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Basic Physiology of Ion Channel Function

Isabelle Baró and Denis Escande[†]

Introduction

It is a common habit that the first chapter of a book on electrical diseases deals with the basic principle of cardiac cellular electrophysiology. It is also common behavior that readers (almost a rule if they are clinicians) skip this chapter to directly enter what they consider the heart of the matter: clinical rhythmology. However, skipping the first chapter would be a great mistake. In cardiac electrophysiology, there is a continuum of concept between the function of ion channel molecules and the clinical phenotype (Figure 1–1). As an example, it would be almost impossible to understand heritable or iatrogenic cardiac channelopathies without knowing what an action potential is and how it is formed. The beauty of cardiac electrophysiology is that it is the same elementary electrical signal arising from billion of single channel proteins that is summed up at the level of a single cell to generate action potentials and is also summed up in time and space to generate a surface electrocardiogram (EKG) signal. Thus cardiac electrophysiology offers the unique opportunity to obtain different levels of view of the same phenomenon either nanoscopically (at the level of a channel pore) or macroscopically (at the level of the whole organ). The present chapter aims to provide the clinical cardiologist specialized in arrhythmias with the very minimum that should be known about ion channel function to understand serenely the mechanisms generating these either acquired or inherited arrhythmias and the fundamentals of antiarrhythmic drug therapy.

[†] Deceased.

Basic Principles of Cardiac Electrophysiology: From Ion Channels to Ion Currents, Action Potentials, and EKG

Ion Channels

The cell membrane is made of lipids and as such is a perfectly hydrophobic milieu, which hydrophilic ions cannot directly cross. To penetrate the cell membrane, ions need to find hydrophilic pathways, which are formed by specialized proteins (the ion channels). It happens that the ion channel pathway is not permanently available, but inversely flips between an open and a closed state. Once a hydrophilic pathway is available (the channel is open), ions move passively across the cell membrane depending on their respective electrochemical gradient (Figure 1–2). If the gradient for a given ion species is directed inward then ions enter the cell. If the gradient is outward then ions leave the cell. "Electrochemical" means that two independent forces can move ions across the membrane: the electrical gradient and the chemical gradient. The chemical gradient makes ions move from a compartment of higher concentration to a compartment of lower concentration (according to their chemical gradient K^+ ions are keen to move from the intracellular to the extracellular compartment; inversely Na^+ ions are keen to move from the extracellular to the intracellular compartment). The electrical gradient makes ions move in the direction of their inverse sign (negative for a cation and positive for

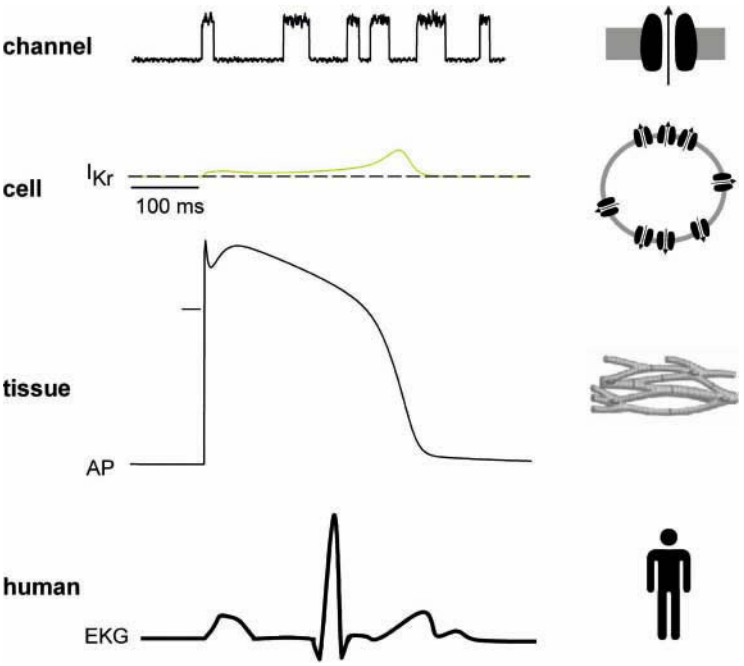

Figure 1–1. Schematic cardiac electrical activity, from molecule to patient. Channel: single channel current versus time recorded using the patch-clamp technique; upward inflection of the signal indicates outward current through a single channel. Cell: outward K^+ current through the open K^+ channels present on the cell membrane. Tissue: action potential resulting from the activity of all the different channels of a myocyte, recorded using the voltage-clamp technique. Human: EKG resuming the electrical activity of the different regions of the heart.

an anion). A negatively charged compartment (i.e., a compartment with a deficit in positive charges) will attract cations but reject anions. In some instances, the electrical gradient and the chemical gradient can oppose each other and eventually be equal; in this situation the force promoting the move of an ion in one direction equals that promoting its move in the reverse direction. An equilibrium is so reached. The electrical gradient depends on the transmembrane potential. Therefore, there is a transmembrane potential value for which the electrical gradient perfectly opposes the chemical gradient and permits the equilibrium of an ion species. In a cardiac cell, the equilibrium potential is around −98 mV for K^+ ions, i.e., at −98 mV (inside negative compared to the outside), the force related to the chemical gradient (directed outward) equals the force related to the electrical gradient (directed inward). The equilibrium (or reverse) potential for each ion species is given by the Nernst equation.*

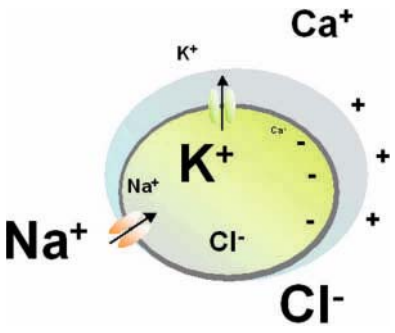

Figure 1–2. Schematic representation of the ion and electrical transmembrane gradient of the cardiomyocyte. Pole size is correlated with the ion concentration.

Membrane Resting Potential

A membrane that would exclusively conduct an ion species (i.e., K^+ ions), polarizes at the equilibrium potential for this ion species (i.e., −98 mV for K^+ ions). It happens that in a cardiac cell, the sodium–potassium pump enriches the cell with potassium and depletes the cell with sodium. It also happens that a cardiac cell at rest is

*$Ei = R\ T/zF$ ln(Extra [i]/Intra [i]) where Ei is the equilibrium potential for ion I, R the thermodynamic gas constant, T is the absolute temperature, z is the charge/valence of ion I, F is the Faraday constant (96485.309 C/mol), Extra [i] is the extracellular concentration of ion i, and Intra [i] is the intracellular concentration of ion i.

predominantly permeable to K^+ and thus the resting membrane potential of −80 mV is closed to the K^+ equilibrium potential. In general, a membrane potential depends on its relative permeability to the different ion species. During the action potential (see below), the cardiac membrane potential is no longer exclusively permeable to K^+ ions but is also permeable to other ion species and therefore diverges from −80 mV.

Ohm's Law

One cannot escape Ohm's law: $U = RI$ where U is the voltage, I is the current, and R is the resistance of the cell membrane. It means that currents generated by ion movements through ion channels surrounded by an electrical insulator (the cell membrane) affect the membrane potential depending on the membrane resistance. It happens that most ion channels expressed in the heart are voltage dependent, i.e., they open or close in relation to variations in the membrane voltage. In most cases, channels open when the cell depolarizes (inside less negative in relation to the outside). However, channel opening is not instantaneous but usually takes time (as much as hundreds of milliseconds in instances). Thus, most cardiac ion channels are both voltage and time dependent.

When an ion channel opens, it generates an ion current, which affects the voltage (Ohm's law). This is turn affects channel opening (voltage dependence) but not instantaneously (time dependence). Thus, the electrical activity of the cell should be considered in a tridimensional space: voltage, current, and time. If positively charged ions (i.e., cations) enter the cell, this movement creates an inward current that depolarizes the cell membrane (the inside is less negative). If positively charged ions leave the cell, this movement creates an outward current, which hyperpolarizes or repolarizes the cell membrane if previously depolarized (the inside is more negative).

Activation/Inactivation

Most cardiac ion channels are governed by two independent processes: activation and inactivation. The cardiac sodium channel is a caricature example (Figure 1–3). At the level of the resting potential (−80 mV), the driving force for sodium ions is clearly in the inward direction (both electrical and chemical gradients are inwardly directed; the equilibrium potential for sodium ions is around +70 mV), but because sodium channels are permanently closed, no current is generated. If the transmembrane potential is brought to values more positive than say −60 mV, sodium channels open (voltage dependence). It is said that they *activate* (a current is generated). Activation is not instantaneous but takes a few milliseconds (time dependence). Sodium channel activation creates an inward current that further depolarizes the cell. This in turn further recruits sodium channel activation (positive feedback). The positive feedback loop is interrupted when Na^+ channels *inactivate*. Indeed, if the membrane stays depolarized, sodium channels do not remain open but close spontaneously. It is said they *inactivate*, a process that is independent of activation. Thus, in response to membrane depolarization, sodium channels undergo rapid *activation* and

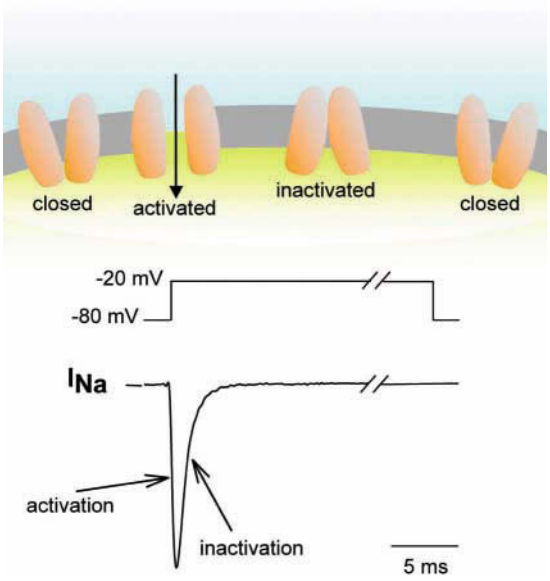

FIGURE 1–3. Activation and inactivation of the Na^+ current linked to the channel conformation. Schematic Na^+ current (bottom) recorded during a voltage pulse (middle). Activation of the current corresponds to an increasing number of Na^+ channels in the open conformation (top) due to the depolarization; inactivation corresponds to an increasing number of channels passed from the open to the inactivated conformation. Repolarization allows the inactivated channels to restore the closed conformation.

then (less) rapid *inactivation*. Because activation is faster than inactivation, sodium channels transiently generate an inward (i.e., depolarizing) current. If the membrane is subsequently repolarized to the resting potential, the activation gate closes (this process is called *deactivation*), whereas the inactivation gate reopens (this process is called *reactivation* or *removal of inactivation*): the channel is ready to open in response to a new depolarization stimulus.

Action Potential

Suppose that for a very few milliseconds the cell membrane is much more permeable to sodium than to potassium ions (a large number of Na^+ channels activates) (Figure 1–4). In this situation, the membrane potential is immediately attracted toward the equilibrium potential for Na^+ ions (about +70 mV). This occurs during the initial phase of the action potential (phase 0) where the membrane potential is abruptly driven to positive values (inside positive relative to outside). The membrane potential crosses the zero line (this is called an *overshoot*). Depolarization caused by activation of the Na^+ channel in turn activates other ion currents such as Ca^{2+} and K^+ currents (Figure 1–4), which have slower activation kinetics than Na^+ currents. Because the driving force for Ca^{2+} is inward (between 0 mV and the highly positive equilibrium potential for Ca^{2+} ions, chemical gradient attracts more Ca^{2+} into the cell than the electrical gradient limits its entry), activation of Ca^{2+} channels in response to depolarization generates an inward current, which maintains depolarization and (with other currents) creates the plateau phase of the action potential. Meanwhile, K^+ currents are also activated. In this depolarized state, the driving force for K^+ ions is outward (above 0 mV, both chemical and electrical gradients attract K^+ outside the cell). Activation of K^+ channels by membrane depolarization creates an outward current that tentatively repolarizes the cell membrane. During the plateau phase (phase 2), inward currents (mainly Ca^{2+}) equal outward currents (mainly K^+); the membrane potential is stable for a few tens of milliseconds. The Ca^{2+} current then progressively inactivates, whereas K^+ currents progressively activate, inducing the cell membrane to repolarize

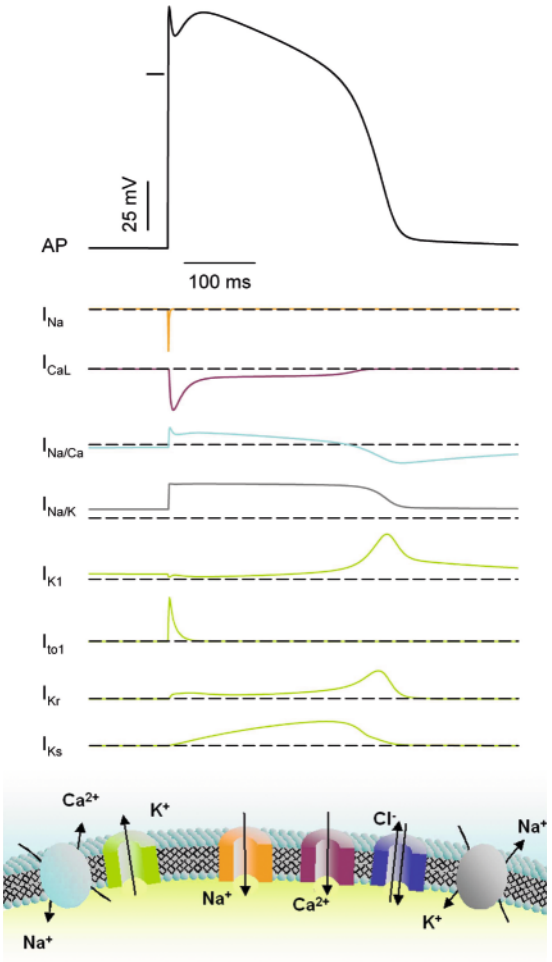

Figure 1–4. Schemes of the cardiac action potential and underlying ionic currents (top). This is a dynamic process: activation of the Na^+ channels generates the voltage upstroke, which activates the other ionic currents. In return, the action potential modulates the shape of the different currents. Bottom: the different ion channels and transporter species. Arrows represent the different current directions in the action potential voltage range.

(between 0 mV and –98 mV the chemical gradient attracts more K^+ outside the cell than the electrical gradient retains it) toward the equilibrium potential of K^+, which is about –98 mV. From what is presented above, it can be understood that if Na^+ channels are made to open (i.e., the membrane potential is brought to values more positive than say –60 mV), an action potential is generated. The membrane potential is brought positive to –60 mV because an adjacent cell electrically connected through gap junctions undergoes its own action

potential process. From action potentials to action potentials in adjacent cells, the electrical signal is conducted from the sinus node down to the ventricular myocardium. The membrane potential can also be brought positive to −60 mV by an external stimulus (e.g., a pacemaker).

Theoretically, generating a cardiac action potential would require an Na^+ current, a Ca^{2+} current, and a K^+ current. This is clearly an oversimplification. Indeed, the cardiac cell expresses much more different ion currents. Concerning K^+ channels, for example, the human genome contains at least 75 genes encoding K^+ channels with different physiological characteristics of which about 35 are expressed in the human heart.[1–3] Thus, the cardiac action potential results from a very complex and finely tuned interplay of an ensemble of ion channels with different relationships to voltage and time. Only sophisticated computerization can approach the complexity of the cardiac action potential (see below).

Excitation–Contraction Coupling

Coupling between the electrical stimulus and contraction is ensured through movement of Ca^{2+} in and out of intracellular stores.[4] The main store for Ca^{2+} in a cardiac cell is the sarcoplasmic reticulum, where Ca^{2+} is buffered on specialized proteins such as calsequestrin. Diastolic free Ca^{2+} in the cytosol is maintained very low, in the order of 10^{-7} M. During the action potential, a small amount of Ca^{2+} entering the cell through L-type Ca^{2+} channels triggers the release of Ca^{2+} from the sarcoplasmic reticulum through Ca^{2+}-release channels (these are intracellular ion channels labeled by their high affinity for the alkaloid ryanodine) located in the sarcoplasmic reticulum membrane (Figure 1–5). This mechanism, called Ca^{2+}-induced Ca^{2+}-released, produces massive release of Ca^{2+} from the sarcoplasmic reticulum and invasion of the cytoplasm with free Ca^{2+}. Increased cytoplasmic Ca^{2+} binds to troponin, opening the myosin binding sites on filamentous actin, and force is produced. Decreased cytoplasmic Ca^{2+} resulting from its recapture in the sarcoplasmic reticulum by Ca^{2+}-ATPase located in the sarcoplasmic reticulum membrane produces relaxation. Because the system is in perfect equilibrium, the exact amount of Ca^{2+} that has entered the cell via L-type channels is now extruded out of the cell through the Na–Ca exchanger.

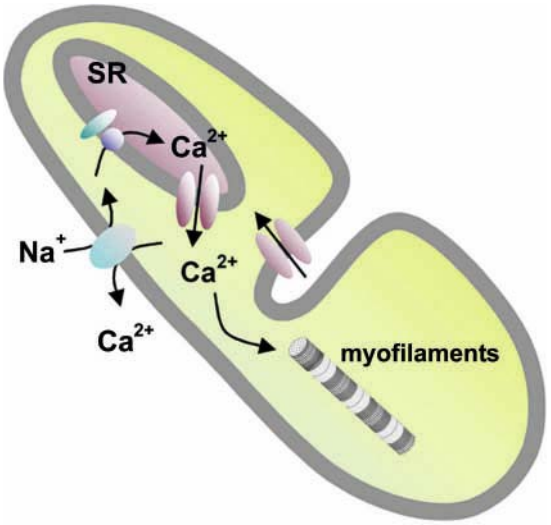

Figure 1–5. Schematic representation of the cellular mechanisms relating the action potential to cardiomyocyte contraction. Ca^{2+} enters into the cell when L-type Ca^{2+} channels are activated. The local intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) increase activates Ca^{2+}-dependent channels localized on the sarcoplasmic reticulum (SR) membrane (ryanodine receptors),which liberate further Ca^{2+} into the cytoplasm. A chain reaction induces a general $[Ca^{2+}]_i$ increase, which induces sarcomeric contraction. At the same time, it induces (1) the Ca^{2+}-dependent inactivation of the sarcolemmal L-type Ca^{2+} channels and (2) the repumping of the intracellular Ca^{2+} toward the sarcoplasmic reticulum (Ca^{2+}-ATPase) and outward to the myocyte (Na–Ca exchanger), resulting in a decrease in $[Ca^{2+}]_i$.

Specialized Tissues

Cardiac cells specialized in automaticity (e.g., nodal cells) or in conduction (e.g., Purkinje fibers) express a different repertoire of ion channels than regular myocardium[5,6] (Table 1–1). Consequently, their action potential has a different shape and characteristics (Figure 1–6). Automatic cells from the sinus node or from the atrioventricular node have a less polarized membrane potential because they express less background K^+ channels. Because of a less negative membrane potential, their Na^+ current is permanently inactivated and the depolarizing phase of their action potential relies on the activation of slow Ca^{2+} currents. As a consequence, the kinetics of the rising phase (a parameter

Table 1–1. Principal ionic currents, channels and auxiliary subunits, and genes expressed in the human heart.

		α-subunit or transporter		Auxilary subunit		
Current	Name	Name	Gene	Name	Gene	Remarks
I_{Na}	Na^+ current	Nav1.5	SCN5A	β1–β3	SCN1B–SCN3B	TTX insensitive channel
		Nav1.7	SCN9A			Channel in Purkinje fibers only
		Nav1.3	SCN3A			In atrial cells only
$I_{to\ fast}$	Fast transient outward K^+ current	Kv4.3	KCND3	KChiP2–KChaP	KCNIP2–PIAS3	KChiP2 gradient in ventricle
$I_{to\ slow}$	Slow transient outward K^+ current	Kv1.4	KCNA4	Kvβ1.2–Kvβ2	KCNAB1–KCNAB2	
I_{CaL}	L-type Ca^{2+} current	Cav1.2	CACNA1C	β2–α2δ1 and 2	CACNB2–CACNA2D1 and D2	Channel absent in ventricle
		Cav1.3	CACNA1D			
I_{CaT}	T-type Ca^{2+} current	Cav3.1	CACNA1G			Absent in ventricle
I_{Kr}	Fast delayed rectifier K^+ current	HERG	KCNH2	MiRP1?	KCNE2?	
I_{Ks}	Slow delayed rectifier K^+ current	KvLQT1	KCNQ1	minK (MiRP3)	KCNE1 (KCNE4)	Gradient in ventricle
I_{Kur}	Ultra-rapid outward K^+ current	Kv1.5	KCNA5	Kvβ1.2–Kvβ1.3	KCNAB1	Channel absent in ventricle
I_{K1}	Inward rectifier K^+ current	Kir2.1	KCNJ2			Mostly in ventricle, absent in node cells
		Kir2.2–Kir2.3	KCNJ12–KCNJ4			Absent in node cells
$I_{K,\ Ach}$	Acetylcholine-dependent K^+ current	Kir3.1–Kir3.4	KCNJ3–KCNJ5			Absent in ventricle
$I_{Na/K}$	Na-K pump current	Na-K pump	ATP1A			Responsible for K^+ and Na^+ gradient
$I_{Na/Ca}$	Na-Ca exchanger current	Na-Ca exchanger	SLC8A1			Contributes to Ca^{2+} gradient
I_f	Pacemaker current		HCN2–HCN4			In nodal tissue

that strongly influences conduction velocity) is much slower than in the myocardium. Another consequence of decreased K^+ current is slow depolarization of the cell membrane during diastole, allowing the membrane potential to spontaneously reach the activation voltage for Ca^{2+} current and thereafter to generate an automatic action potential. Inversely, cardiac cells specialized in

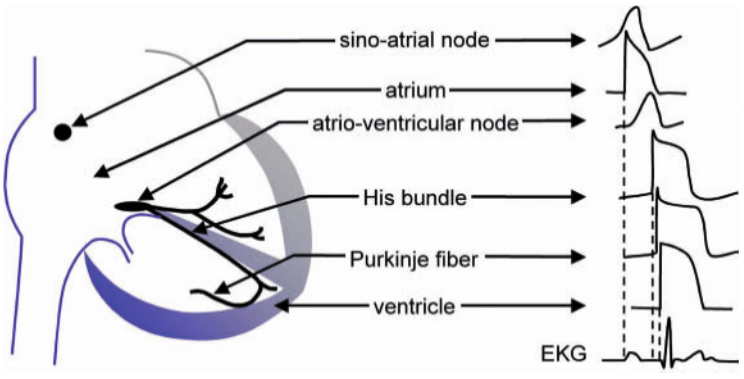

Figure 1–6. Schematic shapes of the action potentials of the different regions of the heart. Dotted lines correlate EKG events with the occurrence of different action potentials.

conduction have a faster rate of rise of phase 0 (dV/dt max) and a faster conduction velocity.

Surface EKG

As already stated in the introduction, the action potential results from the effects on membrane potential of a summation in time of individual channel opening and closing. Multiple action potentials arising from the different segments of the heart are integrated in time and space to generate the extracellular signal known as the surface EKG. Because of this continuum, it might be expected that an alteration in a channel function traduces itself into an anomaly of the action potential shape and finally in the surface EKG. The long QT syndrome is such a situation where a loss-of-function mutation in a K^+ channel gene involved in cardiac repolarization prolongs the action potential duration (less net outward current is available to repolarize the cell membrane) and prolongs the QT interval.

Gene Correlates for Cardiac Ion Channels

The vast majority of the genes encoding cardiac ion channels have been identified and precisely assigned to a chromosomal region. Most ion channel proteins share a common signature. For example, the large potassium channel protein family has two, four, or six transmembrane segments. Voltage-gated K^+ channels possess six transmembrane segments,[1] whereas inward rectifiers[2,3] (this label refers to their capacity to conduct more easily ions in the inward than in the outward direction), which are considered as time independent, have only two transmembrane segments. Six- and two-transmembrane segment K^+ channels share a common pore structure identified by the canonical GYG signature. In 1998, Rod MacKinnon published the structure of a potassium channel obtained by X-ray diffraction studies of a potassium channel crystal and was awarded the 2003 Nobel price in chemistry for his work.[7,8]

Expression of recombinant foreign channel proteins in host cell systems (see below) as well as genetic invalidation of ion channel genes in the mouse have been instrumental in correlating cardiac ion channels (and ion currents) with their encoding genes. In addition, these investigations have also shown that ion channels are often formed by the assembly of α-subunits (the channel pore itself) and β-subunits (an associated protein encoded by a distinct gene that regulates α-subunits) and in occasion with γ- or δ-subunits (see Table 1–1). In general, it is more and more clear that ion channels are not expressed in isolation in the cell membrane but rather in concert with other regulatory proteins within a channel complex. Identification of missing members of the ion channel complex is the subject of active research in various laboratories with the objective of targeting novel candidate genes for cardiac channelopathies.

Technologies to Explore Cardiac Cellular Electrophysiology

Although the first recording of a cardiac action potential was obtained about 50 years ago,[9] it is the discovery and development of the patch-clamp technique (which brought the Nobel prize of medicine to E. Neher and B. Sakman in 1991) that have been the key to our understanding of cardiac cellular electrophysiology.[10,11] The technique is applied to isolated cells either freshly dissociated from cardiac tissues or maintained in culture. Depending on the configuration used, the patch-clamp technique can record the electrical activity of a single channel molecule (amplitudes of a single channel current are in the order of $1\,pA = 10^{-12}\,A$) or the electrical activity of the ensemble of channel protein expressed in a whole cell (current amplitudes may be in the order of $1\,nA = 10^{-9}\,A$). At the level of a single protein recording, the channel appears in a binary situation either closed or open. Voltage-dependent activation is visible because the channel spends more time in the open configuration and thus less time in the closed configuration in response to a voltage step (usually depolarizing). Inactivation is also visible as a progressive decrease in channel firing with time at a stable voltage (see Figure 1–3). In the current-clamp mode, variations in the voltage (action potentials) are measured, whereas in the voltage-clamp mode, ion currents are measured.

Another major technical step in cardiac electrophysiology has been inherited from molecular biology techniques and relates to our capacity to enable host cells (e.g., COS or HEK cells) maintained in culture to express foreign genes. Cells are transfected with ion channel cDNA using routine nonviral methods and then patch-clamped after 24–48 h in culture. Recombinant ion channel proteins (in particular of human origin) are available for physiological and pharmacological investigations.[12] Site directed mutagenesis permits investigations of mutated constructs shading light into genotype–phenotype relations.[13]

Genetic manipulation of ion channel genes and regulators has been successfully achieved in the mouse with either overexpression[14,15] or invalidation of target channels.[16,17] Development of *in vivo* electrophysiological methods adapted to the very small size of this animal (a mouse heart is about 100 mg) has provided investigation capacities similar to those in humans.[18]

More recently, the genomics of ion channel genes has provided information on ion channel expression at a genome scale in various physiological and physiopathological situations.[19]

Cardiac Cellular Electrophysiology of the Human Heart

The patch-clamp technique applied to single cells dissociated from cardiac biopsies sampled during open-heart surgery in association with molecular biology techniques has provided an impressive body of information on the cellular electrophysiology of the human heart.

At the atrial level, the human action potential is initiated by a fast-activating fast-inactivating Na^+ current carried by Nav1.5 channels (encoded by the *SCN5A* gene) in association with its β1 subunit (*SCN1B*).[20,21] Other Na^+ channel α-subunits including Nav1.3 (*SCN3A*),[22] Nav2.1 (*SCN6A*),[23] and β-subunits (*SCN3B*)[24] are also expressed in the human atrium, although at a much lower level than Nav1.5, which is by far the predominant cardiac Na^+ channel. The Nav1.5-carried Na^+ current is responsible for the upstroke of the action potential (phase 0) and carries energy for fast conduction in the atrium. Fast depolarization triggers the activation of transient outward and inward currents. The transient outward current produces initial repolarization of the action potential and a clearly visible notch inscribed prior to the AP plateau. Transient outward current is made predominantly by the Kv4.3 channel (*KCND3*)[25] in association with its regulatory β-subunit KChiP2.[26] Because inactivation of the transient outward current is fast, this current determines the level of the plateau phase and therefore influences the activation of other currents but does not directly influence phase 3 repolarization kinetics. Transient Ca^{2+} currents provide inward current to maintain the depolarized cell during the plateau. Two types of Ca^{2+} currents are operative: L-type (long lasting), which are targets for calcium channel blockers, and T-type (fast inactivated). L-type Ca^{2+} currents are predominantly carried by Cav1.2 (*CACNA1C*)[27] and to a much lower extent by Cav1.3 (*CACNA1D*)[28] channels in conjunction with their auxiliary subunits: Cavβ2,[27] Cavα2δ2,[29] and Cavα2δ1.[30] T-type Ca^{2+} currents are brought by Cav3.1 channels (*CACNA1G*).[31] Repolarization of the action potential (phase 3) is initiated by the delayed rectifier K^+ current, which has two components: a fast activating component termed I_{Kr}, which is carried by HERG channels,[32] and a slow component I_{Ks}, which is carried by KvLQT1 (*KCNQ1*) channels in association with the regulatory β-subunit minK (*KCNE1*).[33] An ultrarapid K^+ current (activation is 2-fold faster than I_{Kr}) is specific of the atrium in human and is carried by Kv1.5 channels (*KCNA5*).[34,35] Final repolarization is achieved by background time-independent currents (also called inward rectifiers), which are also responsible for maintaining a negative membrane polarization during diastole. Kir2.1 channels (*KCNJ2*) are less abundant than in the ventricle, accounting for the less negative resting potential in the atrium.[36] Atrial myocytes also express Kir2.2 and Kir2.3 channels (*KCNJ12* and *KCNJ4*). Specific to the atrium are Kir3.1 and Kir3.4 channels (*KCNJ3* and *KCNJ5*), which open in response to cholinergic stimulation and shorten the duration of the action potential.[32] Other background K^+ currents include TWIK1 (*KCNK1*)[36] and TASK1 (*KCNK3*)[37] currents. Other currents are related to the Na–K pump (*ATP1A1*),[38] which generates an outward current, and the Na–Ca exchanger (*SLC8A1*), which generates an inward current and helps

maintain the plateau. The role of chloride channels in the human heart has still not been ascertained. The electrical connection between cells is ensured by the expression of connexin channel proteins. Connexin 40 (*GJA5*) is specific to the atrium. Cx43 (*GJA1*) and Cx45 (*GJA7*) are also expressed in this tissue.[39]

The ventricular action potential shape differs from that of the atrium. In particular, the initial repolarization phase is less pronounced, the plateau phase is more positive, and phase 3 repolarization is more rapid. As in the atrium, there is no spontaneous depolarization in the contractile myocardium. The ventricle exhibits almost no ultrarapid K^+ current (Kv1.5 channels)[40,41] or T-type Ca^{2+} current.[42] Similarly, Kir3.1 or Kir3.4 channels, which are activated under acetylcholine, are not expressed, whereas the background K^+ current (Kir2.1 channels) is more prominent in comparison with the atrium.[36] Consistent differences are seen between the endocardium and the epicardium with a more pronounced initial repolarization attributed to transient outward current in the epicardium.[43] These differences, which are crucial for the inscription of normal EKG waves, have been linked to a greater KChiP2 expression in the epicardium with no Kv4.3 transmural gradient.[44] Cx40 is not expressed in the regular ventricular myocardium.[39]

Much less information is available on the cellular electrophysiology of specialized regions of the human heart, and most of our knowledge has been obtained from animal models. Action potentials from the sinus node (SAN) have relatively less negative maximum diastolic potential (about −55 mV), a slow rate of rise, and a spontaneous diastolic depolarization than the origin of cardiac automatism. Cholinergic and β-adrenergic stimulations slow and accelerate the spontaneous sinus node, respectively. Many different ion currents are responsible for pacemaking activity with this redundancy being considered as a security system. Cells from the SAN express an inward current that activates when the cell repolarizes. This inward current, sometime called the pacemaker current, is related to the specific expression of HCN channels (*HCN1* and *HCN4* genes).[45,46] Other currents that contribute to depolarizing the cell during diastole including L-type Ca^{2+} currents (*CACNA1D and CACNA1C),* T-type Ca^{2+} current (*CACNA1G*),[6,47] and a delayed rectifier K^+ current that progressively deactivates during diastole (likely made of HERG, KvLQT1, and minK expression).[48–50] Partial depolarization of automatic cells is explained on the basis of the low expression of background Kir2.1 channels (*KCNJ2*).[50,51] Kir3.1 and Kir3.4 channels provide an additional outward current when activated by acetylcholine, leaving less net inward current for diastolic depolarization and thereafter producing bradycardia.[46,52,53] Cholinergic stimulation also decreases the availability of L-type Ca^{2+} channels.[54] β-adrenergic stimulation increases the L-type Ca^{2+} current amplitude[55] and facilitates the activation of HCN channels during repolarization.[56]

The main function of the atrioventricular (AV) node is to slow conduction at the AV junction so as to create a delay between atrium and ventricular contraction. Atrioventricular node cells also have postrepolarization refractoriness, which limits the number of impulses that can activate the ventricle. Finally, AV node cells are the place for accessory pacemaking when the sinus node fails to ensure automatism. These node cells have a faster rate of rise of the action potential than SAN cells, although this value remains much lower in the AV node than in the regular myocardium. As in the SAN, automatism in the AV node is achieved through the expression of specialized channels such as HCN channels.[46] Among delayed rectifiers, I_{Kr} predominates over I_{Ks}.[57] As in the SAN, there is little background K^+ current in the AV node.[58] Also as in the SAN, the AV node expresses more Cav1.3 channels and less KChiP2 and Kv4.x channels than the regular myocardium.[46]

Cardiac cells specialized in conduction belongs to the His-Purkinje system. They have a more negative resting potential than contractile fibers and a greater maximum rate of rise of depolarization. The plateau potential is more negative and the action potential is longer than in the ventricular myocardium.[59] In addition, Purkinje fibers show spontaneous diastolic depolarization responsible for the idioventricular rhythm during atrioventricular dissociation. Human Purkinje fibers express less Cav1.2 (unpublished data), more Cx40, but less Cx43 than regular myocardium.[60] KChiP2 is almost undetectable, whereas Kv4.3 channels are more expressed than in the

ventricle (unpublished data). Kir2.1 and Kir2.2 are lower than in the ventricle, whereas TWIK1 is higher (unpublished data).

Cardiac Cellular Electrophysiology in Other Mammalians

Because in the obvious difficulties in obtaining undiseased human myocardial cells, most electrophysiological and electropharmacological studies have been conducted in animal models. However, the human cardiac cell electrophysiology is unique and clearly differs from that of other mammals. This is seen when a comparison is made with the mouse, a model that has became very popular because of the possibilities offered to manipulate its genome. A mouse heart beats 600 times per minutes, i.e., 10 times faster than a human heart. Accordingly, the mouse has a very abbreviated action potential with virtually no plateau phase.[61] A mouse does not express sizable delayed rectifier (I_{Ks} and I_{Kr})[62] but relies mainly on transient outward currents (Kv4.2 in association with KChiP2 but also Kv1.5 and Kv2.1 channels) to ensure repolarization.[63–65] Thus, the mouse is not an adequate model for human pathology when repolarization is concerned. Inversely, depolarization and conduction have comparable characteristics in the mouse and human hearts.[66] I_{Ks} and I_{Kr} are easily recorded in the guinea pig heart with $I_{Ks} > I_{Kr}$ (the reverse than in human).[67,68] Guinea pigs and pigs inversely express almost no transient outward current.[69,70] The dog has a large endocardium-to-epicardium transient outward current gradient similar to humans, although the biophysical characteristics of this current differ significantly between the two species.[71]

As a consequence, there is no single species that can be used as a convincing model of the human heart. Depending on the problem under study, a different model could be chosen. As an example, ventricular transmural differences in ion channel expression and function are convincingly recapitulated in the dog but not in the mouse.[44,72] In spite of the pronounced differences between the human and mouse heart, a tremendous amount of valuable information has been obtained in this latter species. Finally, it should be kept in mind that *Drosophila* has been the key to identifying the multiple families of K^+ channel genes that later appeared as largely conserved during the evolution from *Drosophila* to human.[73]

Computer Models of Cardiac Cellular Electrophysiology

Each ion current is characterized by its amplitude and relation to voltage and time (activation, deactivation, inactivation, reactivation, etc.) and therefore can be fully described by series of equations and parameters. If the equations and parameters for every ion current expressed in the heart are entered into a computer, a normal action potential can be reconstituted that is related to rate or interpolated stimulus very similar to biological data. Such a complex computerization has been achieved for several animal models[74–77] and for the human atrium[78,79] and ventricle.[80–82] These models have proved of tremendous value not only in understanding the role of each single ion channel function in the global electrical activity of a cell but also, and most importantly, in inferring the consequences of subtle changes in ion current characteristics (as produced, for example, by genetically inherited mutations or drugs). As a matter of fact, the impact of alterations in the characteristics from one ion current to other ion channels contributing to generate the action potential is so complex that it cannot be accurately deduced by a humans without the help of a computer. The computerized action potentials from different cells can also be integrated in two- or three-dimensional thin layers approaching the geometry of the normal heart to reconstitute its global activity on a computerized electrocardiogram.[81]

Conclusion

After decades of electrophysiological studies on animal and human cardiac tissues, many aspects of the molecular basis for cardiac electrogenesis have been unveiled. Although much knowledge of human ionic currents has been gathered, data from human cardiac nodes and conducting tissues are still missing and direct extrapolations from animal data may be hazardous. Furthermore, despite the sequencing of the human genome, a

function assignment for every predicted protein is far from being achieved. As a consequence, the list of yet identified channel auxiliary proteins is largely incomplete. The quest for the Holy Grail assumes that knowledge of every member of the orchestra would provide access to the global cardiac symphony.

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