Basic Cardiac Electrophysiology: Excitable Membranes

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Chapter 2
Basic Cardiac Electrophysiology: Excitable Membranes

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Abstract Cardiomyocytes are excitable cells that have the ability to contract after excitation; therefore, each heartbeat is an event of electrical–mechanical coupling. Cardiac electrical activity at different levels can be measured through variable means and modified by different drugs or medical devices. Understanding the basic mechanisms of cardiac excitation is essential not only to a physiologist, but also to a cardiologist, because cardiac arrhythmias are a major health issue in our society and clinical practice. Diagnosis and therapy of arrhythmias requires understanding the cause or origin of each arrhythmia and making decisions to control or eliminate the arrhythmia. Advances in basic research enhance our understanding of normal cell, tissue, and organ function (physiology) and also disease processes (pathophysiology), and hopefully lead to better clinical diagnosis and improved clinical therapies, either directly or indirectly. Cardiomyocytes are the main component of a heart. Their electrical activity is fundamentally a bioprocess determined by the transmembrane potential, a voltage difference between the intracellular and extracellular compartments. During a normal cardiac cycle, mechanical contraction always follows electrical excitation. This chapter provides a basic overview of membrane excitability of cardiomyocytes and other excitable cells (i.e., neuronal and skeletal).

2.1 Introduction

The most critical job of the heart is to expel blood to the whole body, and transport critical nutrients to and remove waste products from the tissues. The heart is a muscular pump with rhythmic electrical activity and muscle contraction. Cardiac electrical activity results from fluxes of sodium, potassium, calcium, and chloride ions across the cell membrane. These ion fluxes, or membrane currents, can be measured and analyzed at the level of single channel or whole cell by patch clamp, or whole body by ECG.

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The main mechanism of cardiac excitation and contraction is the excitability of the cell membrane, which is the term used to describe the ability of a cell to depolarize or be depolarized to initiate an action potential. Membrane excitability depends on the action of ion channels, ion pumps, and ion transporters or exchangers embedded in the lipid bilayer surrounding a cell. An external stimulus delivered to an excitable cell causes a rise in the resting membrane potential or the voltage gradient across the membrane. Once a critical level, or threshold, is reached, the membrane permeability to ions is altered, causing a depolarizing inward current and initiating an action potential.[1] This property is not unique to cardiac cells but is shared by neurons as well as skeletal and smooth muscle cells. However, non-excitable cells cannot be depolarized to induce an action potential either with or without stimulation.

Before delving into the characteristics of the cardiac action potential, one needs to understand the basic concepts of membrane structures, ion channels, and ion currents contributing to the action potential. The following sections describe how ion channels affect membrane excitability.

2.2 Cell Membrane

The cell membrane is composed of a phospholipid bilayer with its hydrophobic interior and various proteins inserted (Fig. 2.1a). The lipid bilayer itself is impermeable to ions, but embedded channel proteins in the membrane can selectively allow passage of ions.[2] The plasma membrane serves as a barrier to maintain the ionic concentrations in the intra- and extracellular compartment. Due to channel ion selectivity, the compositions of fluids inside and outside the cell are different (Table 2.1). The cell membrane has a charge across it, a transmembrane potential, and is polarized (Fig. 2.1b). The inside of excitable cells is more negative than the outside, resulting in an overall negative potential. Depolarization occurs when the negativity of the transmembrane potential is reduced, while repolarization is the restoration of the resting membrane potential.

Depolarization and repolarization are effectively modulated by a series of ion fluxes across the cell membrane in which are embedded numerous proteins. Some of them span the cell membrane to form pores or channels allowing the passive or active transport of ions. Each type of ion channel is highly selective to permit only one or more specific ions (Fig. 2.1a). Two factors affecting passive diffusion through the membrane are electric potential and ion concentration, or the electrochemical gradient. When the ion concentration is greater in one region, the tendency is for the ions to move to the area of lower concentration due to diffusion forces. Similarly, movement of ions is promoted by electrical forces. Like a capacitor, excitable membranes store energy in the form of electrochemical gradients that can be discharged to transmit electrical signals in tissues. In the electric circuit analogy, the pores form a conductive branch in parallel with the capacitive lipid bilayer (Fig. 2.1b). Ohm's law, which defines the relationship
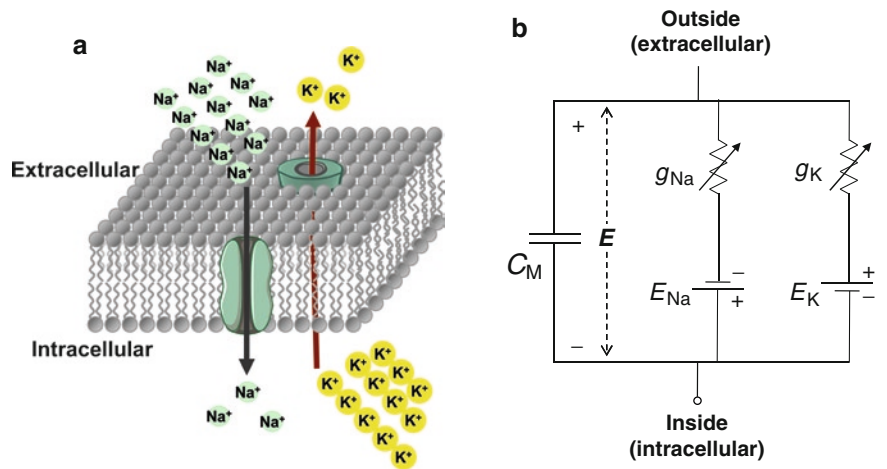
Fig. 2.1 Three-dimensional view of a representative section of a schematic plasma membrane with embedded proteins, such as Na$^+$ channel and K$^+$ channel (**a**) and an equivalent circuit (**b**). The cell membrane acts like an equivalent circuit with a capacitive branch (C_M), representative of the lipid bilayer, and with conductive branches (g_{Na} and g_K), representative of sodium – and potassium-selective pores, as well as the corresponding electromotive forces (E_{Na} and E_K), representative of the sodium and potassium equilibrium membrane potentials

Table 2.1 Major ionic components of intra- and extracellular fluids and equilibrium potentials of major ions

Ion	Intracellular concentration (mEq/L)	Extracellular concentration (mEq/L)	Equilibrium potential (mV)
Na$^+$	15	140	70
K$^+$	135	4	−94
Ca^{2+}	2×10^{-4}	4	132
Mg^{2+}	40	2	
Cl$^-$	4	120	−90
HCO$_3^-$	10	24	
HPO$_4^{2-}$	20	4	
SO$_4^{2-}$	4	1	
Proteins$^-$, Amino acids$^-$, Urea, etc.	152	1	

between current, voltage, and conductance or resistance in an electric circuit, also governs in describing the electrical properties of membranes.[3]

In addition to ion movement down the electrochemical gradient through ion channels, intracellular concentrations of sodium, potassium, and calcium are maintained by active transport via pumps spanning the cell membrane. The sodium–potassium exchange pump is electrogenic, meaning it creates an electrical potential

across the cell membrane and generates a large concentration gradient for sodium and potassium. Sodium is pumped outside the cell at a ratio of 3:2 with potassium being pumped inside the cell, leaving a deficit of positive ions inside the cell. Additionally, calcium pumps and the sodium–calcium exchanger transport calcium ions out of the cytoplasm to achieve a low intracellular calcium concentration.

2.3   Membrane Electrophysiology

2.3.1   Resting Membrane Potential

The origin of resting and action potentials lies in the relative concentrations inside and outside the cell and in the permeability of the membrane to that ion. The resting potential is the transmembrane potential that exists when the electrical and chemical forces are exactly equal and there is no net movement of ions across the cell membrane. In most cardiac cells, the typical resting membrane potential is about −85 to −90 mV (Fig. 2.2). In skeletal muscle and nerve, the resting potential is −95
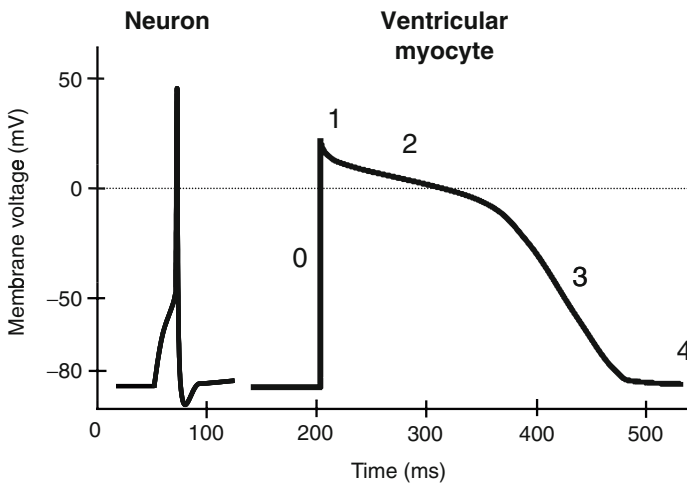
Fig. 2.2 Representative action potentials (APs) of a neuron and ventricular myocyte. Note the morphological differences between the two APs. Regular contractile myocytes have a true resting membrane potential (about −90 mV) at phase 4 in the panel that remains constant near the equilibrium potential for K^+ (E_K). When an AP is initiated, there is a rapid depolarization phase (phase 0) which is caused by activation of fast Na^+ channels. Phase 1 is an initial repolarization due to the opening of transient outward K^+ channels (I_{to}). However, because of the large increase in the inward L-type Ca^{++} currents at the same time and the transient nature of I_{to}, the repolarization is delayed with a plateau (phase 2). This plateau phase prolongs the AP duration and distinguishes the cardiac AP from the much shorter AP in neuron. Continuous repolarization activates another type of K^+ channel (I_K) and, along with the inactivation of Ca^{++} channels, leads to the rapid repolarization (phase 3)

and −90 mV, respectively, while in smooth muscle it is −50 to −60 mV.[4] The terms resting potential and action potential are reserved to describe the transmembrane potential of excitable cells. Non-excitable cells, such as fibroblasts and endothelial cells, cannot initiate and propagate an action potential and thus do not have a corresponding resting potential.

2.3.2   Equilibrium Potential and the Nernst Equation

The flow of ions across the cell membrane is determined by the ion concentration, or chemical gradient, as well as the electrical gradient. For any given ion, the concentration gradient across the cell membrane promotes diffusion of that ion. The electrical gradient across the membrane that prevents the net diffusion of the ion is called the equilibrium potential for that ion. The equilibrium potential is also known as the Nernst potential because it is calculated with the Nernst equation, which is valid for any permeant in a steady state.

$$V = -\frac{RT}{zF} \ln \frac{C_i}{C_o} = -61.5 \cdot \log \frac{C_i}{C_o}, T = 37°C \qquad (2.1)$$

Equation (2.1), Nernst Equation, where V is the membrane potential, R the gas constant, T the absolute temperature, z the valence of the ion, F the Faraday constant, and C_o and C_i the concentration of the ion outside and inside the cell.

When the cell membrane is permeable to multiple ions, the membrane potential that prevents the net diffusion of any of the permeant ions across the membrane is determined by the Goldman–Hodgkin–Katz equation. For a membrane permeable to sodium, potassium, and chloride, the membrane potential is defined as follows:

$$V_m = -61.5 \cdot \log \frac{P_{K^+}[K^+]_i + P_{Na^+}[Na^+]_i + P_{Cl^-}[Cl^-]_o}{P_{K^+}[K^+]_o + + P_{Na^+}[Na^+]_o + P_{Cl^-}[Cl^-]_i} \qquad (2.2)$$

Equation (2.2), Goldman–Hodgkin–Katz equation for the membrane potential across a cell membrane permeable to K^+, Na^+, and Cl^-. P=membrane permeability (cm/s); V_m is in mV.

The equilibrium potentials for the common ions in cardiac muscle are shown in Table 2.1. By evaluating the equilibrium potentials of various ions, we understand where active transport processes come into play. The equilibrium potential for potassium is almost exactly equal to the resting membrane potential, which prevents the movement of potassium outside the cell. However, for sodium, both the concentration gradient and the membrane potential favor movement of sodium inside the cell. Based on the Nernst and Goldman equations, the electrochemical gradients cause diffusion of sodium into the cell, which should lead to steady-state conditions. However, the sodium–potassium exchanger offsets diffusion by continuously pumping sodium out and potassium into the cell.

2.3.3   Ion Channels and Membrane Currents

Ion channels embedded in the cell membrane are proteins that have the ability to transport ions across the membrane, but their permeability to ions is highly selective. Each type of channel only allows passage of one or more specific ions. In excitable membranes, the three major classes of cation-selective ion channels are sodium, potassium, and calcium. Permeability of the channels is controlled by gates that open and close in response to changes in voltage potential (voltage gating) or chemical binding (chemical or ligand gating). While some types of potassium channels are ligand gated, the potassium, sodium, and calcium currents contributing to the action potential in normal hearts are regulated by voltage-gated channels. At the resting membrane potential (−85 to −90 mV for most cardiomyocytes), the majority of potassium channels are in the open state, but sodium channels are closed.[5] Opening of the voltage-gated sodium channel in response to a less negative cytoplasm (threshold) causes a large amount of sodium to enter the cell and is the basis for initiation of an action potential. Table 2.2 summarizes the major electrophysiological properties of excitable membranes of nerve, cardiac myocyte, skeletal, and smooth muscle cells. Chapter 3 further details the gating properties of ion channels.

By convention, inward currents are defined as movement of positive charge, such as Na^+, into the cell or negative charge, such as Cl^-, out of the cell, whereas outward currents are generated by positive charge leaving the cell or by negative charge moving into the cell. Because the inside of the cell is negatively charged at rest, inward currents are considered depolarizing and outward currents are repolarizing. Actually, many different ions dynamically move in and out of a cell at the same time during an action potential. Therefore, the instant membrane potential at a distinct time in an action potential is determined by the sum of all ion movements across the membrane.

2.3.4   Action Potential

The most efficient signal conduction in excitable tissue is the initiation and propagation of action potentials. When an excitatory stimulus causes the membrane potential to become less negative and beyond a threshold level, the permeability of the

Table 2.2 Major electrophysiological properties of excitable membranes

Cell type	Resting membrane potential (mV)	Action potential duration (ms)	Conduction velocity (m/s)
Nerve			
Large myelinated	−80 to −90	0.2–1	100
Muscle			
Cardiac	−85 to −90	200–300	0.3–0.5
Skeletal	−80 to −90	1–5	3–5
Smooth	−50 to −60	10–50	

membrane to ions changes, the cell rapidly depolarizes, and the membrane potential reverses transiently prior to repolarization. This process describes the action potential that includes depolarization and repolarization (Fig. 2.2). Unlike skeletal muscle, cardiac muscle is electrically coupled so that the wave of depolarization propagates from one cell to the next, independent of neuronal input. A significant amount of what we know about action potentials today is based on the seminal studies by A. L. Hodgkin and A. F. Huxley in the squid giant axon in the 1950s[6] and, more recently, by Luo and Rudy[7,8] in the ventricular myocyte. In the classical experiments published by Hodgkin and Huxley, the timing and amplitude of the ionic currents contributing to the squid axon action potential were measured in voltage clamp experiments and characterized mathematically. The Luo–Rudy model was built on the Hodgkin–Huxley model and characterizes the ionic contributions to the ventricular cardiac action potential. These models are discussed further in Chapter 7 of this book.

Morphologically, cardiac action potential has its unique characteristics. Figure 2.2 compares the action potentials of a neuron and ventricular cardiomyocyte. One of the distinguishing characteristics of the cardiac action potential is the plateau and longer action potential duration. In nerve fibers, the action potential is of a short duration (0.2–1 ms), whereas the cardiac action potential is typically 200–300 ms in human cardiomyocytes. Skeletal muscle action potential is similar to the nerve action potential in shape and duration (1–5 ms). These major differences from skeletal and neuronal cells can be explained by contributions of calcium currents in the cardiac action potential. The plateau and longer action potential duration are critical for cardiomyocytes, because myocardial contraction is more dependent on extracellular Ca^{2+} to enter into cells. Table 2.3 summarizes the main ion fluxes across the cardiac plasma membrane during an action potential. In addition, the ionic contributions to the cardiac action potential are discussed in more detail in Chapter 3 of this book.

2.3.5   Refractoriness

The ability of the heart to respond to a stimulus depends on the time that elapsed since its last contraction.[5] There are four states of refractoriness throughout the action potential (Fig. 2.3): (1) absolute refractory period (ARP) or effective refractory period (ERP), which is the period during which an electrical stimulus, no matter how great the stimulus, will not elicit an action potential because the membrane is not sufficiently repolarized and sodium channels have not completely recovered from the inactivated state; (2) relative refractory period (RRP), which requires a larger stimulus to initiate an action potential; (3) supernormal period (SNP), during which the threshold is lower than typically required to generate an action potential; and (4) normal excitability.[5] During RRP and SNP, the cell is slower to depolarize and the amplitude and duration of the action potential are smaller than those elicited after the cell has fully repolarized. Full recovery time, or

Table 2.3 Major ion fluxes across cardiac plasma membrane during action potential

Current	Selective ion	Current direction	Phase	Effect
I_{Na}	Na$^+$	Inward	0	Depolarization
$I_{Ca,T}$	Ca^{2+}	Inward	0	Depolarization
I_{to}	K$^+$	Outward	1	Early repolarization
$I_{Ca,L}$	Ca^{2+}	Inward	2	Plateau
I_{Kr}	K$^+$	Outward	3	Repolarization
I_{Ks}	K$^+$	Outward	3	Repolarization
I_{K1}	K$^+$	Outward	4	Resting potential
I_{f}	K$^+$/Na$^+$	Inward	4	Depolarization/ pacemaking

Source: Modified from Katz[9] and Whalley[10]
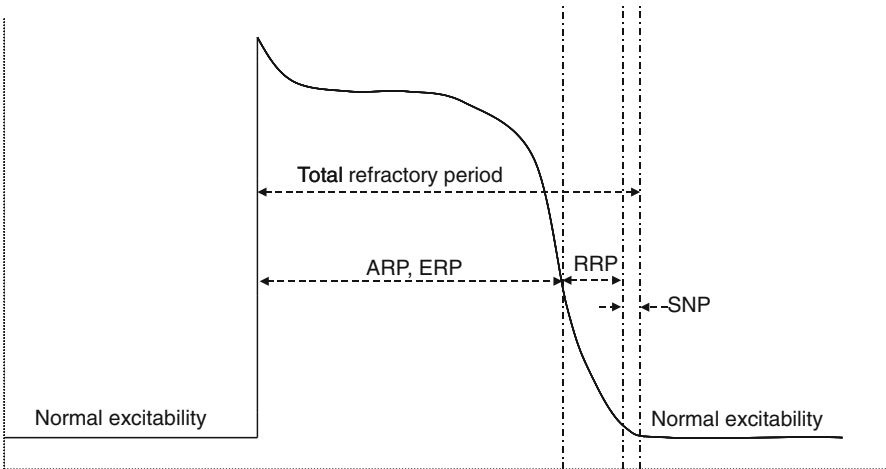
Fig. 2.3 Cardiac AP and refractory period. The total refractory period is comprised of the (1) absolute refractory period (ARP) or effective refractory period (ERP), which is the period during which an electrical stimulus will not elicit an AP because the membrane is not sufficiently repolarized and sodium channels have not completely recovered; (2) relative refractory period (RRP), which requires a larger stimulus; and (3) supernormal period (SNP), during which the threshold is lower than typically required to generate an AP. Normal excitability exists outside of the total refractory period. See Chap. 3 for a detailed discussion of cardiac action potentials

the total refractory period, encompasses the ARP, RRP, and SNP.[9] The membrane responsiveness to electrical stimulus is different in the periods of ARP, RRP, SNP and normal state (Fig. 2.4). The duration of the total refractory period varies with the time course of repolarization, which is shorter in nerve and other types of muscle cells. Duration also varies in the region of the heart with atrial being shorter than ventricular. It also decreases as heart rate increases. The activity of the autonomic system can significantly modify the duration of the cardiac refractory period.
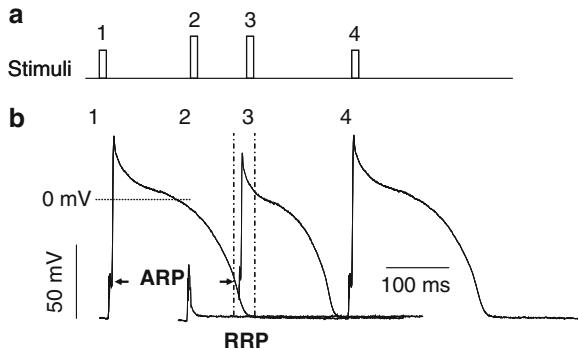
Fig. 2.4 Responsiveness of canine ventricular myocytes to electrical stimulation. (**a**) Marks of stimulation pulses delivered at different time periods (1, 2, 3, and 4) during a cardiac cycle. (**b**) APs initiated by the stimuli delivered at the times corresponding to those in panel (**a**). A pulse stimulus initiated an action potential (1). If a pulse fell in the absolute refractory period (ARP with the *arrows*) of an AP, no AP was induced even with super-threshold stimulation (2). During the relative refractory period (RRP with *dotted lines*), an AP could be generated after super-threshold stimulation (3). After full recovery, a normal AP (4) was initiated with the threshold stimulation as in (1). The notch on the upstroke of the depolarization phase of the AP was a stimulation artifact

2.3.6 Excitation–Contraction Coupling

Excitation of healthy cardiomyocytes always is followed by their mechanical contraction. Excitation–contraction coupling is a biological process in which an action potential triggers a cardiac myocyte to mechanically contract. Therefore, it is a process whereby an electrical signal (i.e., action potential) is converted to a mechanical process (i.e., myocyte contraction). While this process can be explained on a single myocyte level, in normal physiology, the process is orchestrated and coordinated at a whole organ level through the coordinated depolarization and repolarization of the entire heart, which starts with the depolarization of the sinoatrial node and ultimately leads to depolarization and subsequent contraction of the atria and ventricles. This is facilitated by the strong coupling of the cardiac myocytes and the cells of the conduction system via gap junctions. The key contractile proteins in the heart are actin and myosin, while troponins are the key regulatory proteins. Troponin I inhibits the actin binding site of myosin during diastole where cytosolic calcium concentrations are very low. Once an action potential depolarizes the cellular membrane, calcium ions enter the cell during phase 2 of the action potential through voltage-gated (L-type) calcium channels. This calcium triggers further release of calcium to the cytoplasmic compartment from the sarcoplasmic reticulum (SR) through ryanodine receptors. The free calcium then binds to troponin C. Troponin C induces a conformational change in the regulatory complex, thereby enabling troponin I to expose a site on the actin molecule. The actin molecule is then able to bind to the myosin ATPase located on the myosin head. The binding subsequently leads to hydrolysis of ATP, and a conformational change occurs in the
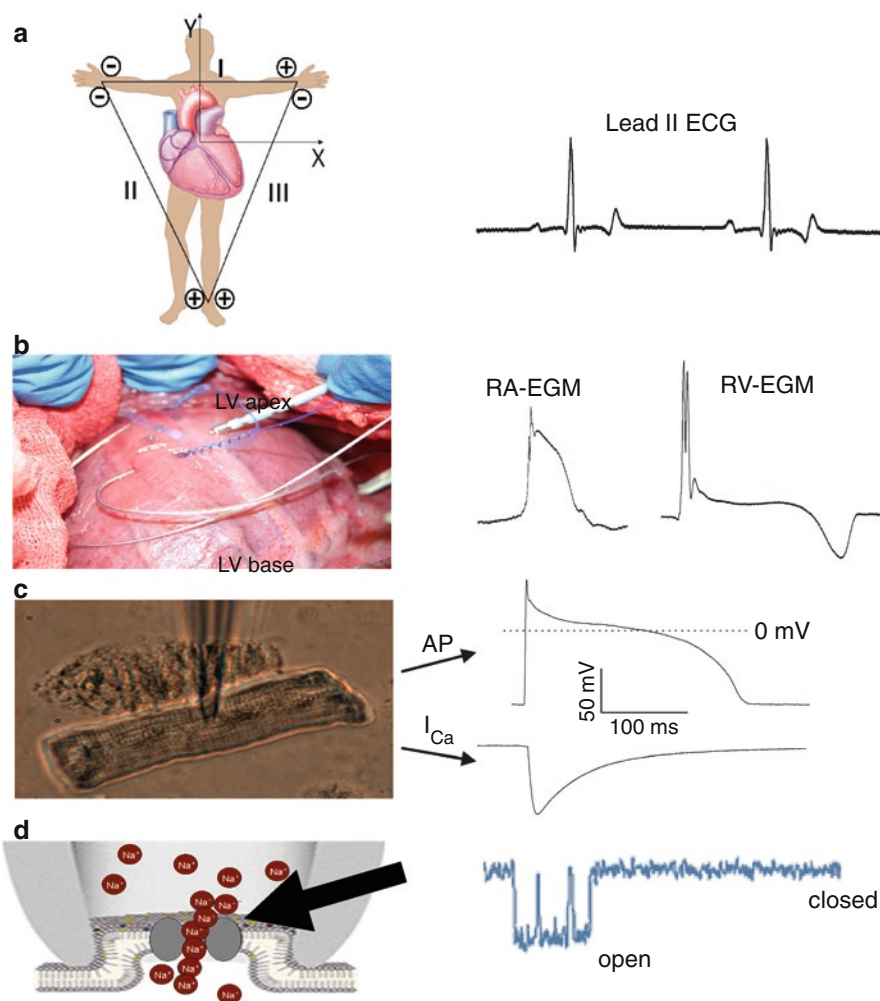
Fig. 2.5 Different levels of cardiac electrophysiological recordings. (**a**) Standard limb lead configuration forming Einthoven's triangle (*left panel*) and electrocardiogram (ECG) signal from Lead II (*right panel*) (adapted from www.cvrti.utah.edu/~macleod/bioen/be6000/labnotes/ecg/figures/limbleads.jpg. (**b**) Epicardial mapping of the canine left ventricle to record local electrograms at the tissue level (*left panel*) and endocardial electrogram recorded from the right atrium (RA-EGM) or from the right ventricle (RV-EGM) with placement of a Medtronic modified 3830 lead with 4-mm tip-to-ring spacing to a porcine heart (*right panel*). (**c**) Whole-cell patch clamp of a human ventricular myocyte (*left panel*) isolated from disposed biopsy tissue; morphology of ventricular AP and inward L-type Ca^{2+} current trace recorded by the whole-cell patch clamp method (*right panels*). (**d**) Schematic diagram of single channel recording configuration and inward single channel currents after a voltage pulse

actin–myosin complex. The result of these changes is a mechanical movement between the myosin heads and the actin, and a shortening of the sarcomere. At the end of the phase 2 of the action potential, calcium entry slows and calcium is

removed from the SR via an ATP-dependent calcium pump (SERCA ATPase). This eventually lowers the cytosolic calcium concentration and removes calcium from troponin C. Calcium is also being removed from the cell by the sodium–calcium-exchanger pump (NCX). A conformational change in the troponin complex is being induced by the reduced cytosolic calcium, leading to troponin I inhibition of the actin binding site. At the end of the cycle, a new ATP binds to the myosin head, displaces ADP, restores the initial sarcomere length, and the cycle starts over.

2.4 Summary

The membrane of cardiomyocytes is excitable. Cardiac electrical activities can be measured and quantified at different levels (Fig. 2.5). The fundamental mechanism of this excitability is due to impermeability to ions of the lipid bilayer and highly selective permeability to ions of the proteins embedded in the membrane. As selective passage of ions across the plasma membrane, the components of ions are differently distributed in the intra- and extracellular compartments. Such imbalanced distribution in the resting state causes more negative than positive ions in the intracellular compartment and results in a transmembrane potential of −85 to −90 mV in most cardiomyocytes. Electrical, chemical, or mechanical stimulation can depolarize cardiomyocytes to a threshold level and initiate an action potential. In the normal heart, each electrical activity is followed by a mechanical contraction. However, abnormalities of initiation and conduction of electrical impulses in a heart can lead to arrhythmias and mechanical failure. More accurate diagnosis and proper therapy of electrical abnormalities of the heart require current knowledge of basic cardiac electrophysiology.

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