An Introduction to the Cardiac Action Potentials

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What You Will Learn in This Chapter

This chapter will begin by examining the origin of the ventricular action potential (AP), its constituent ionic currents and the channels through which these currents flow. Several examples will be used to help appreciate the clinical implications of modifying this excitation system. Finally, we will introduce the pacemaker potential and the contemporary theories surrounding its generation.

Learning Objectives

- Analyse the five phases of the cardiac AP, state the main ionic currents active in each phase and the channels through which these currents flow.
- Provide three examples of the clinical relevance of the aforementioned channels.
- Consider the contemporary theories of pacemaker potential generation.

5.1 Introducing the Cardiac Action Potential

The cardiac AP provides the electrical component of excitation-contraction coupling, using voltage changes across the cell membranes. The length of the AP plays a role in determining the strength of cardiomyocyte contraction. To ensure effective pumping function, cardiac muscle requires a long, slow contraction, and thus the ventricular AP is relatively long (~350 ms) compared with the excitatory events characteristic of the peripheral nervous system (~2 to 3 ms). The shape and duration of the AP varies in different parts of the heart largely determined by the size and type of ionic currents that flow. In turn, ionic currents are regulated by the levels of expression of ion channels through which they flow [2]. The initial cardiac AP (termed the 'pacemaker potential') is generated both autonomously and rhythmically by the cells of the *sinoatrial node* (*SAN*): a diffuse collection of specialised pacemaker cells located around the border between the superior vena cava and the right atrium [1]. These cells have different characteristics and so, we will discuss the pacemaker potential separately from the ventricular AP.

5.2 The Ventricular Action Potential

The ventricular AP can be considered as the summation of five phases, each of which possesses a unique ionic profile based on the transient activation of ion transporters. This creates the broad morphology shown in Fig. 5.1 [3]. The five phases are:

- ϕ 0 – Upstroke
- ϕ 1 – Early Repolarisation
- ϕ 2 – Plateau
- ϕ 3 – Final Repolarisation
- ϕ 4 – Resting Membrane Potential (RMP)

5.2.1 ϕ 0 – Upstroke

The arrival of an action potential (AP) from a neighbouring cell causes a small depolarisation of the cardiomyocyte. If this depolarisation is sufficient to bring the membrane potential to a threshold level sensed by a large number of voltage-gated Na^+ channels,

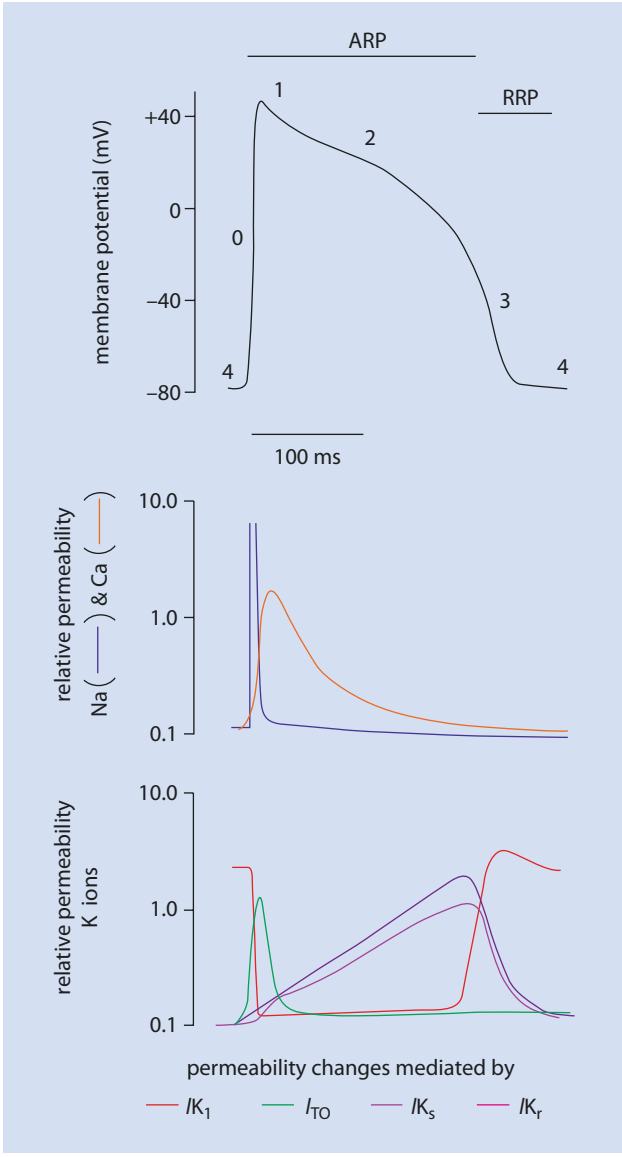

Fig. 5.1 A typical AP from a ventricular myocyte showing phases and the approximate lengths of the absolute (ARP) and relative (RRP) refractory periods . The main changes in the relative permeability of the myocyte membrane to ions carried by the main currents during each phase are shown

these open with the subsequent influx of Na^+ ions leading to a rapid depolarisation of the cell towards the Na^+ equilibrium potential [4]. This is known as the upstroke phase.

The inward Na^+ current is usually abbreviated as I_{Na} and influx of ions occurs via the $Na_{v1.5}$ channel, encoded by the *SCN5A* gene [2]. $Na_{v1.5}$ is a large transmembrane voltage-gated channel made up of four repeated domains, with each domain comprising six subunits (S1–S6). S4 is positively charged and acts as the voltage sensor [2].

Movement of S4 can induce a conformational change in S5 and S6 that allows access to the pore-forming region through which Na^+ flows. Hence the configuration state of the channel, i.e. open or closed, is voltage-dependent.

The inactivation of $Na_{v1.5}$ occurs in both a voltage and time-dependent manner. Inactivation is the rapid block of Na^+ movement through the channel and must occur to allow the cell to repolarise. Once inactivated, channels must return to their closed state before their reactivation is possible. This change of state (from inactivated to closed) is achieved by repolarisation of the membrane [5]. The two-step process for reactivation is responsible for the action potential refractory period. The absolute refractory period is the time during which another action potential cannot be generated because the majority of Na^+ channels are in the inactive state and thus cannot be re-opened [5]. The relative refractory period occurs as the cells repolarise more so that a larger portion of Na^+ channels have recovered from inactivation and have returned to their closed state. Under these conditions a stronger than normal stimulus may depolarise the cell sufficiently to trigger channel opening that may lead to the production of a premature depolarisation [5].

5.2.1.1 Clinical Implications

Genetic mutations in the *SCN5A* gene may cause functional changes in the $Na_{v1.5}$ channel, as seen in type 3 Long QT and Brugada syndromes [6]. In both cases, the disruption of Na^+ channel inactivation leads to prolongation of the action potential, increasing the risk of arrhythmogenesis [6]. This is discussed further in ► Chap. 8.

Unlike many voltage-gated sodium channels in the human body, $Na_{v1.5}$ is not inhibited by low concentrations of tetrodotoxin; it is considered 'insensitive' to this compound [7]. Tetrodotoxin poisoning, occassionally a result of eating Japanese Fugu fish, is associated with respiratory arrest but few cardiac implications [7].

5.2.2 ϕ 1 – Early Repolarisation

Manifesting as the distinctive notch in ventricular APs, phase 1 results from the action of two transient outward currents, I_{to1} and I_{to2}, that summate to cause initial repolarisation of the cell [8]. This early repolarisation helps to prevent the cell depolarising to E_{Na}, and assists in the timely activation of channels involved in ϕ 2. Changes to the currents involved in this phase affects AP duration. The two transient outward currents are:

I_{to1} The efflux of potassium through voltage-gated K^+ channels generates a repolarising outward current. The main channels involved in humans are isoforms of the rapidly inactivating A-type K^+ channels, $K_{v4.2}$ and $K_{v4.3}$, coded by the *KCND2* and *KCND3* genes respectively [9]. Due to these voltage-gated channels undergoing both rapid activation and inactivation, I_{to1} only participates in early repolarisation. Inhibition of these channels using 4-aminopyridine is associated with action potential prolongation [10].

I_{to2} An outward current generated by the influx of Cl^- in a Ca^{2+}-mediated process, repolarises the cell towards E_{Cl}. The precise identity of these channels is uncertain but they open in response to changes in intracellular Ca^{2+} concentration [9]. Activation and inactivation times are slower compared with I_{to1}.

5.2.3 ϕ 2 – Plateau

During the plateau phase, the membrane potential changes very slowly due to a fine balance between inward Ca^{2+} currents and outward K^+ currents. The Na^+/Ca^{2+} exchange ion transporter (NCX) can contribute to the inward current because of the exchange stoichiometry of $3Na^+$ and $1Ca^{2+}$. Under normal physiological circumstances, during the plateau NCX will expel one Ca^{2+} ion from the myocyte in exchange for the inward movement of three Na^+ ions producing a net +1 inward current [5].

5.2.3.1 Inward Current (I_{Ca})

Following depolarisation, the membrane voltage range enables Ca^{2+} channel activation and therefore Ca^{2+} influx: an absolute requirement of myocyte contraction [11]. The activation of L-type Ca^{2+} channels (LTCCs), encoded by the *CACNA1C* gene, is responsible for the inward Ca^{2+} flux that occurs early in the AP and continues during the plateau [12]. Ca^{2+} influx triggers a complex process known as '*calcium-induced calcium release*', which will be discussed in the ▶ Chap. 6. The L-type Ca^{2+} channel, also known as $Ca_{v1.2}$, is composed of 5 subunits, with the $\alpha 1$ component containing the voltage sensor responsible for detecting membrane potential and forming the conducting pore [9, 12]. The **ε** subunit is responsible for the Ca^{2+}-dependent inactivation process.

LTCC inactivation is dependent on both membrane potential and intracellular Ca^{2+} concentration. Mechanistically, the voltage-dependent inactivation involves cytoplasmic loops locking with the cytoplasmic end of S6 to block calcium flux. In contrast, Ca^{2+}-dependent inactivation occurs as the sub-sarcolemmal Ca^{2+} concentration increases, due to influx across the surface membrane and preliminary release from the sarcoplasmic reticulum (SR). This form of inactivation presents a negative feedback loop [12].

Clinical Implications

The LTCC is inhibited by both dihydropyridine Ca^{2+} channel antagonists, e.g. nifedipine, and also non-dihydropyridine antagonists such as verapamil [12]. Although this drug class will be discussed in greater detail in ▶ Chap. 15, clinically, the effect of LTCC inhibition can produce antiarrhythmic and antihypertensive effects depending on the location of the channels being acted upon [13]. Dihydropyridines modulate LTCCs in the vasculature and therefore have antihypertensive effects. Non-dihydropyridines (also referred to as phenylalkylamines) modulate LTCCs located in the heart itself and have been demonstrated to exert antiarrhythmic effects [14].

5.2.3.2 Outward Currents (I_{Kur} I_{Kr} I_{Ks})

Depolarisation induces the gradual activation of a series of K^+ currents known as delayed rectifiers [9]:

- I_{Kur} – ultra rapid (present only in the atria)
- I_{Kr} – rapid
- I_{Ks} – slow

During the plateau, there is a delay in the onset of the opening of these channels, with outward I_{Kr} and I_{Ks} activating progressively as LTCCs inactivate. The combination of a decline in inward current and an increase in outward current repolarises the cell, eventually bringing the plateau to an end and starting the more rapid phase of repolarisation [5, 9]. Following the plateau, as membrane potential becomes more negative, a large increase in membrane permeability to K^+ occurs from the opening of a different type of K^+ channel that behaves in an unusual way (see Sect. 5.2.4) [5].

The delayed rectifier K^+ channels consist of four subunits that form a tetrameric structure with a central pore. Each subunit contains six transmembrane segments, with the S4 segment containing the voltage sensor whereby a change in voltage results in S4 rotation. This pulls on the S4-S5 linkage, triggering a conformational change in the S5-S6 'glycine hinge' that causes the channel to open [15]. This encapsulates the concept of voltage-gated channels, whereby a channel involves a gate and a sensing device that controls the gate by voltage. Multiple genes encode the wide variety of K^+ channel isoforms [15]. For the delayed rectifiers [9, 16]:

- *KCNA5* encodes the channel $K_{v1.5}$ producing I_{Kur}
- *KCNH2* encodes the channel $K_{v11.1}$ producing I_{Kr}
- *KCNQ1* encodes the channel K_{vLQT} producing I_{Ks}

Clinical Implications

Class III antiarrhythmic drugs are the principal K^+ channel inhibitors, specifically I_{Kr}. An example is amiodarone, which acts to block the K^+ channel, decreasing the channel conductance to this ion and inhibiting the outward flux of K^+ [17]. As a result, phase 2 of the AP is prolonged, increasing the duration of the refractory period. This therpeutic approach is commonly used to terminate arrhythmic re-entrant circuits. Importantly however, prolongation of the plateau phase predisposes to early afterdepolarisations (EADs), an abnormal depolarisation of the myocyte during phase 2 or 3 of the AP [17]. This illustrates the potential arrhythmic effect of antiarrhythmics in certain situations.

5.2.4 ϕ 3 – Fast Repolarisation

I_{K1} is the outward current responsible for the final rapid repolarisation, fully terminating the action potential and stabilising the resting membrane potential [9]. Often referred to as an '*inward rectifying channel*,' it is generated from the co-assembly of the $K_{ir}2.1.x$ subfamily of proteins ($K_{ir}2.1$ [main ventricular isoform], 2.2, and 2.3) [9, 18].

During the plateau phase [2] of the ventricular AP, a combination of the time-dependent inactivation of LTCCs and increasing delayed rectifier current activation results in a repolarisation of membrane potential to approximately −10 mV [5, 11].

At this voltage inward rectifier channels start to open increasing the magnitude of I_{K1} and thus K^+ conductance across the membrane [18]. This results in the membrane potential becoming more negative, increasing the probability of K_{ir} channel opening. With K_{ir} channels capable of passing large amounts of outward current, their activation quickly repolarises the membrane, hence the name 'fast repolarisation' [18]. Moreover, once the resting membrane potential has been reached, the channel produces an outward current that 'pulls' the membrane potential towards the K^+ equilibrium potential and establishes a stable potential [18].

It is of note that the channels that produce I_{K1} do not behave in the same way as many other K^+ channels and have a different structure. These channels comprise four subunits that form a tetramer with a central pore similar to K_v channels [9]. Importantly however, $K_{ir}2.x$ subunits differ in that each has two transmembrane segments, M1 and M2, instead of the six seen in K_v subunits. With no voltage-gated S4 (typical of standard K^+ channels), these channels use a different mechanism to confer voltage sensitivity [9]. I_{KACh} and $I_{K\text{-}ATP}$ are two other examples of inward rectifier currents with a similar channel structure to I_{K1}.

With inward rectifier channels lacking an S4 segment, K^+ movement through the channel is hindered (and facilitated) by the binding (and unbinding) of so-called 'blocking molecules' such as magnesium and voltage-sensitive (−10 to 20/30 mV) polyamines [19]. Blocking molecules enter the channel pore to obstruct the efflux of K^+. At negative voltages less than −10 mV, the blocking molecules leave the channel pore, allowing the flow of potassium. For this reason, I_{K1} does not pass current during early repolarisation/phases 1 and 2, as the voltage is more positive than the −10 mV required for the displacement of blocking molecules [19].

5.2.5 ϕ 4 – Resting Membrane Potential

Phase 4, also referred to as 'resting membrane potential (RMP)', results primarily from I_{K1} and I_{KACh} currents, which together increase the membrane K^+ conductance. As previously discussed, these two currents conduct at negative E_m and mediate an efflux of K^+ that maintains polarisation of the cell in an attempt to reach the K^+ equilibrium potential, E_K. This high K^+ permeability is partially opposed by fluxes of other ions (e.g. some Na^+ and Ca^{2+} influx) across the membrane.

The consequence of this is that the RMP in cardiac myocytes does not reach E_K, and is slightly more positive [20]. Theoretically, if channels in the membrane were only permeable to K^+, the membrane potential would be equal E_K [5]. Importantly however, the foremost determinant of stabilising RMP is the passive flux of K^+ mediated by the high conductance I_{K1} [5].

Also of note is the activity of ATPase transporters, the most notable of which is the sodium-potassium pump (Na^+/K^+-ATPase). These consume ATP to actively move three Na^+ out of the cell against their concentration gradient whilst simultaneously moving two K^+ in. This process is essential for the maintenance of concentration gradients [5].

5.2.5.1 Other Currents

Late I_{Na} Carried by a subset of Na^+ channels that do not inactivate rapidly and produce a current that persists late into the AP. While this inward current is small in magnitude compared with the main Na^+ current, it can prolong the AP duration by opposing the outward current generated by delayed rectifiers. Its pathological effects are seen in heart failure, where its current is increased – a phenomenon that may derive from the hyperphosphorylation of Na^+ channels, increasing their open probability [21]. In these circumstances, the enhanced late I_{Na} is associated with the development of arrhythmias in two main ways [21]:

1. By prolonging the action potential. If AP duration increases, some LTCCs are able to recover from inactivation and will reopen because the correct voltage range is maintained. This results in Ca^{2+} influx into the cell, producing a substantial inward current that promotes EAD formation.

2. By increasing the intracellular Na^+ concentration. The enhanced late Na^+ current can cause delayed afterdepolarisations (DADs), because increased cytosolic Na^+ load leads to NCX reverse mode and Ca^{2+} influx.

I_{KACh} Acetylcholine (ACh) released by the parasympathetic vagus nerve binds to M2 muscarinic cholinergic receptors, triggering a G-protein cascade that activates $I_{K,ACh}$ channels, causing the efflux of K^+ ions and hyperpolarisation of the membrane [22]. This results in the threshold potential being reached more slowly, decreasing the AP firing rate and therefore heart rate [22].

$I_{K,ATP}$ Sarcolemmal ATP-sensitive K^+ (K_{ATP}) channels aid in protecting the heart against ischaemia via metabolo-electrical coupling [23]. Closed under normal circumstances, inhibition of cellular metabolism (such as during ischaemia) decreases cellular ATP concentration. This increases the open probability of K_{ATP} channels, augmenting K^+ efflux [23]. The AP duration is therefore shortened, decreasing contraction and preserving the cell's energy status. However, the decreased refractory period also provides a substrate for re-entry, promoting arrhythmogenesis [24].

$I_{K,ATP}$ activation has also been shown to limit Ca^{2+} entry during ischaemia by maintaining a negative and stable resting membrane potential, decreasing NCX-mediated Ca^{2+} influx and hence preventing Ca^{2+} overload [23, 24].

5.2.5.2 The I_f (Funny Current) in Cardiac Pacemaking

Cardiac pacemaking occurs from spontaneous diastolic depolarisations (DDs) that arise in the SAN. This relies on complex interactions between a nexus of interdependent currents, the precise mechanism of which remains unclear. That said, a vital current associated with cardiac pacemaking is I_f (the funny current), which is localised to pacemaking cells and is distinguished by three factors [25, 26]:

1. I_f occurs when cells are hyperpolarised.
2. I_f is a non-specific inward cationic current carried by K^+ and Na^+.
3. I_f flows through channels activated by both hyperpolarisation and cyclic AMP (cAMP). They are known as hyperpolarisation-activated, cyclic nucleotide-gated (HCN) channels.

In this way, variation in the concentration of cAMP facilitates modulation of cardiac pacemaking, and thus heart rate [27]. HCN is encoded by four genes (HCN1-4) with contrasting cAMP sensitivity and channel kinetics. HCN4 is the predominant SAN isotype, with high cAMP sensitivity and slow gating [28]. Despite having considerable structural commonality with voltage-gated potassium (K_v) channels, HCN4 displays higher sodium conductance at physiological voltages, facilitating a net inward current critical in DD [29]. Due to its activation by hyperpolarisation, this current constitutes the focal point of the so-called 'DiFrancesco' theory of cardiac pacemaking, named after its key proponent, Dario DiFrancesco [30].

5.3 What We Don't Know: The Mechanism of Cardiac Pacemaking

Two primary hypotheses exist for the spontaneous depolarisation observed in pacemaking cells: Dario DiFrancesco's I_f channel and Edward Lakatta's Ca^{2+} clock model. The first school of thought centres around the I_f channel, which is activated on hyperpolarisation

and provides a pathway for the non-specific influx of cations (mainly Na^+ but also K^+) that depolarises the cell bringing the membrane potential closer to the activation potentials of T-and-L-type Ca^{2+} channels [30]. Evidence for this theory is provided by the contribution of I_f to spontaneous AP firing [30].

An alternative theory has been proposed by Lakatta et al., who suggest that spontaneous AP firing results from the summated interactions of two clocks: membrane (M) and Ca^{2+} [31]. The M-clock contains L-and-T-type Ca^{2+} currents, while the Ca^{2+} clock refers to SR ryanodine receptors (discussed in later chapters). Evidence for this theory is provided in the form of membrane potential investigations by Bogdanov et al., who demonstrate the critical period of exponential voltage rise during DD results from the synergistic interactions of membrane Ca^{2+} currents and sub-sarcolemmal Ca^{2+} release by ryanodine receptor (RyR) clusters [32]. The importance of this clock interplay is seemingly reinforced by studies implementing ryanodine-mediated block of RyR, causing a subsequent negative chronotropic effect in SA nodal cells [33, 34].

Overall however, there is a lack of definitive experiments that implicate one or other mechanism. Pacemaking may be, in fact, an amalgamation of both mechanisms, with I_f contributing to the membrane clock that triggers the Ca^{2+} clock and thereby spontaneous activity.

Take-Home Message

- The differences in action potential profile between ventricular, atrial and SA nodal cardiac myocytes arise from variance in the magnitude of specific ionic currents.
- This results from differences in the degree of expression of relevant ion channels.
- Changes in the above can have profound arrhythmogenic consequences.

Phase		Current	Direction	Channel
0	Upstroke	I_{Na}	Inward	$Na_{v1.5}$
1	Early repolarisation	I_{to1}	Outward	$K_{v4.3}$, $K_{v4.2}$
		I_{to2}	Outward	
2	Plateau	I_{Ca}	Inward	$Ca_{v1.2}$
		I_{Kur}	Outward	$K_{v1.5}$
		I_{Kr}	Outward	$K_{v11.1}$
		I_{Ks}	Outward	K_{vLQT}
3	Fast repolarisation	I_{K1}	Outward	$K_{ir}2.1/2.2/2.3$

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