CHAPTER 1

# 1 Bioelectricity

The movement of selected ions across biological membranes generates changes in the intracellular environment that, either directly or indirectly, result in the contraction of the muscle cell. This passage of ions can be studied from a variety of perspectives. A practical approach is to take advantage of the fact that ions carry an electrical charge. As such, the flow of ions across cell membranes can be studied using equipment designed to measure electrical flow, and the properties of excitable membranes can be modeled after the behavior of electric devices. In fact, the subject of electrophysiology is borne out, to a certain extent, by the similarities that can be established between the flow of ions across membranes and the behavior of electrical currents moving through cables. As an introduction to the subject of cardiac electrophysiology, we will first define some basic concepts of bioelectricity to establish the fundamental principles that govern electric currents across cell membranes.

## On the Electricity of Biological Membranes

### Charge

Most elements in nature tend to maintain an equal number of protons and electrons. However, occasionally electrons are transferred more or less permanently from one element to another, thus creating an imbalance. For example, sodium, potassium, and chloride ions have an unequal number of protons and electrons. This imbalance turns the element into a charged particle. Particles that are charged positively are called cations. Negatively charged particles are called anions. The unit of charge is the coulomb (C). Electricity is created by the attraction of charged particles of opposite sign.

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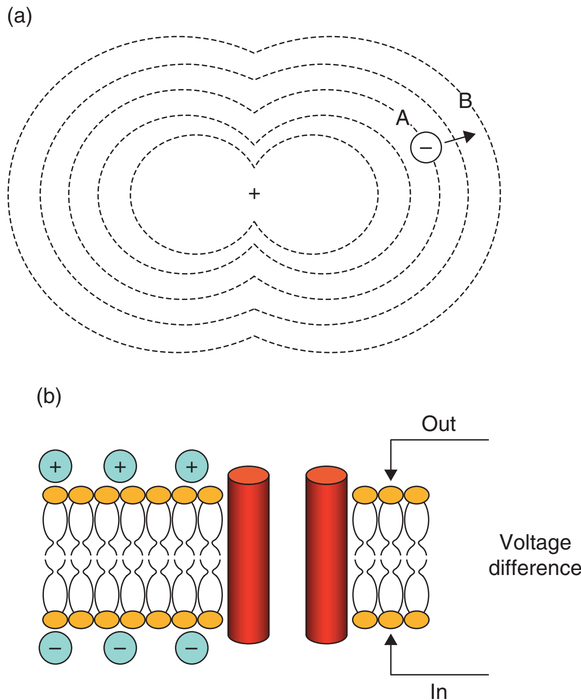



**Figure 1.1** Voltage difference. (a) Dotted lines represent the distribution of an electric field around a positive charge. Voltage is the work involved in moving a charge along the electric field (e.g., from point A to point B). (b) The voltage difference across the cell membrane results from the uneven distribution of charges between the inside and the outside of the cell.

## Voltage Difference

The attracting (or repelling) force generated by a charged particle in space is called the electric field. If a negative charge is free to move within a given electric field, it will be strongly attracted to a positive charge, and the field will eventually become electroneutral. There is therefore a certain amount of work involved in keeping the negative particle from rejoining its positive counterpart. More formally, we say that the work involved in moving a charge from point "A" to point "B" in an electric field (Figure 1.1a) is called potential difference (or voltage difference). In more practical terms, from the point of view of the electrophysiologist, potential differences are created when charges accumulate unequally across an insulator. For example, a potential difference is created across the membrane of cardiac myocytes because more anions are present inside than outside the cell (Figure 1.1b).

## Current

As illustrated in Figure 1.2a, when the two ends of a source of voltage are separated, the potential difference is maintained. If a conductive pathway is

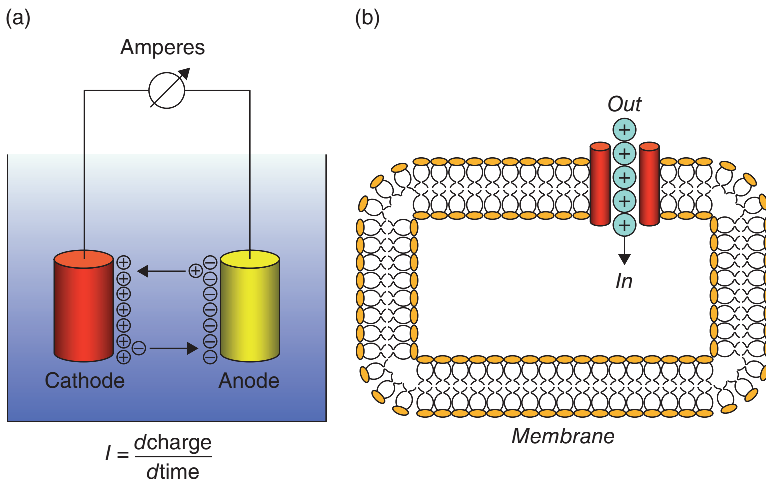



**Figure 1.2** Concept of electric current. (a) If a positively charged and a negatively charged electrode (anode and cathode, respectively) are placed inside a conductive medium, charge will flow from one to the other along the gradient. Current is defined as the magnitude of the charge that moves along a cross section of the conductor per unit time. (b) Hydrophilic channels allow for the flow of charge through cell membranes. The direction of the current follows the direction of the positive charges.

placed between them, charge will flow from the positive to the negative end. The negative plate will attract cations and is therefore referred to as the cathode (red). Conversely, the positively charged plate, which attracts anions, is called the anode (yellow). This movement of charges along a conductor is known as electric current. The unit of measure for electric current is the ampere (A). Current is more formally defined as the amount of charge passing through a conductor per unit time. By convention, positive current refers to the movement of cations toward the cathode.

In the cardiac cell (as in most living cells, for that matter) ions are constantly moving across the membrane, thus generating electric current. Ionic current is conceptualized as the flow of charge moving through selective hydrophilic pores or channels (Figure 1.2b). In the past, ion channels were studied only as functional entities, without any clear structural or biochemical correlate. Nowadays we know that channels are formed by integral membrane proteins that traverse the lipid bilayer and form a pathway for the transfer of selected ions between the intra- and extracellular spaces (see Chapter 2). Channels are conceptualized as electric resistors that connect the intra- and extracellular compartments. In the following section, we will describe the basic behavior of resistors in electric circuits. These concepts should be helpful in our subsequent review of the electrophysiological properties of the various ion channels in the membrane of the cardiac cell.

## Resistance

### Ohmic Resistors

All conductors offer a certain resistance ($R$) to the flow of current (if the flow of a fluid is used as an analogy, it can be said that a hose offers resistance to the flow of water). The unit of resistance is the ohm (Ω). Often, the properties of conductors are defined not by their resistance, but by their conductance. Conductance ($G$) is simply the inverse of resistance (i.e., $G = 1/R$) and it is expressed in siemens (S). The simplest resistors are those whose behavior is independent of time or voltage. These resistors are called "ohmic" because they follow Ohm's law:

$$I = V/R \tag{1.1}$$

where $I$ is current and $V$ and $R$ represent the magnitude of the voltage difference and the resistance, respectively. Ohm's law establishes that, given an increase in voltage across a constant resistance, there would be a linear increase in the amplitude of the current flowing through the circuit. Moreover, in an ohmic resistor, the time course of the change in current should be equal to the time course of the change in voltage. An example is illustrated in Figure 1.3. As shown by the simple circuit in panel (a), when a resistor ($R$) is placed between the anode and the cathode of a source of voltage (i.e., a battery), and the circuit is then closed by a switch, current flows toward the cathode. Moreover, a sudden increase in voltage induces an equivalent step in the amplitude of the current, as illustrated in panel (b). The bottom tracings represent three superimposed positive voltage steps of different magnitudes. The top tracings show recordings of the current flowing through the circuit in response to each voltage step. Thus, if current is plotted as a function of voltage (panel c), a linear function, of slope $1/R$ (or G) is obtained. This linear current–voltage ($I$–$V$) relation would be the same for both positive and negative voltage steps.

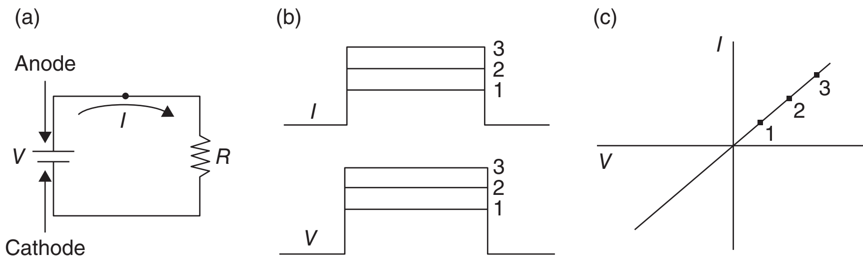



**Figure 1.3** Current through an ohmic resistor. (a) An electrical circuit composed of a voltage source ($V$) and a resistor ($R$). Current ($I$) flows through the circuit. (b) The amplitude of the current is directly proportional to the magnitude of the voltage pulse. (c) A plot of current as a function of voltage (an IV plot) yields a straight line. Resistance is equal to the inverse of the slope of the line (slope = $1/R$).

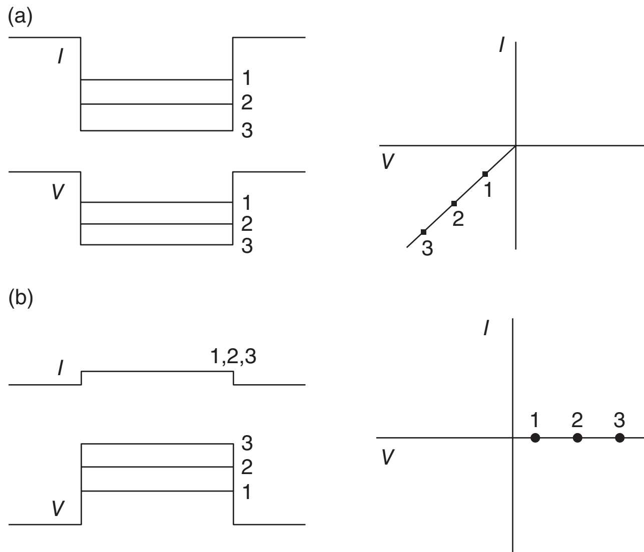



**Figure 1.4** Rectification. The ability of some resistors to pass current depends on the voltage applied to the circuit. (a) In this example, current can flow in the negative direction. Negative voltage pulses 1, 2, and 3 generate progressively increasing currents of amplitudes 1, 2, and 3, respectively. (b) Positive voltage pulses do not elicit currents of increasing amplitudes. As a result, the *I–V* plot in the positive direction shows a horizontal line.

## Non-ohmic Resistors: Rectification

It is common to find that the resistance of a conductor varies with the polarity of the current that flows through it. An example is illustrated in Figure 1.4. Panel (a) shows three superimposed negative tracings of current (top) obtained from our electric circuit in response to voltage steps of negative polarity (bottom). A linear relation similar to the one obtained from a purely ohmic resistor (Figure 1.3) is obtained. However, as shown in Figure 1.4b, a different behavior is observed for pulses of positive polarity. In that case, voltage steps induce only a small current step whose amplitude is essentially constant for any voltages being applied. This property of some conductors to allow the passage of current only (or largely) in one direction is called rectification. Rectification is one example of voltage dependence.

## Slope Resistance and Chord Resistance

Some cardiac membrane channels rectify. In most cases, the channel allows the passage of current more effectively in the inward (i.e., from the extra- to the intracellular space) than in the outward direction. For this reason, this property is called inward-going rectification. Figure 1.5 shows the example of a current–voltage relation of an inward-rectifier cardiac membrane channel (in this case, the potassium current $I_{K1}$; see Chapter 2). It is clear that in this case the resistance of the channel is not constant. Indeed, the slope of the *I–V*

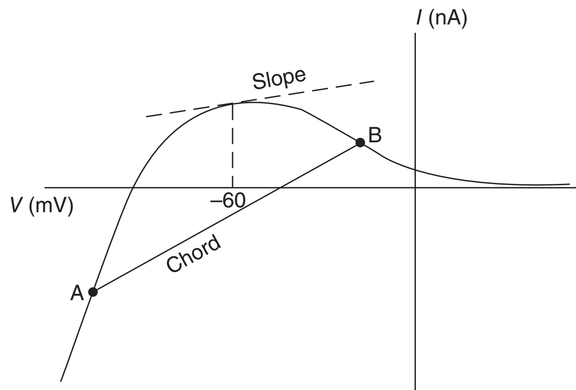



**Figure 1.5** Current–voltage relation of an inward-rectifier channel. The diagram illustrates the concepts of slope resistance and chord resistance. Chord resistance is the inverse of the slope of a line joining points A and B. Slope resistance is the inverse of the slope of the line that is tangential to a specific point of the *I–V* curve. For a rectifying channel, slope and chord resistances can be different.

relation changes with the voltage. There are basically two approaches to evaluate the conductive properties of these channels. One is to determine the slope resistance. This is done by calculating the slope of a line that is tangential to the *I–V* relation at a certain point. In the case of Figure 1.5, the slope of the dashed line that touches the *I–V* function at a voltage of –60 mV is the slope conductance of that channel at that particular voltage. The inverse of the slope conductance is the slope resistance. Clearly, in a nonlinear *I–V* relation, the slope resistance varies appreciably, depending on the voltage at which it is measured. The other approach is to measure the chord resistance. In that case, two specific points (A and B) are chosen, and resistance is measured from the slope of the line (or "chord") that joins those two points. In a linear *I–V* relation, slope resistance and chord resistance are the same; however, in a nonlinear *I–V* relation, the two parameters may be different from each other, and their individual values should depend on the points chosen for measurement.

## Time Dependence

Thus far, we described the properties of resistors that respond instantaneously to the changes in voltage. However, in some cases, the amplitude of the current in response to a voltage change may vary also as a function of time. An example is illustrated in Figure 1.6. In this case, a sudden change in voltage causes a progressive increase in the amplitude of the current. Because the voltage is constant, the increase in current is not due to voltage changes but rather to the intrinsic ability of the conductor to allow the passage of varying amounts of current as a function of time. Many cardiac membrane currents are time-dependent. In some cases, the current progressively increases during a voltage step, whereas, in other cases, the current decreases, and yet

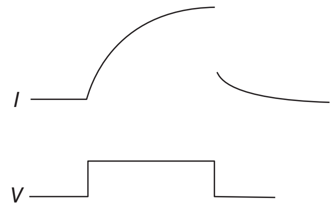



**Figure 1.6** Time-dependent current. In this case, the ability of the conductor to pass current changes with time. Thus, the current amplitude increases progressively while the voltage is held constant.

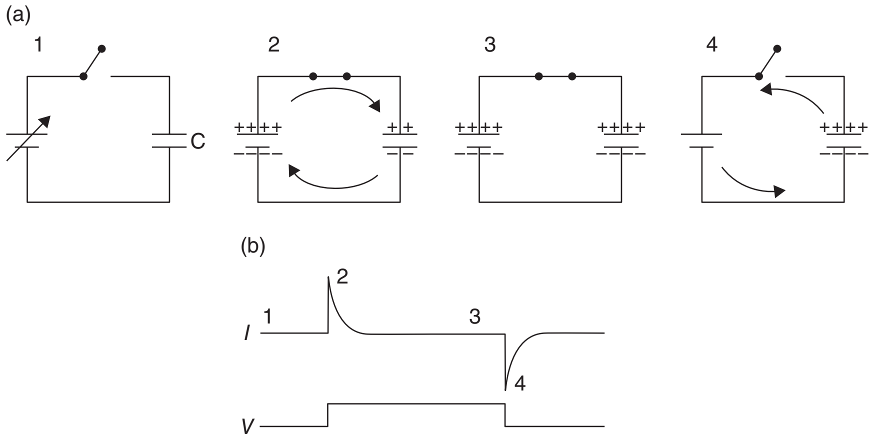



**Figure 1.7** Capacitive current in an equivalent circuit consisting of a switch, a variable voltage generator, and a capacitor (C). (a) The diagrams illustrate the charge distribution along the circuit at four different times (1–4). (b) Current (*I*) generated by a voltage (*V*) square pulse. The small numbers in panel (b) correspond to the four frames in panel (a). The switch is open; no current flows across the circuit (frame 1). When the circuit is closed and a voltage step is applied from the baseline, the current surges rapidly (frame 2) (from point 1 to point 2) but then decreases to zero once the capacitor is fully charged (frame 3). An identical surge of opposite polarity is elicited when the voltage step is returned to baseline or the circuit is open (frame 4).

in other instances, completely disappears even if the voltage step is held constant for an extended period of time.

## Capacitance

Capacitance is the property of an electric nonconductor that permits the storage of energy as a result of electric displacement when opposite surfaces of the nonconductor are maintained at a different potential. The measure of capacitance is the ratio of the change in the charge on either surface to the potential difference between the surfaces. Thus, a capacitor is formed when two conducting materials are separated by a thin layer of nonconducting material, an insulator (or dielectric). Cell membranes are capacitors in that the thin lipid bilayer (which is a very poor electric conductor) behaves like a dielectric interposed between the intracellular and the extracellular spaces, both of which are capable of conducting electricity. As opposed to resistors (ion channels in the case of cells), a voltage step imposed through a capacitor causes only a temporary current. This is illustrated in Figure 1.7. In panel (a),

the top diagram shows an electric circuit consisting of a variable voltage generator (i.e., a battery of variable voltage), a capacitor, and a switch. Initially (step 1), the switch is off and no voltage difference is set between the anode and the cathode. When the switch is turned on, a voltage difference is established across the circuit (step 2), charge travels toward the cathode until it encounters the capacitor. Because the conductive pathway is interrupted by the dielectric that separates the two plates of the capacitor, positive charge accumulates at the plate that is closer to the anode. A steady-state condition is rapidly reached (step 3), and the flow of current stops. The tracings in panel (b) show the time course of positive capacitive current in response to voltage in this circuit. The voltage step elicits a rapid surge of current; however, the current rapidly returns to zero. When the voltage difference is switched back to zero (step 4), the capacitor is gradually discharged (i.e., charges now flow in the opposite direction) and a negative capacitive current is observed.

Capacitive current ($I_C$) is thus defined as

$$I_C = C\,dV/dt \tag{1.2}$$

where $C$ is the capacitance and $dV/dt$ represents the first derivative of voltage with respect to time. The latter can be roughly thought of as the rate at which voltage changes. When voltage is constant, $dV/dt$ is zero (because voltage is not changing), and the amplitude of the capacitive current is also zero.

It is important to note that the capacitive properties of the cell are essential for the maintenance of a voltage difference across the membrane. Indeed, the lipid bilayer allows for the separation of charge. The voltage difference across the membrane is established by the fact that charge is unequally distributed. Therefore, the magnitude of the membrane potential reflects the extent of the disparity in charge distribution across the capacitor. Changes in membrane potential occur when ions, normally moving through the membrane channels, charge or discharge the membrane capacitance, thus changing the number of charges in the intra- and the extracellular spaces.

## Parallel RC Circuits

In the previous sections, we described the behavior of the lipid bilayer of the membrane as a capacitor. We also equated membrane channels with resistors, because they allow the movement of ionic currents. Because the channels are formed by proteins that span the membrane, they are usually modeled in equivalent circuits as resistors in parallel with the membrane capacitor.

Therefore, the basic membrane circuit is that of a resistor and a capacitor in parallel (Figure 1.8a) and is usually referred to as an RC circuit. In an RC circuit, the total current flow ($I_t$) is equal to the sum of the current that moves through the capacitor ($I_C$) and the current that flows through the resistor ($I_R$).

$$I_t = I_C + I_R \tag{1.3}$$

Consequently, if one combines Equations 1.1, 1.2, and 1.3, then

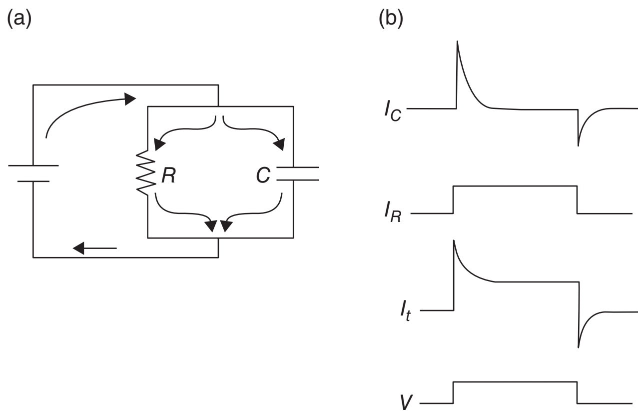



**Figure 1.8** Parallel RC circuit. (a) A resistor (*R*) and a capacitor (*C*) in parallel are connected to a voltage source. (b) A voltage step (*V*) generates a total current ($I_t$), which is the sum of the current flowing through the resistor ($I_R$) and that flowing through the capacitor ($I_C$).

$$I_t = C\,dV/dt + \mathrm{V/R} \tag{1.4}$$

Figure 1.8b depicts the change in current in response to a voltage step in a parallel RC circuit (assuming that the resistor shows no time-dependent properties). The current flowing through the capacitor ($I_C$) has the properties depicted in Figure 1.7, whereas the current moving through the resistor ($I_R$) is directly proportional to the voltage step itself (as in Figure 1.3). Because both currents add, the total current ($I_t$) in Figure 1.8b shows an initial transient change, which is due to the flow of capacitive current, but rapidly reaches a steady state. The steady state corresponds to the magnitude of the current flowing through the resistor, and it is maintained for as long as the voltage step is maintained. Termination of the voltage step elicits the discharge of the capacitor, and then the current trace returns to the baseline value.

As noted earlier, cell membranes are modeled as parallel RC circuits. Accordingly, when a voltage change is imposed across the cell membrane, there is an initial transient surge of capacitive current, also called the capacitive transient. In the case of a square voltage pulse, the capacitive current rapidly drops to zero. Hence, all currents recorded after the end of the capacitive transient are currents that move through ion channels.

## Origin of the Membrane Potential

Electrical current is driven by the voltage difference across a conductor. In the case of cells, this driving force is generated by the unequal distribution of electric charges and ion concentrations across the membrane. In other words, the membrane potential is electrochemical in origin. The physical basis for the establishment of electrochemical potentials is defined by the Nernst equation.

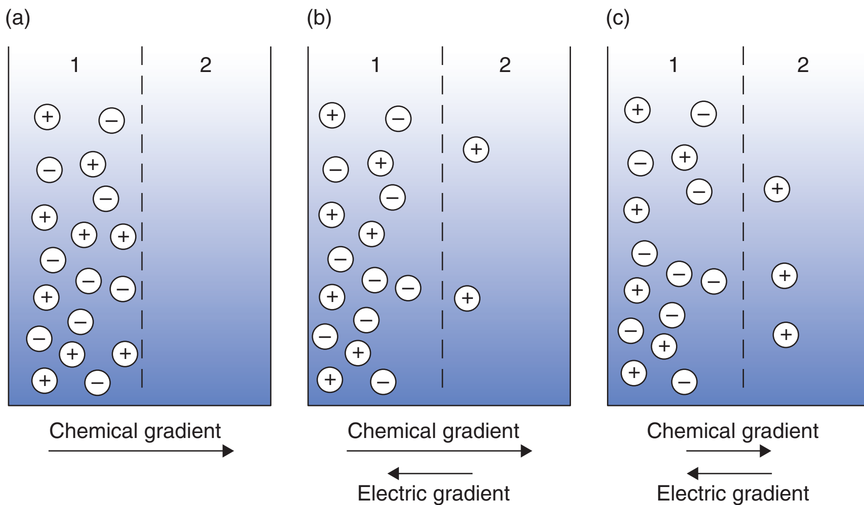



**Figure 1.9** Electrochemical potential. A vessel is divided into two compartments (1 and 2) by a membrane that is permeable to positive ions but impermeable to negative ions. (a) Placing an ionizable solution into compartment 1 creates a chemical gradient for the flow of ions toward compartment 2. (b) As positive ions move across the membrane, they leave negative ions behind, generating an electric gradient whose direction is opposite to the chemical gradient. (c) Steady state is reached when the magnitude of the chemical and electric gradients are equal.

The principles of the Nernst equation can be illustrated by the example shown in Figure 1.9. A vessel is divided into two compartments (1 and 2) by a semipermeable membrane. The membrane allows for the passage of cations, but not anions. In panel (a), at the onset of the experiment, a solution of potassium chloride is placed in compartment 1. Both ions now tend to move to side 2, following the respective concentration gradients. Because the membrane is permeable to cations only, every time a potassium ion crosses to side 2 (following its concentration gradient), it leaves a negative charge (a chloride ion) behind. Two opposing forces are therefore created: (1) chemical, which pushes the potassium ions along their concentration gradient and (2) electric, which is created by the attraction that the negatively charged chloride ions exert over the cations (panel b). At steady state, a dynamic equilibrium is reached in which the magnitude of the chemical force is equal and opposite to the magnitude of the electric force (panel c). Consequently, the concentration of potassium differs in the two compartments, while at the same time a voltage difference is created. Mathematically, this equilibrium is expressed by the Nernst equation, as follows:

$$E_K = RT/F \ln [K]/[K]_1 \qquad (1.5)$$

where $T$ is temperature, $R$ is a constant derived from the gas law, $F$ is the Faraday constant, and $[K]_2$ and $[K]_1$ are the final concentrations of potassium

in compartments 2 and 1, respectively. $E_K$ is the equilibrium potential for potassium. That is, the voltage difference imposed across this semipermeable membrane is a result of the selective conductance of the membrane for potassium. If the cell membrane were exclusively permeable to potassium (and impermeable to all other ions), one could draw its equivalent circuit as a resistor in parallel with a capacitor, with the driving force (i.e., the source of membrane voltage) created by the electrochemical gradient of potassium, as predicted by the Nernst equation. In that case, the resistor represents the potassium channels in the membrane.

The concentration of potassium inside most mammalian cells is significantly larger than outside. In a cardiac ventricular myocyte, for example, the intracellular concentration of potassium is approximately 150 mM, whereas the concentration of potassium in the extracellular space is about 5 mM. Solving for the Nernst equation (Equation 1.5), one would predict a resting potential for a ventricular myocyte (kept at 37°C) of about –90 mV. The actual value, however, is slightly less negative than that. The reason is that other conductances with more positive equilibrium potentials may also contribute to the resting potential. For example, in some cells, a small permeability to sodium can be detected at rest. The concentration of sodium in the extracellular space is much larger (~140 mM) than in the inside (~10 mM); consequently, the sodium equilibrium potential is more positive than zero. A small conductivity to sodium would therefore tend to bring the resting potential to a less negative level. In the case of the ventricular myocyte, the resting potential is much closer to $E_K$ because the membrane is much more permeable to potassium than to sodium. The final resting potential of a cell is therefore established by the balance between the equilibrium potentials for those ions to which the cell is permeable and the conductivity that the membrane may have for that ion. Mathematically, for a cell that is permeable to sodium, potassium, and chloride, the resting potential ($V_m$) can be predicted by the Goldman–Hodgkin–Katz (GHK) equation as follows:

$$V_m = RT/F \ln\{P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i / P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o\} \quad (1.6)$$

where $P_{Na}$, $P_K$, and $P_{Cl}$ are the permeabilities of the membrane to sodium, potassium, and chloride, respectively. Hence, a complete electric diagram of the cell membrane at rest should include several sources of voltage, each providing a driving force for current across highly specific resistors. The magnitude of each source of voltage corresponds to the equilibrium potential for individual ions, and each specific resistor represents the ion channel that is specific for the ion in question. The magnitude of each resistor at rest is different. In the case illustrated in Figure 1.10, a hypothetical equivalent circuit of a cell is drawn that shows selective permeability to potassium, sodium, and chloride. In this case, the resting potential $V_m$, which is maintained across the cell capacitor, is established by the solution of the GHK equation (Equation 1.6) where all pertinent equilibrium potentials and conductivities are taken into account.

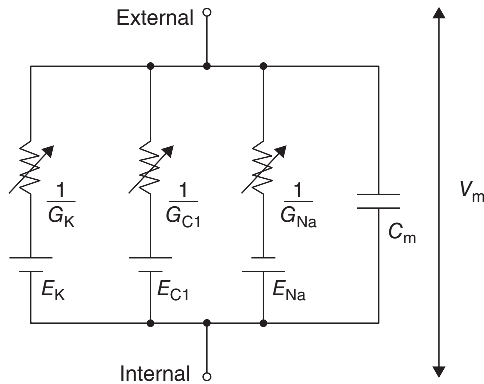



**Figure 1.10** Simplified electric circuit of an excitable membrane. Three conductances in parallel are represented ($G_K$, $G_{Cl}$, and $G_{Na}$), each one connected to an electrochemical voltage source ($E_K$, $E_{Cl}$, and $E_{Na}$) created by the different concentrations of the individual ion species across the membrane. The lipid bilayer is represented by the capacitor $C_m$. A voltage difference ($V_m$) can be recorded across the membrane.

## Active Currents

Most membrane conductances are not ohmic. The specific time- and voltage-dependent characteristics of various ion channels are reviewed in Chapter 2. However, it is important to note here that the conductivity of various channels changes during cardiac excitation. The action potential is therefore generated by variations in the magnitude of individual ion channel conductances, thus creating ionic currents that flow as a result of the difference between the actual membrane potential and the equilibrium potentials for the ions involved in the excitation–recovery process. As new charges enter or leave the cell, the capacitor is charged or discharged accordingly, thus displacing the resting potential. As shown in Figure 1.11a, when a positively charged ion (e.g., sodium or calcium) enters the cell, the membrane potential becomes more positive (or less negative); i.e., the cell is "depolarized." Conversely, a positive ion leaving the cell (e.g., potassium; see Figure 1.11b) increases the negativity of the cell interior. The flow of positive ions into the cell is referred to as "inward current." Inward currents are depicted as downward deflections and are represented by a negative sign. Positive ions flowing from the inside to the outside of the cell generate an "outward current." Outward currents are depicted as upward deflections and are represented by a positive sign. The amplitude and duration of the action potential results from the balance among the amplitude, time course, and direction of the specific currents involved.

Ionic currents flow through hydrophilic pores formed by membrane channel proteins. Electrically, these channels can be thought of as resistors. Thus, we can use Ohm's law to define the amplitude of the current that moves through a given channel, as follows:

$$I_x = (V_m - E_x)G_x \quad (1.7)$$

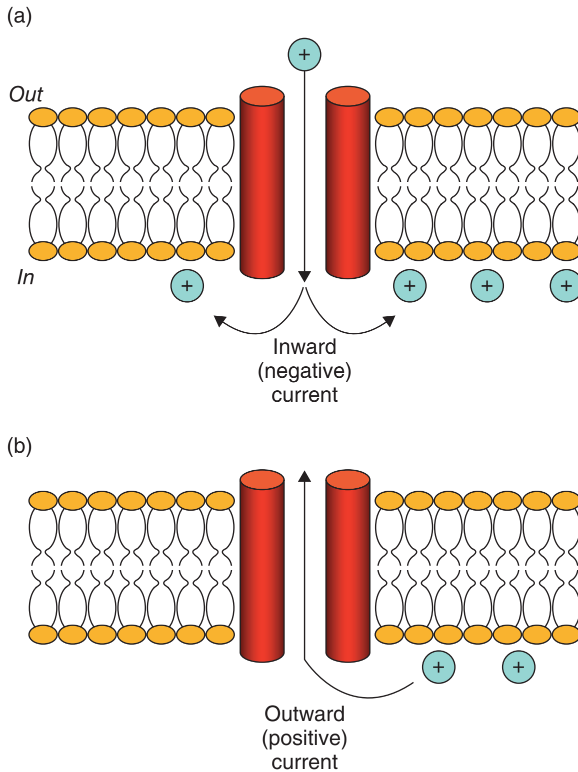



**Figure 1.11** Direction of current across the membrane. (a) A positive ion entering the cell generates inward current and is represented by a negative deflection. (b) A positive charge leaving the cell creates an outward (positive) current.

Equation 1.7 indicates that the amplitude of the current flowing through "$x$" type of channels is a function of the channel conductance ($G_x$) and the driving force. The latter parameter is defined by the difference between the membrane potential ($V_m$) and the equilibrium potential for the ion ($E_x$). If the membrane potential is equal to the equilibrium potential, no current will flow (even if the channels are conductive). A current will be elicited only if the membrane potential is different from $E_x$ and the channel is conductive. This relation also shows that, when the equilibrium potential is more negative than the membrane potential (e.g., the potassium conductance during repolarization), the current has a positive sign. Conversely, if the equilibrium potential is more positive than the membrane potential (e.g., sodium during depolarization), the current is of negative sign. Finally, it is important to note that, for time- and/or voltage-dependent channels, the value of $G_x$ can change constantly. For example, the sodium conductance ($G_{Na}$) is almost zero at rest. As a result, no sodium current is present in a quiescent cell. However, if the cell reaches a critical (threshold) value, $G_{Na}$ increases transiently, thus allowing for a large influx of sodium and consequent depolarization of the cell. On the other hand,

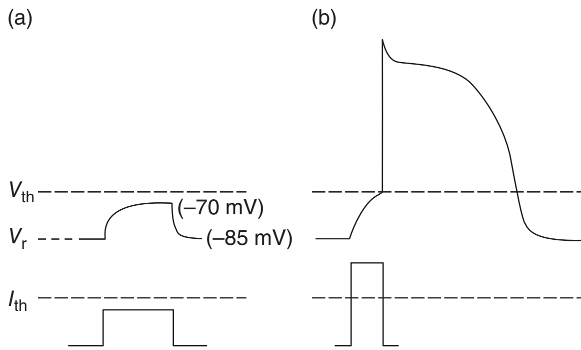



**Figure 1.12** Cell excitability and the action potential. The membrane potential of a ventricular cell (top trace) stays at its resting level ($V_r$) if undisturbed by a current input (bottom trace). (a) A depolarizing current pulse of amplitude lower than a certain threshold value ($I_{th}$; bottom dotted line) will only change the membrane potential from rest (–85 mV in this example) to a constant level (–70 mV), and the membrane potential will return to rest when the current input is switched off. (b) A current pulse larger than the critical $I_{th}$ amplitude will force the membrane potential to reach the voltage threshold ($V_{th}$) at which an "all-or none" action potential is elicited. In that case, the membrane potential goes through an entire cycle of changes in ionic currents that occur regardless of whether the initial input is maintained.

the amplitude of the inward-rectifier potassium current ($I_{K1}$) at rest is also close to zero, but in this case, the lack of measurable current is consequent to the fact that, at the level of the resting potential, there is no driving force for potassium. (Yet, the channels remain conductive.) Depolarization of the membrane elicits an outward potassium current because a driving force ($V_m - E_K$) is created for the ion. The importance of these issues will become clearer as we define the ionic bases of the action potential and discuss some of the ionic mechanisms that may be involved in cell excitability and refractoriness.

## Excitability

Cardiac cells are "excitable" because they are able to generate action potentials that transfer information between cells. In Figure 1.12 we have plotted the membrane potential of a cardiac ventricular myocyte as a function of time. These types of measurements are obtained by introducing a fine-tipped glass microelectrode into the cell, and comparing the recorded potential against a reference electrode kept in the extracellular space. If no external stimulus is delivered to the cell, the membrane remains at its resting potential of about –85 mV. Panel (a) shows the changes in membrane potential that result from depolarization induced by a square electric current pulse of "subthreshold" amplitude applied through the microelectrode. In this case, the externally applied current causes the membrane potential to depolarize to a level of approximately –70 mV. After reaching that level, the membrane potential remains constant at –70 mV for the duration of the pulse. At the end of the pulse, the membrane potential returns to the resting level, and no further change is observed. However, as shown in panel (b), a stimulus of slightly

larger amplitude triggers a regenerative response (an action potential) that lasts much longer than the duration of the current pulse itself. The level of voltage at which the cell needs to be depolarized to elicit the action potential is called the threshold voltage ($V_{th}$). Similarly, the minimum amount of current needed to trigger the active response is referred to as the threshold current ($I_{th}$). Threshold voltage and current are very useful variables for defining quantitatively the excitability of the cells. For instance, to measure excitability, investigators usually apply depolarizing pulses of progressively larger amplitude until threshold is reached. The larger the current needed to achieve threshold, the lower the excitability; i.e., excitability = 1/threshold current.

For the most part, excitability is determined by the availability of inwardly directed currents to depolarize the cell membrane. However, it is important to remember that outward currents are also essential determinants of excitability. Consider, for example, the case of an isolated ventricular myocyte. As noted earlier, the resting potential is established primarily by the presence of the inward-rectifier $I_{K1}$ conductance. These highly selective potassium channels maintain the resting membrane potential close to the equilibrium potential for potassium. Figure 1.13b shows a representation of the current–voltage relation of $I_{K1}$, and Figure 1.13b shows the expected values of membrane

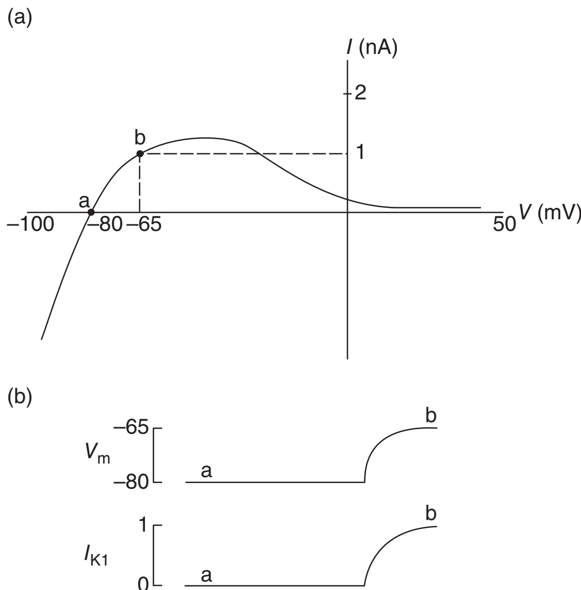



**Figure 1.13** Role of the background current $I_{K1}$ in determining the response to an externally applied subthreshold current pulse. (a) *I*–*V* relation of $I_{K1}$. (b) Subthreshold changes in membrane potential ($V_m$) and $I_{K1}$ amplitude. Cell depolarization from point "a" (–80 mV) to point "b" (–65 mV) causes an increase in $I_{K1}$ that is proportional to the magnitude of the depolarization, as predicted by the *I*–*V* curve. This outward current opposes the depolarizing influence of the excitatory input and, under extreme conditions, can interfere with the generation of the action potential.

potential ($V_m$; top) and $I_{K1}$ amplitude (bottom) in quiescence (a) and during subsequent depolarization (b) induced by an external current source (not shown). At rest, a negligible amount of ionic current flows through these channels because the resting potential is very close to $E_K$. However, depolarization moves the membrane potential away from the rest and establishes the driving force ($V_m - E_K$) for the outward potassium current. This outward (i.e., repolarizing) current opposes cell depolarization; the larger the outward current, the lesser the depolarization and the more difficult it will be for the membrane to achieve threshold. In other words, excitability is determined not only by the number of sodium channels available to depolarize the cell but also by the balance between the availability of active inward currents and the amplitude of the outward conductance that opposes depolarization by the excitatory input.

Under normal conditions, $I_{K1}$ may be a small factor in the regulation of excitability and conduction. However, conditions such as acute ischemia can induce the opening of other potassium channels ($K_{ATP}$) that generate large outward currents at subthreshold values of potential. Given their ability to oppose depolarization, these currents may be important in the regulation of excitability during ischemia.

## Cardiac Action Potential

The action potential of a cardiac ventricular myocyte presents four distinct phases (see Figure 1.14, top panel): phase 0 corresponds to the rapid depolarization (or action potential upstroke) that ensues once the cell reaches the voltage threshold. Phase 1 corresponds to the brief, rapid repolarization that is initiated at the end of the action potential upstroke and that is interrupted when the cell reaches the "plateau level" or phase 2. During the plateau, repolarization progresses slowly; but eventually a final phase of rapid repolarization, or phase 3, ensues. Finally, phase 4 is the period between the last repolarization and the onset of the subsequent action potential. In atrial or ventricular myocytes, phase 4 corresponds to the resting membrane potential. As noted earlier, all of these changes in membrane potential are the result of the transmembrane movement of charges flowing through ion-specific, time- and voltage-dependent channels. A more detailed discussion of specific ion channel proteins is presented in Chapter 2; at this point it is appropriate to introduce some basic concepts relating the individual ionic currents to the various phases of action potential in a "generic" non-pacemaking cardiac cell.

### Phase 0: Action Potential Upstroke

At rest, the dominant membrane conductance is provided by the potassium channels. However, once the cell reaches a threshold level of approximately –65 mV, membrane sodium channels suddenly open. Hence, at threshold, the membrane rapidly switches from being mostly permeable to $K^+$ to being

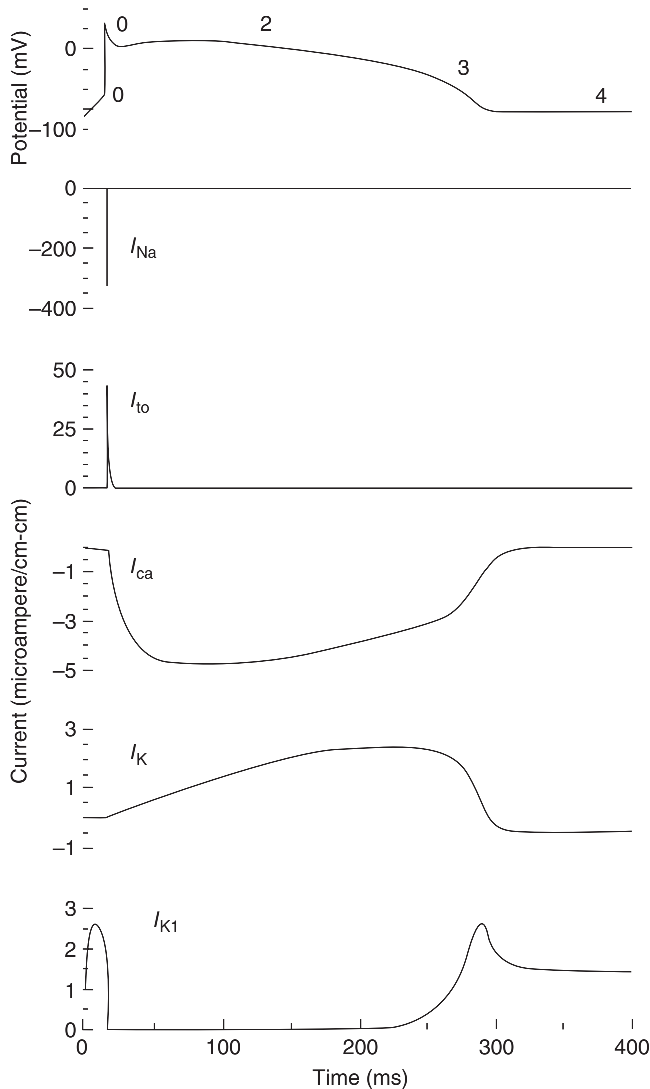



**Figure 1.14** Ionic basis of the ventricular action potential. A simulated action potential (top panel) is shown in parallel with the various membrane currents that originate it (see Chapter 2 for a detailed description of ionic currents). The phases of the action potential are noted by the small numbers. $I_{Na}$ and $I_{Ca}$ are inward (downward deflections) sodium and calcium currents, respectively. $I_{to}$, $I_K$ and $I_{K1}$ are outwardly directed (upward deflections) potassium-dependent currents. For illustration purposes, actual magnitudes of currents shown are not scaled.

largely permeable to $Na^+$. The concentration of $Na^+$ in the extracellular space (~140 mM) is significantly larger than in the intracellular space (~4 mM). Figure 1.14 shows a computer simulation of the cardiac action potential (top) and the major ionic current components. Clearly, the sodium current ($I_{Na}$) represents a very large, yet rapid transition (note that each current has a

different vertical scale). When the conductance to sodium suddenly increases, the large transmembrane gradient of $Na^+$ leads to a rush of ions into the cell in the form of an inwardly directed negative sodium current ($I_{Na}$). These ions accumulate in the intracellular side of the lipid bilayer and thus charge the membrane capacitor. Because sodium ions are positively charged, the membrane rapidly becomes less negative; i.e., it depolarizes, and results in the upstroke, or phase 0 of the action potential. In fact, given that during phase 0 the cell is mostly permeable to $Na^+$, the membrane potential becomes transiently positive as it moves toward $E_{Na}$ (approximately +40 mV). However, the increase in sodium conductance is very brief. After a few milliseconds, the sodium channels enter a nonconductive state. The membrane potential does not quite reach the sodium equilibrium potential, but stops at approximately +20 mV and then begins to repolarize.

### Phase 1: Rapid Repolarization

As shown in Figure 1.14, for the most part, the end of the action potential upstroke is brought about by the inactivation of the sodium channels. During the initial phase of repolarization of the action potential, or phase 1, potassium channels provide the dominant membrane conductance. Although there can be important differences in the ionic currents that are activated at this stage, depending on the region of the heart from which the cells originate (and also depending on the animal species studied), in most cases the so-called "transient outward current" ($I_{to}$) provides most of the repolarizing charge. This rapidly activating potassium conductance turns on during the action potential upstroke. When the sodium channels enter their nonconductive state, repolarization begins in earnest, with the membrane potential heading toward the reversal potential for potassium. $I_{to}$ inactivates also very rapidly, and its contribution to the repolarizing process during phases 2 and 3 of the action potential is somewhat less than that observed during phase 1.

### Phase 2: Action Potential Plateau

Other voltage-dependent membrane channels are also activated by cell depolarization, although they activate at a slower rate (Figure 1.14). Consequently, these channels provide a sizable current only several milliseconds after the end of the action potential upstroke. The two dominant currents during the plateau phase of the action potential, or phase 2, are the inward calcium current ($I_{Ca}$) and the delayed rectifier potassium outward current ($I_K$). For the sake of simplicity, we labeled as $I_{Ca}$ all current components that are calcium-dependent (including, for example, the L-type calcium current and electrogenic sodium–calcium exchanger). As discussed in more detail in Chapter 2, the potassium current $I_K$ includes at least two separate components: a rapid component ($I_{Kr}$) and a slow component ($I_{Ks}$). Given the concentration gradient for $Ca^{2+}$, opening of calcium channels leads to movement of calcium from the extracellular to the intracellular space (i.e., an inwardly directed, depolarizing current). Potassium ions, on the other hand, move in the opposite direction.

The end result is that, while the calcium channels remain open, repolarization by potassium currents is prevented by the presence of a calcium current that is moving positive charges into the cell. Thus, during the plateau, the membrane potential depends on the balance between inward $I_{Ca}$ and outward $I_K$ currents. Although in some cells (e.g., Purkinje fibers) outward currents dominate and the plateau tends to have a consistently negative slope, in others there may be an actual slight depolarization before repolarization continues (in a "domelike" shape).

#### Phase 3: Final Repolarization

Inactivation of the calcium channels leads to the end of the plateau (Figure 1.14). Only potassium conductances remain active; consequently, the membrane potential returns relatively rapidly toward $E_K$. The delayed rectifier currents ($I_{Kr}$ and $I_{Ks}$) tend to close as the cell repolarizes, and thus the inward-rectifier current ($I_{K1}$) predominates.

#### Phase 4: Diastolic Potential

In atrial and ventricular muscle cells, the resting potential remains constant throughout the diastolic interval. In these cell types, the inward-rectifier current $I_{K1}$ remains the dominant conductance at rest and it is largely responsible for setting the resting membrane potential. An additional small background conductance, with a more positive equilibrium potential, keeps the resting potential slightly more depolarized than the value estimated by the potassium equilibrium potential. Atrial and ventricular myocytes remain at this constant level of potential until a new excitatory stimulus brings the membrane potential to threshold, thus eliciting a new active response.

## Basic Action Potential Parameters

Several parameters are commonly used by cellular electrophysiologists to quantify the time course and magnitude of changes in the action potential as a result of changes in the activation frequency, ionic concentrations, temperature, pH, or drug effects. Some such parameters are illustrated in Figure 1.15. Action potential amplitude (APA) is measured from the resting potential to the peak of action potential depolarization. Action potential overshoot refers to the magnitude of membrane potential reversal from negative to positive values. Action potential duration (APD) is usually measured between the onset of the action potential upstroke and the point at which the cell repolarizes to a certain fraction of the maximum. For example, $APD_{50}$ refers to the APD measured at 50% repolarization and $APD_{90}$ is the APD at 90% repolarization. In pacemaker cells, where the membrane potential is not constant during diastole, the term "maximum diastolic potential" is used to identify the most negative value of membrane potential reached after repolarization. Finally, the maximum action potential upstroke velocity (i.e., the fastest rate of depolarization measured during phase 0) is often used as a rough indicator of the

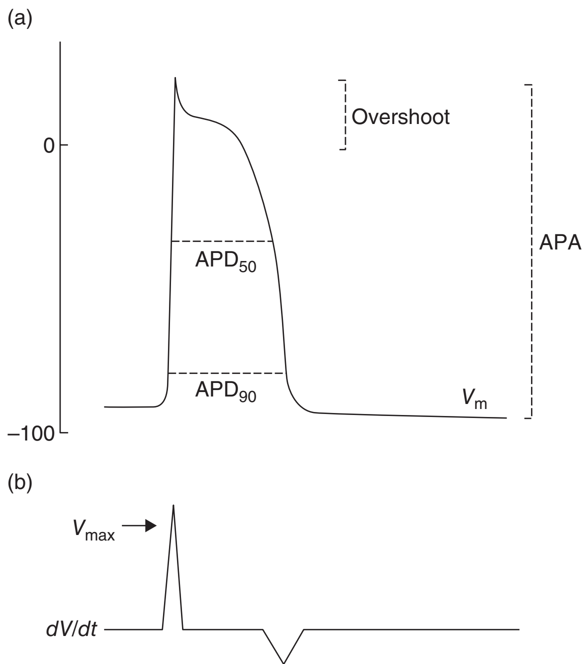



**Figure 1.15** Action potential parameters. (a) A ventricular action potential. $APD_{50}$ and $APD_{90}$ refer to the action potential duration measured at 50% and 90% repolarization, respectively. APA stands for action potential amplitude. The overshoot refers to the segment of the action potential where the inside of the cell is positive with respect to the outside. (b) First derivative of the action potential. The maximum rate of rise of membrane potential ($V_{max}$) is, within certain limits, an indirect measure of the conductance of the sodium channels.

magnitude of the active inward current mediating phase 0 (see Figure 1.15b). Upstroke velocity can be estimated from the first derivative of the change in voltage as a function of time. Thus, the maximum upstroke velocity ($V_{max}$ or $dV/dt_{max}$) is equal to the maximum positive value of the first derivative of the action potential.

## Refractory Period

A characteristic feature of excitable cells is that, once the cell is activated, there is a period of time during which a second action potential cannot be generated. The interval of time during which the cell cannot be re-excited is called the refractory period (RP). The RP of cardiac cells is long compared with other excitable cells such as neurons and skeletal muscle cells. An important functional advantage of having a long RP is that cardiac cells cannot be activated again before enough time passed for at least partial relaxation from the previous contraction. Consequently, cardiac myocytes do not undergo tetanic contraction (i.e., tonic contraction). Although tonic contraction is indeed very important for the normal physiology of skeletal muscle, it would have fatal consequences if it were present in the heart.

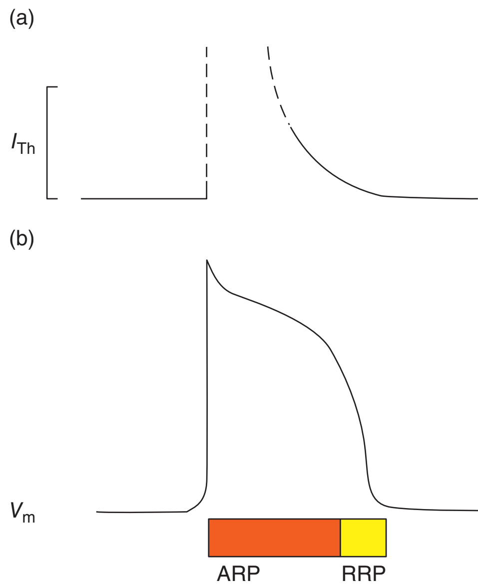



**Figure 1.16** Refractory period. (a) Changes in the amplitude of the threshold current ($I_{th}$) during the action potential. (b) Voltage recording during the action potential. From the moment of rapid depolarization and until the end of the plateau phase (red bar), a second action potential cannot be generated regardless of the amplitude of the current input (absolute refractory period; ARP). Toward the end of phase 3 (yellow bar), an additional action potential can be elicited, but the current required is larger than during control (relative refractory period; RRP).

The recovery of excitability of a cardiac myocyte develops gradually after an action potential. Figure 1.16a shows a plot of the threshold current ($I_{th}$, i.e., the minimum current magnitude necessary to elicit an active response) at different times during an action potential. The membrane potential of a ventricular myocyte is shown in panel (b). An action potential is elicited after a long period of quiescence. Thus, the magnitude of current required to elicit the response reflects a baseline (or "control") level of excitability. At the onset of the action potential upstroke, there begins a period of time (marked by the solid bar at the bottom) during which $I_{th}$ grows to infinity and a second response cannot be elicited, regardless of the magnitude of the excitatory input. This interval marked in red is known as the absolute refractory period (ARP). As the cell repolarizes, it once again becomes excitable. However, there is a period of time during which the amount of current needed to trigger the active response is much larger than the control $I_{th}$. This period, illustrated by the yellow bar at the bottom, is known as the relatively refractory period (RRP). As the membrane repolarizes toward resting levels, $I_{th}$ decreases gradually, and excitability is recovered, achieving control values several milliseconds after the completion of repolarization.

The ARP occurs because, once an action potential ensues, active inward currents are no longer available to depolarize the membrane. Indeed, as

discussed in Chapter 2, sodium channels become inactive (nonconductive) during depolarization. A period of recovery from inactivation (which is initiated by repolarization) is required so that these channels can become conductive once again.

The outward currents also participate in the RRP. As discussed earlier, the total membrane current results from the balance between the inward and outward currents. Outward currents oppose the depolarizing influence of inward currents. During repolarization (and briefly during diastole) the delayed rectifier channels are conductive. To re-excite the cell, the depolarizing current must overcome the repolarizing influence of the outward currents. Thus, the duration of the RRP is determined in a complex manner by the time course or reactivation of the inward currents as well as by the time course of deactivation (or "closure") of the delayed rectifier outward conductance.

### Supernormal Excitability

Under normal circumstances the recovery of excitability after an action potential follows a smooth exponential time course. However, in Purkinje fibers and, under certain conditions, in working cardiac muscle as well, there is a brief period of time at the end of repolarization in which the cells are actually more excitable than at rest. This period of "supernormal excitability" (labeled "SN" in Figure 1.17) occurs at least in part because, as the membrane potential returns toward resting levels, it does so at a time when enough sodium channels are reactivated and the threshold for activation is sufficiently close to normal. As shown in Figure 1.17, as the membrane repolarizes and crosses

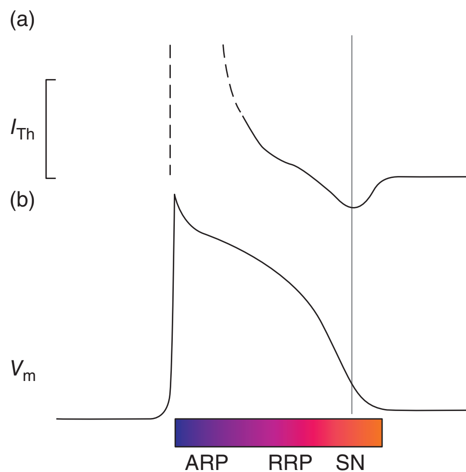



**Figure 1.17** Supernormal excitability (SN). (a) Changes in the amplitude of the threshold current ($I_{th}$) during the action potential. (b) Voltage recording during the action potential. In some cell types, the relative refractory period (RRP) is followed by a time interval during which $I_{th}$ is actually smaller than in the control. ARP, absolute refractory period.

the threshold for sodium channel activation (dashed vertical line), stimulation during this period requires less current than normal for a new action potential to occur. The period of supernormal excitability may be important in the generation of cardiac arrhythmias, as it allows for the new action potential to be triggered at a vulnerable time in the cardiac cycle for reentrant excitation to occur.

### Action Potential Morphology in Specialized Cardiac Tissues

The preceding paragraphs describe the general concepts on the ionic currents mediating a generic cardiac action potential in the atria or ventricles. However, the magnitude and time course of action potentials vary from one cardiac region to another. In this section, we describe some of the major features in the action potentials of sinoatrial (SA) and atrioventricular (AV) nodal as well as Purkinje cells.

#### Sinoatrial Nodal Cells

Cardiac activation normally starts in the SA node. The specialized muscle cells in this area are capable of spontaneous (also called pacemaker) activity (see Figure 1.18), and their intrinsic frequency of discharge is, under normal conditions, faster than that of any other pacemaker in the heart. In true pacemaker cells of the SA node, the diastolic membrane potential does not remain constant. Instead, there is a spontaneous (phase 4) depolarization that brings the membrane potential slowly from the most negative value achieved at the end of the preceding repolarization (i.e., the maximum diastolic potential) to the threshold for activation, thus eliciting a new active response. It is important to note that the maximum diastolic potential of SA nodal cells is less negative (between –50 and –65 mV) than that of ventricular cells. Nodal cells have a lower permeability to potassium than ventricular cells at rest. Consequently, the repolarization process stops at less negative levels; furthermore, in nodal cells, the threshold for activation stands at approximately –35 mV because in these cells the action potential upstroke results from the inward flow of calcium through channels that are similar to those mediating the $I_{Ca}$ current of ventricular cells. Sodium channels play a negligible role in the action potential upstroke of SA nodal cells. Therefore, the maximum rate of depolarization during the action potential upstroke is relatively slow in these cells (~1 to 10 V/s).

The slow diastolic (phase 4) depolarization that characterizes spontaneously active nodal cells results (at least in part) from activation of a hyperpolarization-activated current, commonly referred to as the "funny current" or $I_f$. Indeed, in the voltage range of phase 4 depolarization, the $I_f$ channels allow for the entry of positive charges into the cell. As opposed to other channels mentioned earlier in this section, $I_f$ channels open at voltages more negative than –60 mV (see Chapter 2). Consequently, the channels are closed during most of the action potential. Yet, when the cell repolarizes from a previous discharge, $I_f$ channels activate. The entry of cations leads to gradual cell

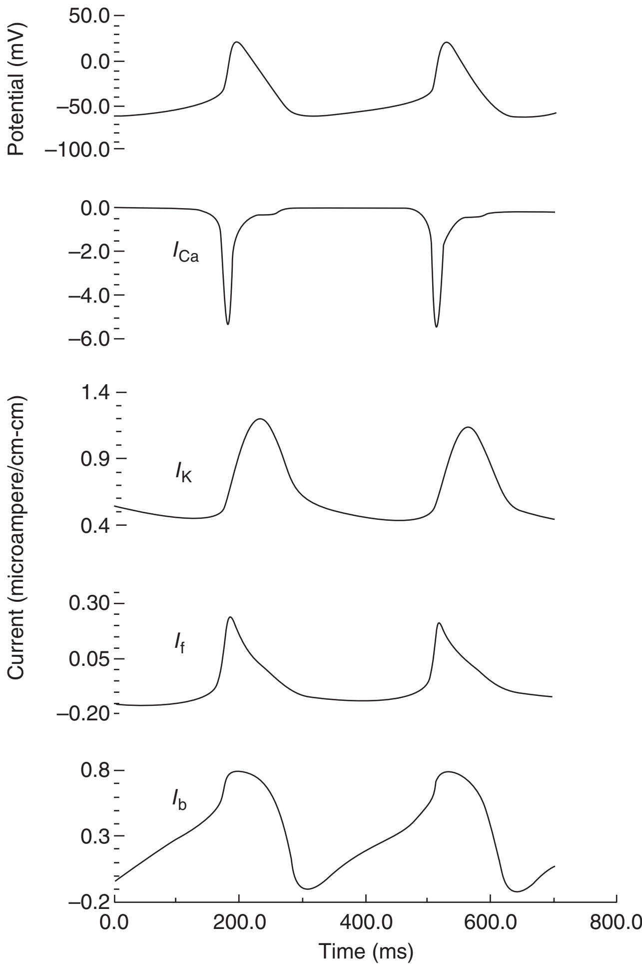



**Figure 1.18** Ionic bases of the sinus nodal action potential. The simulated electric activity of the nodal cell (top) was generated by a set of differential equations describing the different relevant ionic currents. As opposed to the action potential of atrial working muscle, or ventricular tissues, in this case sodium current does not play a role. The upstroke is mediated by the calcium current ($I_{Ca}$). The potassium current ($I_K$) dominates the repolarization phase. Two additional currents are shown. The $I_f$ current activates upon repolarization, and contributes inward current to the pacemaker potential. A time-independent background current ($I_b$) also changes polarity during the cardiac cycle and contributes to the different phases of the action potential.

depolarization (Figure 1.18). Depolarization progresses slowly until the membrane reaches the threshold for activation of calcium currents, at which time a sinus nodal action potential ensues. Depolarization then causes the $I_f$ channels to close. However, during the subsequent repolarization, the $I_f$ channels activate once again, allowing for a new entry of positive charges, thus repeating the process.

Although the presence of $I_f$ in sinus nodal cells has been clearly documented, some authors have argued that the current provided by $I_f$ in the voltage range of the sinus nodal pacemaker potential is not large enough to solely account for spontaneous pacemaker depolarization. An additional current during phase 4 depolarization may result from the combination of the closure of potassium channels and the activation of calcium currents. The latter hypothesis presumes the existence of a background (time-independent) current with a reversal potential near the equilibrium potential for sodium (i.e., in the positive voltage range). According to this hypothesis, the total membrane current results from the balance of currents moving through these opposing conductances. During repolarization, the outward current is larger than the background inward component. However, the progressive closure of potassium channels during repolarization is thought to unmask the depolarizing force of the background inward current. Depolarization then progresses following largely the time course of potassium current deactivation. Calcium channels are thus activated by the membrane depolarization, eventually eliciting an active response.

Of course, the mechanisms postulated here are not mutually exclusive. All individual currents ($I_f$, $I_K$, $I_{Ca}$, and $I_b$) depicted in Figure 1.18 may participate during the process of pacemaker activation. Moreover, because the SA node is a highly heterogeneous region, multiple action potential morphologies may be recorded, depending on the specific region of the node, including the center or the periphery. Thus, it is possible that pacemaking in cells with more negative diastolic potential is more dependent on $I_f$ than in other cells where the maximum diastolic potential is never larger than −60 mV.

### Atrioventricular Nodal Cells

Action potential morphology in the AV node varies widely, depending on the nodal region from which the action potentials are obtained. In the center or "N region" of the AV node, action potentials present characteristics similar to those described for the sinus nodal action potential. Briefly, as depicted in Figure 1.19, the maximum diastolic potential is more depolarized than in the ventricular or atrial cells, the action potential upstroke is slower and mostly dependent on $I_{Ca}$, and a pacemaker potential can be detected. The spontaneous rate of activation of the AV node is slower than that of the sinus node. Yet, in the absence of the sinus node, AV nodal cells can discharge spontaneously and act as the pacemaker for the entire heart. As illustrated in Figure 1.19, closer to the atrium, within the AN region of the AV node, the action potential morphology is somewhat intermediate between atrial and N cells, whereas in the NH region the action potential shape is intermediate between the N and His bundle cells.

### Purkinje Fibers

An important characteristic of cells within the His bundle and Purkinje network is their ability to generate spontaneous action potentials. The

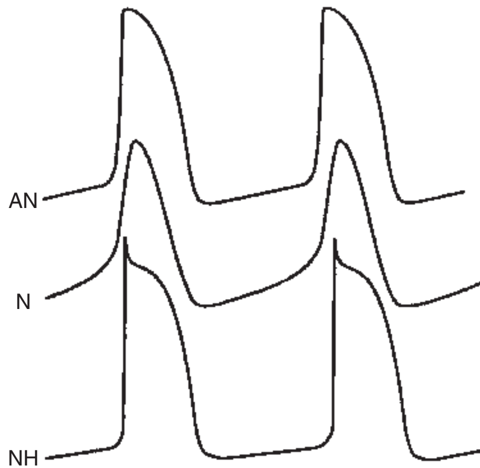



**Figure 1.19** Atrioventricular (AV) nodal action potential morphology. At least three different regions of the AV node can be identified electrophysiologically: the atrio-nodal (AN), the nodal (N), and the transitional region between the node and the His (NH). Action potential morphology varies among those regions.

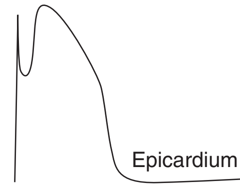
frequency at which His or Purkinje fibers fire their spontaneous discharges is significantly slower than in the nodes. These slow pacemaker cells can command the ventricles when activation of supraventricular origin fails. There is general agreement that the major pacemaker current of Purkinje fibers is $I_f$. As opposed to nodal cells, Purkinje cells are highly polarized; their action potential upstrokes are very fast and mediated by the fast sodium current.

### Regional Variations in Action Potential Morphology

Differences in action potential morphology may be detected between different regions of the ventricles. In particular, ventricular subendocardial cells have longer action potentials than subepicardial cells (Figure 1.20). These and other functional differences within the heart may help to explain regional differences in the electrophysiological effects of certain antiarrhythmic drugs as well as some arrhythmic patterns that result from reentrant circuits in ventricular tissue. The implications that ventricular heterogeneities may have in the generation of cardiac rhythm disturbances are discussed in Chapter 6.

## Voltage Clamp

In the preceding section, we discussed how, during the cardiac cycle, currents flow across membrane channels repeatedly changing the membrane potential. Study of the actual time and voltage dependence of individual transmembrane currents under such conditions is complicated by the dynamic nature of the voltage changes. The voltage-clamp technique allows for the control of



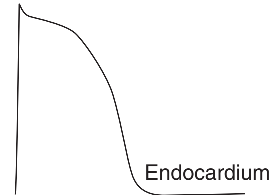




**Figure 1.20** Diverse action potential morphology in the ventricles. The shape of the action potential, as well as some of its intrinsic electric properties, varies across the myocardial wall. Most noticeable, the transition between phases 1 and 2 of the action potential varies between a "spike-and-dome" morphology in the epicardium to a smooth, progressively downward deflection in the endocardium.

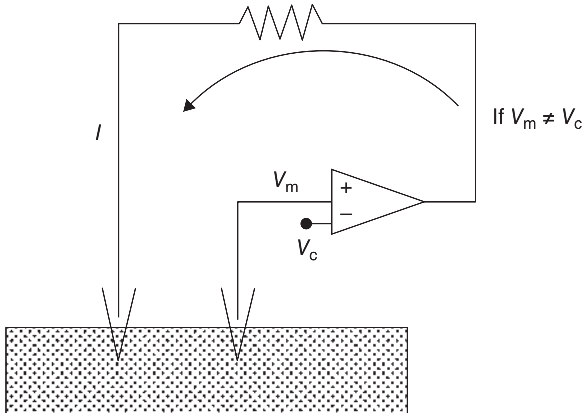



**Figure 1.21** The voltage-clamp circuit. A single cell (hatched area) is impaled by two microelectrodes. One records the membrane potential ($V_m$); the other delivers current (*I*). The recorded voltage is compared against a command potential ($V_c$). If $V_m$ is different from $V_c$, current is delivered into the cell. The current delivered by the electrode is equal and opposite to the current that moves through the cell membrane during a particular voltage pulse.

the membrane potential during specific periods of time and at preselected levels so that the biophysics of individual channels can be studied in detail.

Conceptually, the procedure is simple, and it is best explained using the configuration called "two-electrode voltage clamp" (see Figure 1.21). A fine-tipped glass microelectrode is introduced inside the cell to measure the voltage difference across the membrane ($V_m$). The reading of membrane potential is then compared, in an amplifier, with the value of potential chosen by the operator. This potential is usually referred to as the command voltage, or $V_c$. The ultimate goal is to make $V_m$ equal to $V_c$. Thus, if there is a difference

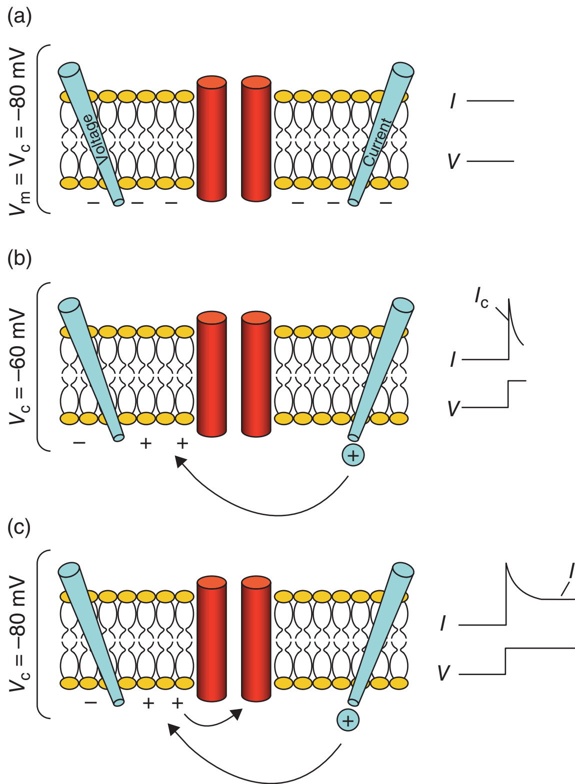



**Figure 1.22** Current transitions during a voltage-clamp step. A hypothetical cell membrane, with a hydrophilic channel in the center, is illustrated in all panels. (a) The cell is impaled by two electrodes (labeled voltage and current). Magnitudes of measured voltage and delivered current are shown on the right. $V_m$ equals the command potential, and no compensatory current is delivered by the circuit. (b) A voltage step to –60 mV requires the charge of the cell capacitor ($I_c$). (c) As positive charges leave the cell through the channel, they are compensated by the amplifier. Thus, the current delivered by the amplifier at this time is a reflection of the current escaping through the resistive channel ($I_r$).

between the two variables, the amplifier delivers current to the cell so that the membrane potential is clamped to the level of $V_c$. In the diagram, this current ($I$) is delivered by a second microelectrode placed also inside the cell.

Consider the simplest case of a cell membrane whose ion channels behave like ohmic resistors, as illustrated in Figure 1.22. Before the voltage clamp is turned on (panel a), the voltage electrode reads the membrane potential ($V_m$), at –80 mV. The investigator switches the voltage-clamp amplifier to exactly –80 mV. In this condition, $V_m$ and $V_c$ are identical, and, therefore, no current is necessary to make $V_m$ equal to $V_c$. The operator now changes $V_c$ to –60 mV (panel b). The amplifier reads the voltage difference and "injects" charge into the cell through the current electrode to force the membrane potential to

–60 mV. The current delivered by the amplifier will be used first to charge the membrane capacitance (recall that the membrane potential is the result of an electrochemical gradient across the membrane's lipid bilayer, which acts as the cell capacitor).

Most of the initial current injected by the amplifier therefore reflects the magnitude of current ($I_c$) needed to charge the membrane capacitance so that the voltage difference across the membrane is equal to –60 mV. In other words, the first change in the current tracing will be the capacitive transient. Current will also be moving through the ohmic resistor (the channel). The charge that moves across the channel would tend to discharge the capacitor, thus changing the membrane potential. Because the amplifier constantly compares $V_m$ with $V_c$, it injects current in response to any minor deviation. Thus, the constant "leak" of charges through the resistor is immediately compensated by an injection of current by the amplifier to maintain the membrane capacitor charged to the desired voltage (panel c). The same principle applies to the case of a channel that is non-ohmic. If, for example, the channel activates slowly with time (as in Figure 1.6), progressively more charge will leave the cell through the channel, thus displacing the membrane potential. In response, the amplifier compensates by injecting more current. Again, the current injected by the amplifier is equal to the current leaving the cell through the membrane channel. In other words, once the capacitive transient is over, the current flowing through the amplifier is equal to the current flowing through the ion channels in the membrane.

A clear limitation of the voltage-clamp technique becomes apparent from the previous discussion. Indeed, most of the current injected by the amplifier at the onset of the voltage clamp is used to charge (or discharge) the cell capacitor. Therefore, ionic currents that occur during this brief interval are not properly measured. This was a major stumbling block in measuring rapid events that take place across some ion channels (see Chapter 2). Fortunately, new technology as well as the use of various alternative voltage-clamp methods helped to partially solve this important problem.

Another factor to consider when designing voltage-clamp experiments is that the entire cell membrane under study must be fixed to the desired potential. If during the voltage clamp a voltage difference exists between one region of the cell (or fiber) and another, currents will flow not only across the membrane but also between the two regions that are not isopotential. Consider the extreme example presented in Figure 1.23. A long Purkinje fiber is impaled with voltage ($V$) and current ($I$) electrodes that are relatively close to each other. During a voltage-clamp step from –80 to –60 mV, the current injected by the amplifier changes the potential of the patch of membrane surrounding the voltage electrode; however, because of the length of the fiber, the membrane potential distal to the site of impalement may be different from that near the electrode. As a consequence, current moves axially along the fiber in an effort to maintain (unsuccessfully) the entire membrane at the same level of potential. In other words, the current injected by the amplifier not only

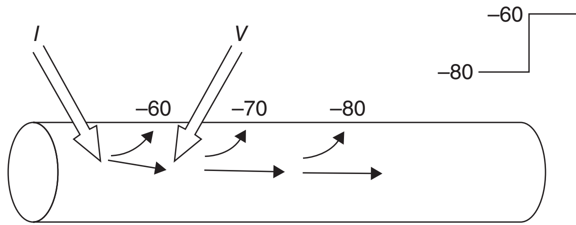



**Figure 1.23** Voltage clamp requires spatial voltage control. A segment of a long fiber is voltage clamped between a voltage (*V*) and a current (*I*) electrode. The command step is from –80 to –60 mV. Although the amplifier may measure –60 mV at the site of recording, the membrane potential would not be homogeneous throughout the fiber, due to the spatial decay (see "Cable Equations" in Chapter 4). A longitudinal voltage gradient would create a longitudinal current. Accordingly, the amplifier would provide current not only to compensate for the membrane current between the electrodes but for that moving horizontally along the fiber. This condition indicates poor voltage control.

reflects the current moving through the membrane, but also the undetermined amount of current that is moving along the fiber. We shall discuss the axial flow of current in greater detail in our discussion on propagation, in Chapter 4. Suffice to say here that the lack of isopotentiality in the preparation (i.e., the lack of spatial control) is an important problem in voltage clamping. Adequate spatial control is an essential requirement of success in a voltage-clamp experiment.

In small cells, it is possible to use voltage-clamp amplifiers that both measure potential and pass current through the same electrode. This technique, called single-electrode voltage clamp, offers the great advantage of requiring only one electrode impalement. The technique is limited by the magnitude of current that can be passed across the circuit in the absence of artifacts. Large cells (e.g., *Xenopus laevis* oocytes) require large amounts of current to achieve adequate voltage control. In those cases, a two-electrode voltage clamp (Figures 1.21–1.23) is required.

## Methods of Resolving Currents through Ion Channels

The ionic basis of cardiac electrical activity intrigued scientists for over a century. The initial attempts to understand the ionic bases of the cardiac impulse relied on microelectrode work carried out in isolated cardiac preparations. Although these initial studies provided important qualitative data on ionic currents, several limiting factors, for example, inadequate voltage-clamp conditions, made it difficult if not impossible to assign any meaningful functional significance to the currents measured.

Later advances, such as the development of the single-electrode voltage-clamp technique and the procedure for isolating single cardiac cells, made it possible to measure the current flowing across the membrane of an individual cardiac cell or from a single ion channel localized to a membrane patch. In

the single-electrode voltage clamp, a glass capillary tube is pulled over heat to a fine tip of approximately 1–4 μm in diameter, filled with saline, and connected to a high-gain, low-noise amplifier. This recording electrode or "patch pipette," as it is often referred to, is placed on the surface of the cardiac cell and a very high-resistance (in giga-ohms, GΩ) "seal" is formed between the pipette and a patch of the cell membrane, essentially excluding ions in the extracellular space from the patch pipette contents.

Several modalities of the single-electrode voltage-clamp technique were developed through the years. The most commonly used when studying ion currents across the entire cell is the whole-cell patch-clamp technique. In this approach, after the seal is formed between the electrode and the cell membrane, a gentle suction is applied, rupturing the cell membrane in the patch and gaining access to the cytoplasm (Figure 1.24a). Consequently, the internal pipette solution enters in direct contact with the intracellular space. The membrane of the entire cell is voltage clamped, and the recorded currents are a composite of the current flowing through all the active channels. A variation of the same procedure is the perforated-patch technique (Figure 1.24b). Here, the idea is to introduce in the pipette a specific pharmacologic agent (nystatin, amphotericin B) that creates large channels in the membrane under the patch. The seal, therefore, is not broken. Instead, the electrode gains electric access to the intracellular compartment through the channels perforated by the drug. Yet, the intracellular space is not disturbed.

In another modality, a glass pipette is sealed to the membrane but, in this case, the patch is not broken (Figure 1.24c). This procedure is commonly referred to as the cell-attached patch-clamp technique. In this case, the operator controls the membrane potential only of the patch of membrane sealed inside the diameter of the microscopic glass pipette tip. The great advantage of this procedure is that the recording is obtained only from a few channels (sometimes only from a single channel). In the cell-attached configuration, the intracellular space is kept intact. Although this is ideal for studying the channel in a more "physiological" condition, it does not allow direct manipulation of the concentration of ions or other molecules that may regulate the channel under study. The latter can be done by pulling the patch of membrane out of the cell without breaking the seal (Figure 1.24d). This variant, called the cell-excised patch clamp (or inside-out patch clamp, referring to the fact that the intracellular face of the membrane is now facing the outside solution), gives the investigator direct access to the cytoplasmic side of the channel. The drawback is that the channel studied under this condition is no longer in contact with its natural intracellular environment.

Finally, yet another modality of the patch-clamp technique is called outside-out patch clamp. In this case, a pipette is sealed to the membrane, and the membrane within the patch is broken, as in the whole-cell mode. However, once the whole-cell recording is formed, the pipette is gently pulled away from the cell (Figure 1.24e). The membrane that is sealed to the glass pipette retracts, and the ends of the retracted membrane fuse. The ultimate

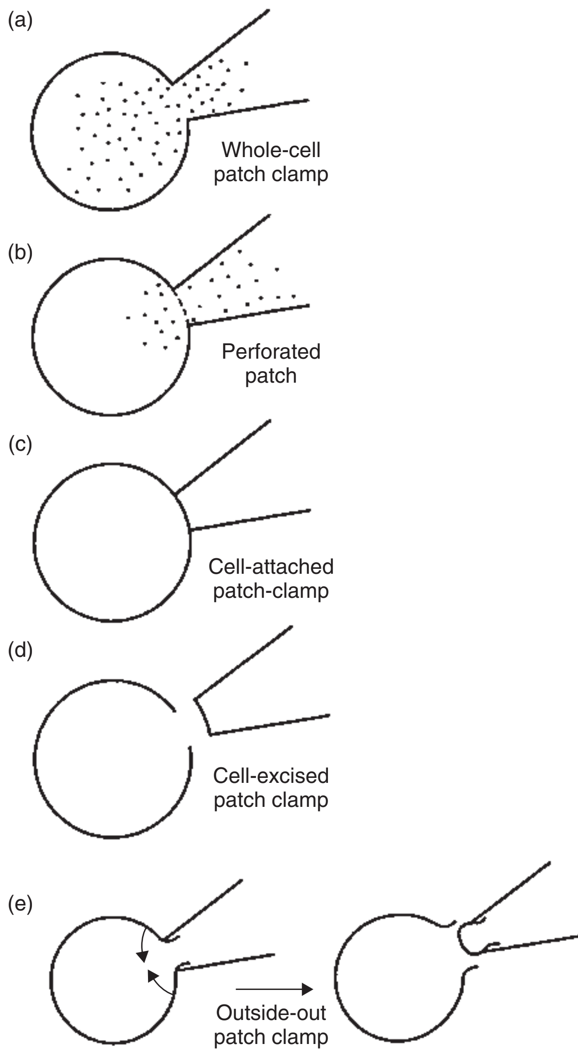



**Figure 1.24** Single-electrode voltage-clamp configurations. (a) In whole-cell patch clamp, there is continuity between the intracellular space and the pipette-filling solution. (b) Dialysis of the cytoplasm is avoided when the perforated-patch technique is used. (c) When the seal between the electrode and the cell membrane is not broken, the amplifier records the electric activity of the patch of membrane within the area under the electrode; single channel recordings are thus feasible (cell-attached patch clamp). (d) The patch can also be separated from the cell (cell-excised patch clamp), thus giving access to the cytoplasmic side of the channels under recording. (e) Finally, gently pulling the pipette in a whole-cell configuration may allow for the fusion of the membrane at the edges and formation of a small patch. In this case, the "outside" phase of the membrane faces the bath (outside-out patch clamp).

consequence is that a patch of membrane is isolated from the rest of the cell and the intracellular side exposed to the pipette-filling solution.

Each of these patch-clamp configurations has specific applications in the study of ion channels. They all have advantages as well as limitations. When properly used, such techniques can provide (and have provided) highly accurate information about the physiology and pharmacology of ion channels in the cardiac cell membrane.

## Some Basic Terminology

Now that we have explained the idea of the cell membrane as an RC circuit and have described some basic concepts on the study of the movement of electric currents across cell membranes, it is appropriate to define some basic terms that are used commonly in the field of cellular electrophysiology.

### Direction of Current Across Cell Membranes

As noted earlier, ions move constantly across the cell membrane and generate transmembrane currents. To define whether a current is inward or outward, we use the same convention as in electricity, i.e., we follow the direction of the positive charge. Thus a positively charged sodium ion (i.e., a cation) that moves from the outside to the inside of the cell creates an inward current. Similarly, a positively charged potassium ion (also cation) that moves from the inside of the cell to the outside creates an outward current because in this case the positive charge is moving outwardly. Inward or outward currents therefore indicate whether the direction of movement of positive charges is inward or outward, respectively. The issue can be a bit confusing when discussing chloride currents. In this case, a negatively charged ion, or anion, is moving across the membrane. Because by convention we follow the direction of positive charges, in the case of a chloride ion, the direction of current is opposite to the actual direction in which the ion moves. Thus, a chloride ion that moves from the inside of the cell to the outside creates an inward current (i.e., a negative charge leaving the cell is equal to a positive charge entering the cell). When tracings of cell currents are shown, inward currents are depicted as downward deflections, whereas outward currents are conventionally depicted as upward deflections.

### Components of a Voltage-Clamp Protocol

The most commonly used waveform to study ion currents is the square voltage pulse. Usually, pulses of varying amplitudes and/or durations are used to study the time and/or voltage dependence of the currents under investigation (e.g., see Figure 1.25). The membrane potential at which the cell is maintained between two consecutive pulses is referred to as the holding potential ($V_h$). Usually (though some exceptions apply), the cell is held at a membrane potential value at which the channel under study is closed and ready to be activated. The test potential, or command potential ($V_c$) is the

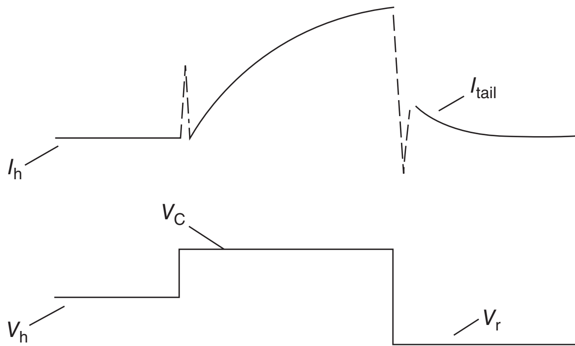



**Figure 1.25** Voltage-clamp parameters. A voltage command ($V_c$) is applied from a holding potential ($V_h$). The holding current ($I_h$) is the current recorded while the holding potential is maintained. The voltage command may be returned to a voltage ($V_r$) different from the holding potential. The time-dependent current after the end of the voltage command is referred to as the tail current ($I_{tail}$).

voltage at which the cell is clamped during the pulse. At the end of the pulse, the cell is voltage clamped to a return potential ($V_r$). In many instances, the return potential is the same as the holding potential. However, in some cases, such as that illustrated in Figure 1.25, the membrane potential is clamped at an intermediate value for a certain fraction of the time before it is brought back to $V_h$.

The current flowing through the membrane at the level of $V_h$ is referred to as the holding current ($I_h$); similarly, "pulse current" is the term used to describe the current that is activated by the test pulse. In many cases, when the membrane is switched back to the return potential, a remnant of current is observed. This remaining current is often called "tail current." Tail currents are often used to determine the conductance of the channel at the end of a specific test pulse.

## What Are the Differences Among Activation, Inactivation, Reactivation, and Deactivation?

These four terms are sometimes a source of confusion. In the case of whole-cell currents, activation refers to the increase in current amplitude during a voltage-clamp pulse. It is commonly used as an indirect measure of the opening of channels following a change in membrane potential. The term "inactivation" refers to a decrease in conductance that either follows or, in some instances, occurs concomitantly with activation of the channels. Activation and inactivation are well exemplified in the case of sodium currents, as illustrated in Figure 1.26. In this hypothetical experiment, a cell is voltage clamped from a holding potential of –100 to –20 mV. The membrane current recorded shows a rapid inward deflection (activation); even though the voltage pulse is still maintained, the current spontaneously returns to the baseline. Investigators refer to that phase of current reduction as inactivation.

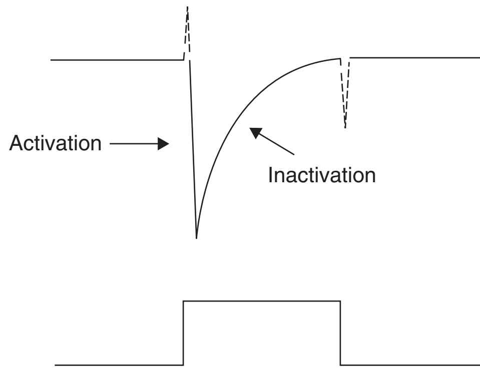



**Figure 1.26** Activation and inactivation. Top trace, the membrane current; bottom trace, voltage step. The dotted lines represent the capacitive transients. The increase in current amplitude with time is referred to as "activation." A spontaneous decrease in amplitude despite the presence of the same voltage gradient is called "current inactivation."

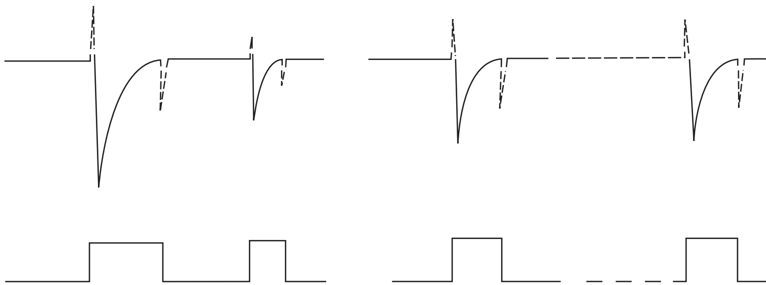


**Figure 1.27** Reactivation of a membrane current. Top trace, membrane current; bottom trace, membrane potential during a voltage-clamp protocol. Left panel: two voltage steps applied with short delay. Because of its prematurity, the amplitude of the current elicited by the second step is smaller than that generated by the first one. Right panel: prolonging the delay between the two voltage commands yields two currents of equal amplitude. The process of recovery of a membrane current from inactivation is referred to as "reactivation."

On the other hand, reactivation is the repriming of the channel from its inactivated state to a state where it can be activated again. This is illustrated in Figure 1.27, where we also use the sodium current as an example. When a second pulse is applied immediately following the first one, the amplitude of the elicited current is smaller because the channels are not ready to be activated again. However, when the delay between the first and second pulses is prolonged, the amplitude of the elicited current is the same for both pulses. Once again, this process is called reactivation. Finally, some currents, such as those shown in Figure 1.25, do not seem to inactivate (at least not completely). In that case, a deactivation process is observed ($I_{tail}$) when the membrane voltage is returned from the pulse potential, to a voltage value in which the channel would not be activated (e.g., the holding potential).

## Summary

We discussed some of the basic principles for applying electric concepts to the movement of ions across cell membranes. We established that the transmembrane flow of ions leads to electric currents and the displacement of charges across the cell membrane capacitor establishes the membrane potential. Some fundamental principles that determine the electric properties of the cell at rest, and during activation, were also reviewed. In the chapters that follow, we make repeated use of these concepts when discussing the properties of various membrane currents and the propagation of currents along tissues in the normal as well as the diseased heart.