms) does not reflect the length of time that individual channels remain open. Instead, it indicates a slow decay in the probability of channel opening. The processes of activation (m^3) and inactivation (h) represent first an increase and then a decrease in the *probability* that a channel will open for a brief period. Their product (m^3h) describes the time course of the overall probability change. The probability increases rapidly near the beginning of the pulse, reaches a peak, and then decreases with time. In any given trial an individual channel may open immediately after the onset of the pulse, at any subsequent time during the pulse, or not at all.

A second point of interest is illustrated by referring again to Figure 6.9: The movement of charge is virtually complete in 0.5 ms, before sodium current reaches its peak—that is, before most of the channels have opened. This means that the conformational change in the channel protein associated with the charge movement is distinct from the conformational change associated with channel opening itself. Thus, it is not accurate to think of the charges as being connected with some kind of "handle" that opens a gate in the channel directly. Instead the charge movement simply increases the probability that the channel structure will enter the open (or the inactivated) state.

MOLECULAR MECHANISMS OF ACTIVATION AND INACTIVATION

Gating of Voltage-Activated Channels

Structural studies of voltage-activated channels suggest that channel gating occurs near the cytoplasmic end of the pore (Chapter 2). One question not yet answered is how the gate itself is coupled to changes in membrane potential. For voltage-activated gating to occur, there must be charged elements within the channel protein that are displaced by membrane depolarization. It is this charge displacement that is responsible for the gating

currents. One structure that has attracted particular interest in this regard is the S4 helix, which, as it winds through the plane of the membrane, contains a string of positively charged lysine or arginine residues located at every third position (Figure 6.11). This feature, which is highly conserved within the superfamily of voltage-activated channels, has led to the idea that the S4 helices comprise the voltage-sensing elements that link changes in membrane potential to the gating mechanism.[20] Thus, application of a positive potential to the inside of the cell membrane (depolarization) would displace the positive charges outward, causing outward movement of the helix (Figure 6.11B), and (by steps unknown) a consequent increase in the probability of channel opening.

To test this idea, mutations were directed at S4 regions of rat brain sodium channel.[21] Neutral or acidic amino acids were substituted for one or more of the basic residues to determine the effect on channel activation. It would be expected that when positive charges were removed from the helix a greater voltage change would then be required to produce a response. This expectation was realized for substitutions near the cytoplasmic end of the helix, but removal of charge from the extracellular half had the reverse effect—an increased voltage sensitivity. Similar equivocal results have been obtained with mutants in the S4 region of potassium A-channels.[22]

Biochemical experiments on mutated channels also support the idea that activation is accompanied by translation of the S4 segment.[23,24] In these experiments residues at either end of the helix were replaced by cysteine. The accessibility of the cysteine sulfhydryl groups to hydrophilic reagents was then tested (Chapter 3). Residues inaccessible from outside the cell at rest became accessible when the membrane was depolarized; conversely, residues accessible from the inside at rest became inaccessible upon depolarization, suggesting outward movement of the helix.

In summary, although the experimental evidence is incomplete, it seems safe to assume that outward movement of the S4 helix is the first step in the gating process. At rest, the internal negativity holds the helix toward the cytoplasmic end of the channel protein. When depolarization occurs, the reduction in internal negativity allows the helix to move outward. The movement of S4 then sets in motion additional conformational changes that ultimately allow the gate to open.

[20]Sigworth, F. J. 1994. *Q. Rev. Biophys.* 27: 1–40.

[21]Stühmer, W., et al. 1989. *Nature* 239: 597–603.

[22]Papazian, D. M., et al. 1991. *Nature* 349: 305–349.

[23]Yang, N., George, A. L., and Horn, R. 1996. *Neuron* 16: 113–122.

[24]Larsson, H. P., et al. 1996. *Neuron* 16: 387–397.

[25]Armstrong, C. M., Bezanilla, F., and Rojas, E. 1973. *J. Gen. Physiol.* 62: 375–391.

Sodium Channel Inactivation

Hodgkin and Huxley's experiments with prepulses suggested that inactivation was a distinct phenomenon, separable from the activation process. A subsequent experimental observation supporting this idea was that pronase, a mixture of proteolytic enzymes, when perfused through the inside of a squid axon, led to a delay in onset of inactivation and, eventually, to its abolition.[25] The enzyme was ineffective when applied in the same concentration to the outer surface. It appeared, then, that pronase had degraded a portion of the cytoplasmic end of the sodium channel associated specifically with the inactivation

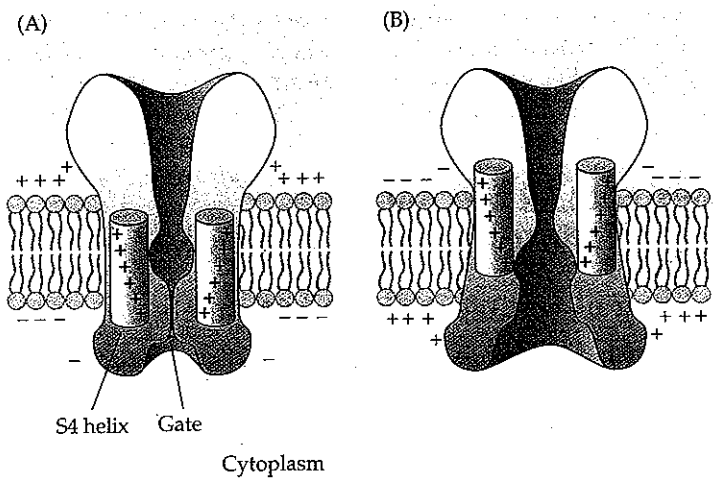


FIGURE 6.11 Proposed Shift of S4 Helices by membrane depolarization. Charged S4 helices are represented in two of the four domains of a voltage-activated sodium channel. (A) At the resting potential the helices are held against the inner end of the channel and the channel-gating elements are closed. (B) Depolarization causes outward movement of the positively charged helices, allowing the gate to open.

process. This led Armstrong and Bezanilla to propose a "ball and chain" model whereby an intracellular blocking particle (the ball), tethered to the cytoplasmic end of the channel by a flexible link (the chain), swings in to block the channel during inactivation.[26]

Experiments with sodium channels have identified the intracellular loop of amino acids between domains III and IV as being closely involved in the inactivation process. The loop is about 45 residues in length, and it is envisioned as a hairpin that swings into the inner vestibule of the channel to block the pore. In experiments with rat brain channels expressed in oocytes, three adjacent amino acid residues in the middle of the loop have been identified as essential for inactivation to occur.[27,28] When they were removed or replaced by site-directed mutagenesis, inactivation was severely attenuated or abolished. Similar experiments also identified groups of glycine and proline residues at either end of the loop involved in inactivation. These are assumed to be "hinge" regions that allow the hairpin to flip into the vestibule.[29]

Sodium channel activation and inactivation are also affected by a group of lipid-soluble toxins, including veratridine, an alkaloid from plants of the lily family, and batrachotoxin from the skin of South American frogs. They virtually eliminate inactivation so that the channels remain open indefinitely.[30] In addition, the voltage dependence of activation is shifted so that the channels are open at the normal resting potential.

Inactivation of Potassium A-Channels

The identification of an intracellular structure associated with inactivation was made first on potassium A-channels from *Drosophila* (Chapter 3) that, unlike delayed rectifier channels in squid axons, inactivate during maintained depolarization. Experiments on this channel provided evidence that a particular intracellular string of amino acids is associated with inactivation, and revived the "ball-and-chain" model proposed earlier for sodium channel inactivation. The model is illustrated in Figure 6.12. The ball is a clump of amino acids and the chain a string of residues tethering it to the main channel structure. Upon depolarization, the ball binds to a site in the inner vestibule of the channel, thereby blocking the pore.

This model of inactivation was tested in potassium A-channels by examining the behavior of channels formed in oocytes from mutant subunits (recall that the A-channel is a tetramer rather than a single polypeptide). Mutations and deletions were made in the 80 or so amino acids between the amino terminus and the first (S1) membrane helix.[31] Channels formed by mutant subunits with deletion of residues 6 through 46 showed virtually no inactivation, suggesting that some or all of these residues were involved in the normal inactivation process. When a synthetic peptide matching the first 20 amino acids in the N-terminal chain was simply added to the solution bathing the cytoplasmic face of the membrane, inactivation was restored with a linear dose dependence over the concentration range of 0 to 100 μM.[32] This amazing observation provides unusually strong support for the idea that in potassium A-channel subunits, the first 20 or so amino acid residues constitute a blocking particle responsible for inactivation of the channel. Because

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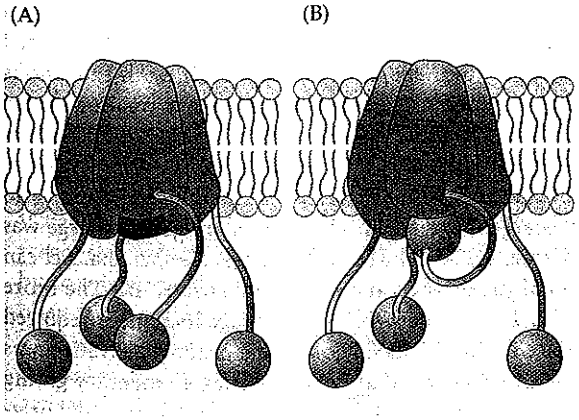


FIGURE 6.12 Ball-and-Chain Model of Inactivation of a voltage-activated potassium channel. The figure shows the complete channel, with one ball-and-chain element tethered to each of the four channel subunits. (A) Gating elements at the inner (cytoplasmic) end of the channel are open. (B) One of the four inactivation balls enters the inner vestibule to block the open channel.

it involves the N-terminal structure, this type of inactivation in potassium channels is often referred to as N-type inactivation. Some potassium channels also display a slower C-type inactivation, originally suspected to involve the carboxy terminus but later found to be related to structures near the outer mouth of the pore.[33,34]

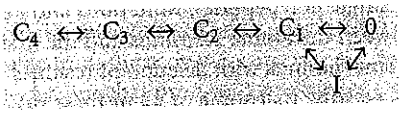
Kinetic Models of Channel Activation and Inactivation

From their observations that the time courses of activation of the sodium and potassium currents were best fitted by exponential functions raised to the third and fourth powers (m^3 and n^4), Hodgkin and Huxley suggested that activation could be explained by the independent displacement of three or four charged particles in the membrane. For example, we might imagine that a voltage step has to produce displacements of the S4 helices in all four domains of the potassium channel before the channel can open. Similarly, we might suppose that at least three such displacements are necessary for sodium channel activation. Further, we might suppose that in the sodium channel one or more of the displacements also leads ultimately to inactivation. A parallel model of this kind has been proposed by Keynes.[35,36]

The idea that four separate events (such as S4 helix displacements) are necessary for channel opening gives rise to the possibility of 16 different channel states: no displacement (one state), one displacement in any one of four domains (four possible states), two displacements in any two domains (six possible states), three displacements in any three (four possible states), and displacements in all four (one state). If the steps are independent and kinetically identical, then this reduces to five states: no displacement, displacement in any one domain, in any two, in any three, and in all four. On this basis, the transition from closed to open can be represented as follows:

$$C_4 \leftrightarrow C_3 \leftrightarrow C_2 \leftrightarrow C_1 \leftrightarrow O$$

where C_4 represents the state of the channel at rest, C_3 and so on represent a series of closed states into which the channel can be driven by depolarization, and O is the open state. For sodium channels we must add the inactivation process. Measurements of both macroscopic and single-channel currents suggest that the sodium channel can be inactivated whether or not it has opened previously.[37,38] Thus, inactivation (I) can occur both from the open state and from one or more of the closed states:



Many variations of this kind of model have been proposed with more or fewer steps and with more than one inactivated state.[39,40] They differ from the original model of Hodgkin and Huxley in the sense that activation and inactivation are envisioned as sharing a number of sequential events, rather than proceeding in parallel as independent processes. In addition, although progression through any number of the steps may depend on membrane potential, the final steps leading to activation and inactivation need not themselves be voltage-dependent.[41,42]

How many states really exist? This is not known for certain, but it appears that sodium channel activation involves at least three discrete charge displacements. Conti and Stühmer[43] reached this conclusion by measuring gating currents in large cell-attached membrane patches ("macro patches") on *Xenopus* oocytes injected with exogenous mRNA coding for rat brain sodium channels. The size of the elementary gating charge movement was deduced by measuring the mean and variance of a large number of individual gating currents. The procedure is analogous to using noise measurements for determining the size of single-channel currents (Chapter 2): The variance/mean ratio gives the size of the elementary charge movements. The elementary gating charge was calculated to be 2.3 electronic charges (2.3e). The total charge transfer per channel can be estimated from the steepness of the activation curve (see Figure 6.7B): The more charges there are on the affected structure, the smaller the voltage increment required to change its conformation. These considerations suggested that channel activation was associated with a charge transfer of 6e to 8e—that is, three of the elementary gating charges. This finding is remarkably consistent with the activation model proposed orig-

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inally by Hodgkin and Huxley. Conti and Stühmer note that it is attractive to identify the charge transfers with structural transitions in three of the four sodium channel domains, and to ascribe inactivation to interaction of these three with the fourth. Such findings, of course, take no account of additional state transitions that are electrically silent.

In summary, the kinetic models suggest that depolarization initiates a series of stepwise conformational changes that lead eventually to channel opening, with one or more alternate steps leading to inactivation. Although we can imagine in a very general way how such structural changes might occur in the protein, it is difficult to specify them precisely (Chapter 3). An initial step has been made in relating inactivation to particular groups of amino acids in the sodium channel and in potassium channel subunits. Further correlations will no doubt be forthcoming when the molecular anatomy is understood in greater detail.

Properties of Channels Associated with the Action Potential

The conductance of the voltage-activated sodium channel has been determined directly by patch clamp measurements to be about 20 pS in cultured rat muscle fibers (see Figure 6.10) and 14 pS in rat spinal motoneurons.[44] The density of sodium channels has been determined in a number of tissues by measuring the density of TTX-binding sites. Using tritiated tetrodotoxin, Levinson and Meves[45] estimated that in squid axon an average of 553 molecules were bound to each square micrometer of membrane. Values in other tissues have been found to range from a low of 2 molecules/μm^2 in neonatal rat optic nerve[46] to 2000 molecules/μm^2 at the node of Ranvier in rabbit sciatic nerve.[47] Sodium channel densities in skeletal muscle have been measured by depolarizing small areas of the membrane with a focal extracellular pipette and measuring inward sodium currents through the underlying membrane. The currents varied from one patch to the next, indicating variations in channel density. Sodium channels were found to be most concentrated near the end-plate region, and to decrease in density with distance from the end plates, reaching a low of about 10% of their maximum density near the tendons.[48] In addition, sodium channels in the muscle fiber membrane were found to be distributed in clusters rather than uniformly.[49]

The conductance of the potassium channels underlying the late current has been measured in cut-open squid axon.[50] Delayed rectifier channels were found to have conductances of 10, 20, and 40 pS, with the 20 pS channel predominating. Potassium channels in frog muscle, like sodium channels, are distributed in clusters.[36] However, the sodium and potassium channel clusters are not co-localized. Delayed rectifier channels appear to be totally absent at nodes of Ranvier in rabbit myelinated nerve, since depolarization produces no late outward current.[51] During the action potential, repolarization is achieved by a large leak current after rapid inactivation of the sodium channels.

Other Potassium Channels Contributing to Repolarization

In addition to potassium channels associated with the delayed rectifier current, neurons have a number of voltage-activated potassium channel types,[52] some of which can contribute to action potential repolarization. One voltage-activated channel is the potassium A-channel, which is activated rapidly by depolarization. The contribution of A-channels to action potential repolarization is minimal for two reasons: They inactivate rapidly, and in most cells activation occurs only after a preceding hyperpolarization; that is, they are usually inactivated at rest. Two other voltage-sensitive potassium channels, M-channels (Chapter 16) and S-channels, are similar to delayed rectifier channels in that they open in response to depolarization. M-channels have the additional feature of being inactivated by acetylcholine through *muscarinic* ACh receptors (hence their name). S-channels are open at the resting membrane potential and inactivated by *serotonin*.

Calcium-activated potassium channels can also contribute to action potential repolarization.[53] During the action potential, calcium ions enter the cell through voltage-activated calcium channels (see the next section). In many cells this inward calcium cur-

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rent stimulates an increase in potassium conductance that contributes to repolarization and produces a subsequent hyperpolarization. The calcium-activated potassium channels have at least three subtypes with very large (200 pS), intermediate (30 pS), and small (10 pS) conductances. Their presence can be demonstrated experimentally by raising intracellular calcium—for example, by injection from an intracellular micropipette.[54] Following such an injection the membrane conductance of the cell increases rapidly and the resting membrane potential approaches the equilibrium potential for potassium. The resistance and potential then return to their control levels as the excess calcium is removed from the cytoplasm by internal buffering mechanisms and outward transport. Still other potassium channels are activated by intracellular sodium.[55,56] Their activation by sodium influx during the action potential may contribute to repolarization in some cells.[57]

THE ROLE OF CALCIUM IN EXCITATION

Calcium Action Potentials

The membranes of nerve and muscle fibers contain a variety of voltage-activated calcium channels (see Chapter 3 for calcium channel classifications and properties). Calcium ions enter the cell through such channels during the action potential, and this entry plays a key role in a variety of processes (Chapters 9 through 12). For example, a transient increase in intracellular calcium during the action potential is responsible for secretion of chemical transmitters by neurons and for contraction of muscle fibers.

In some muscle fibers and some neurons, calcium currents become sufficiently large to contribute significantly to, or even be solely responsible for, the rising phase of the action potential. Because g_{Ca} increases with depolarization, the process is a regenerative one, entirely analogous to that discussed for sodium. The participation of calcium in the action potential process was first studied in invertebrate muscle fibers by Fatt and Ginsborg[58] and subsequently by Hagiwara.[59] Calcium action potentials occur in cardiac muscle, in a wide variety of invertebrate neurons, and in neurons in the vertebrate autonomic and central nervous systems.[60] Such action potentials occur in nonneural cells as well, including a number of endocrine cells and some invertebrate egg cells. The voltage-dependent calcium currents can be blocked by adding millimolar concentrations of cobalt, manganese, or cadmium to the extracellular bathing solution. Barium can substitute for calcium as the permeant ion; magnesium, on the other hand, cannot. A particularly striking example of the coexistence of sodium and calcium action potentials in the same cell is found in the mammalian cerebellar Purkinje cell, which generates sodium action potentials in its cell body and calcium action potentials in the branches of its dendritic tree.[61,62]

Calcium Ions and Excitability

Calcium ions also affect excitation: A reduction in extracellular calcium increases the excitability of nerve and muscle cells; conversely, increasing extracellular calcium decreases excitability. Frankenhaeuser and Hodgkin[63] used voltage clamp experiments to examine these effects in the squid axon and found that when extracellular calcium was reduced, the voltage dependence of sodium channel activation was shifted so that smaller depolarizing pulses were required to reach threshold and to produce sodium currents equivalent to those in normal solution. The reduction in depolarizing pulse amplitudes was constant throughout the range of excitation and depended on calcium concentration. A fivefold reduction in extracellular calcium resulted in a 10 to 15 mV reduction in the required depolarization.

Frankenhaeuser and Hodgkin suggested that the effect might be associated with screening by calcium ions of negative charges fixed to the outer surface of the membrane. Such charges at the membrane surface can arise, for example, by glycosylation of membrane proteins with carbohydrate chains that include negatively charged sialic acid. The eel sodium channel itself has over 100 sialic acid residues.[64] As long as the charges were screened, the potential gradient across the membrane would be the same as the measured

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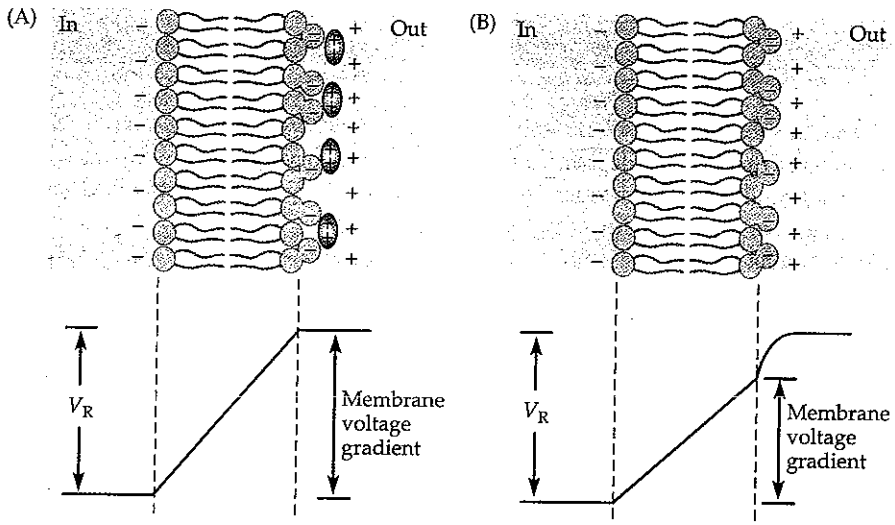

FIGURE 6.13 Effect of Surface Charge on Membrane Potential, proposed to explain the effects of calcium on action potential threshold. (A) The membrane structure includes negatively charged elements on the outer surface whose charge is neutralized by divalent cations. The resting membrane potential, V_R, produced by ionic charge separation, is determined by the composition of the intracellular and extracellular fluids. (B) When the fixed negative surface charges are unscreened (e.g., by removing calcium from the extracellular solution) the resting potential is unchanged, but the shape of the potential profile is altered by the surface negativity, reducing the potential gradient across the membrane.

resting potential (Figure 6.13A). Upon removal of calcium the unscreened charges would add an increment of negative potential to the outer surface, thereby reducing the potential gradient across the membrane (Figure 6.13B). This idea introduces a new concept with regard to membrane potential: The potential between the intracellular and extracellular solutions is determined by extracellular and intracellular ion concentrations and ion permeabilities, as discussed in Chapter 5. However, the shape of the potential gradient may depend on charged molecules fixed to the membrane surface. This can have a pronounced effect on voltage-sensitive components in the membrane, since they sense only the voltage gradient in their immediate vicinity.

One problem with the surface charge idea is that reducing the potential gradient across the membrane by removing extracellular calcium should affect not only activation but also inactivation of sodium channels, as well as activation of potassium channels. A reduction in the depolarization required for sodium channel inactivation and potassium channel activation should lead to a *decrease* in excitability. In fact, for reasons that are not clear, calcium removal has a much smaller effect on these parameters than on sodium activation.[64,65] It is perhaps not surprising that local changes in the potential gradient across the membrane should have varying effects on different channel molecules, or even on different regions of the same molecule, depending on the location of the voltage-sensitive element relative to the surface charges. Whatever the reason for these disparities, the net effect of calcium is to stabilize the membrane, maintaining a margin of safety between the resting membrane potential and the threshold for action potential initiation.

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SUMMARY

■ The action potential in most nerve cell membranes is produced by a transient increase in sodium conductance that drives the membrane potential toward the sodium equilibrium potential, followed by an increase in potassium conductance that returns the membrane potential to its resting level.

■ The increases in conductance occur because sodium and potassium channels in the membrane are voltage-dependent: Their opening probability increases with depolarization.

■ Voltage clamp experiments on squid axons have provided detailed information about the voltage dependence and time course of the conductance changes. When the cell membrane is depolarized, the sodium conductance is activated rapidly, then inactivated. Potassium conductance is activated with a delay and remains high as long as the depolarization is maintained.

■ The time course and voltage dependence of the sodium and potassium conductance changes account precisely for the amplitude and time course of the action potential, as well as other phenomena, such as activation threshold and refractory period.

■ The activation of sodium and potassium conductances by depolarization requires, in theory, charge movements within the membrane. Appropriate charge movements, called gating currents, have been measured.

■ Patch clamp experiments on voltage-activated sodium and potassium channels are consistent with voltage clamp experiments and reveal new details about the process of excitation. For example, sodium channels open for a relatively short time, and the probability that they open during a depolarizing step first increases and then decreases, corresponding to activation and inactivation of sodium conductance in the whole cell. Various kinetic models have been proposed for channel activation and inactivation.

■ Calcium plays an important role in excitation. In some cells calcium influx, rather than sodium influx, is responsible for the rising phase of the action potential. In addition, membrane excitability is controlled by extracellular calcium concentration. As extracellular calcium decreases, excitability increases.

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