CELL MEMBRANE AND ION CHANNELS

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Contents

Summary

The current mathematical modeling of the cell membrane and the ion channels therein started in the 1950s with the seminal work of Hodgkin and Huxley on the squid giant

axon. In their electrical equivalent of the nerve cell membrane, the cell membrane is thought of as a capacitor, representing the lipid bilayer that constitutes the cell membrane, in parallel with a resistor that represents the permeability of the cell membrane to specific ions. In the present chapter it is shown how this concept introduced by Hodgkin and Huxley has evolved into today's mathematical models of the cell membrane and the ion channels therein. To provide the modeler with some insight into the electrophysiological techniques that have been used to obtain the experimental data on which the mathematical models are based, a brief overview of these techniques and their particular strengths and shortcomings is given. Also, some numerical issues are addressed, including the public availability of model source code and simulation environments to compile and run this code.

1. Introduction

In mathematical physiology, the description of the cell membrane and ion channels therein traces back to the seminal work of Alan Lloyd Hodgkin and Andrew Fielding Huxley on the giant axon of squid from the late 1930s to the early 1950s, for which they were awarded the Nobel Prize in Physiology or Medicine in 1963. In 1937, J.Z. Young had reported that the squid *Loligo* had exceptionally large 'giant nerve cells', with an axonal diameter up to almost one millimeter, controlling the contraction of the squid's mantle, which the animal uses to squeeze out sea water and thus propel back in case of danger.

The giant axon of *Loligo* allowed Hodgkin and Huxley to insert a glass capillary with two separate silver wires wound around it in spirals and carefully isolated from each other. One of these wires was then used to inject current and the other to record voltage, such that—with the use of a dedicated 'feed-back amplifier'—the transmembrane potential could be clamped at any desired level ('voltage clamp', see Section 2.3 below). This allowed the detailed characterization of the two main time- and voltage-dependent current systems present in the membrane of the *Loligo* giant axon, i.e. a fast inward sodium current and a slower potassium outward current, which are responsible for the rapid upstroke and the subsequent repolarization of the action potential, respectively.

In the final paper of the famous 1952 series on their voltage clamp experiments, Hodgkin and Huxley summarized their experimental results and analyzed these in terms of an electrical equivalent of the nerve membrane. Specifically, they fitted equations to the dependence of the permeability of the nerve membrane to sodium and potassium ions as functions of membrane potential and time under voltage clamp conditions. This resulted in a set of non-linear differential equations that could predict the quantitative behavior of the model nerve under a variety of free-running (non-voltage-clamped) conditions.

It is important to note here that Hodgkin and Huxley were aware of the bilipid structure of the cell membrane, which had only recently been established at the time of their discoveries, but that they did not know that the cell membrane contained specific proteins that we now know as ion channels, which can flip between open and closed states at rates that depend on the transmembrane potential, or membrane potential, i.e.

the voltage between the cytoplasm, which is considered to be isopotential, and the extracellular fluid. With respect to the voltage-dependent changes in membrane permeability to specific ions, Hodgkin and Huxley noted that "*details of the mechanism will probably not be settled for some time, but it seems difficult to escape the conclusion that the changes in ionic permeability depend on the movement of some component of the membrane which behaves as though it had a large charge or dipole moment*", thus correctly suggesting the action that takes place if an ion channel opens or closes. Also, they realized that "*some additional process must take place in a nerve in the living animal to maintain the ionic gradients which are the immediate source of the energy used in impulse conduction*", thus pointing to the requirement of an 'ion pump'. It was Jens Christian Skou who discovered the Na^+/K^+-ATPase, or sodium-potassium pump, which maintains the sodium and potassium gradients by pumping three sodium ions out of the cell for every pair of potassium ions pumped in, thus generating a steady net outward current. The energy for this pump comes from the decomposition of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and a free phosphate ion. For his important discovery, which he first described in 1957, although not fully realizing the importance of his findings at that time, Skou received the Nobel Prize in Chemistry in 1997.

In the following, we will explain and discuss how the seminal work of Hodgkin and Huxley has evolved into today's mathematical models of the cell membrane and the ion channels therein.

2. Experimental Techniques

It is important that 'modelers' have some insight into the experimental techniques that have been used to obtain the experimental data on which their mathematical models are based. Therefore, a brief overview of these techniques and their particular strengths and shortcomings will be given in this section.

2.1. Ion Concentrations

First of all, it should be noted that the electrical activity of all excitable cells relies on the existence of a 'chemical gradient' between the intracellular and extracellular fluids that are effectively separated by the lipid bilayer that we know as the cell membrane. This chemical gradient results from a difference in ionic composition of the intracellular and extracellular compartments, in particular a ratio of approximately 1:15 for sodium ions and 30:1 for potassium ions, as diagrammed in Figure 1 for mammalian cardiac cells.

A common misunderstanding is that (intracellular) ion concentrations change considerably during the course of an action potential. This is only true for the intracellular free calcium concentration, which is several orders of magnitude lower, in the nanomolar range, than that of sodium and potassium, which is in the millimolar range. Due to the large capacitance of the cell membrane, the movement of a relatively small number of ions is sufficient to cause a significant electrical effect, i.e. an action potential, without causing any noticeable effect on the concentration of these ions.

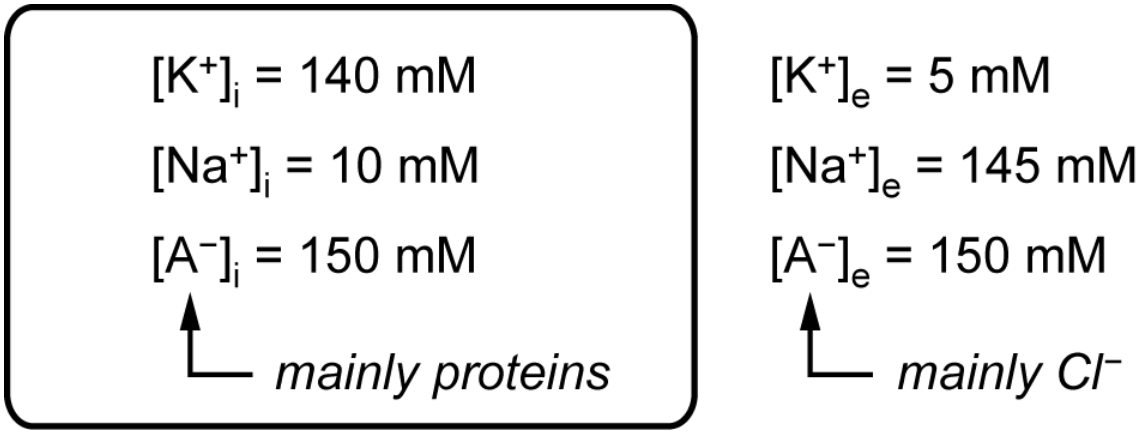


Figure 1. Composition of the intracellular and extracellular fluid in mammalian cardiac cells. A denotes anions.

2.2. Whole-Cell Recording

In today's cardiac cellular electrophysiology, 'patch clamp' is the common technique to record the electrical activity of single cardiac cells, e.g. the spontaneous electrical activity of pacemaker cells isolated from the sinus node, or sinoatrial (SA) node, or secondary pacemaker cells isolated from the atrioventricular (AV) node. Initially, the ionic mechanism of cardiac electrical activity was studied in small tissue preparations, using a double microelectrode voltage clamp technique. This technique yielded useful qualitative information on this ionic mechanism, but precise quantitative data could not be obtained due to, among other things, nonuniformity of the voltage clamp and extracellular accumulation and depletion of carrier ions. For these reasons, and also because of the technical difficulty of obtaining these data, i.e. of maintaining two separate microelectrode impalements in contracting muscle, this technique has been superseded by whole-cell recording from single cells that are enzymatically dissociated from cardiac tissue.

Cardiac myocytes can be isolated from (fragments of) the hearts of laboratory animals by dedicated enzymatic isolation techniques. Cell suspensions are then put into a recording chamber on the stage of an inverted microscope and continuously superfused with an extracellular Tyrode's solution, i.e. a salt solution with a composition in accordance with the interstitial fluid of the intact heart. Pharmacological blockers of specific ion channels may be added to this bath solution.

Also, the composition of the bath solution may be changed to facilitate the recording of a specific ion current, e.g. sodium ions may be replaced with non-permeating choline ions, as in some of the experiments by Hodgkin and Huxley. Apparently healthy spindle or elongated spindle-like nodal cells or rod-shaped working myocardial cells with clear cross-striations (Figure 2A) are then selected for electrophysiological measurements. If not stimulated, pacemaker cells isolated from SA or AV nodal tissue show regular rhythmic contractions, whereas cells isolated from the working myocardium, i.e. from atrial or ventricular tissue, are quiescent.

For electrophysiological recording, pipettes are pulled from small borosilicate glass capillaries and heat polished. A pipette is filled with a salt solution mimicking the intracellular fluid. When filled with this 'pipette solution', the pipette typically has a 2–3 MΩ resistance, contributing to an unwanted 'series resistance', which can be electronically compensated by the 'patch clamp amplifier'.

With the use of a micromanipulator that holds the pipette, the myocyte is approached with the recording pipette (Figure 2B). When the pipette tip is in close vicinity of the myocyte (Figure 2C, left), a little suction is applied and an omega-shaped seal is obtained (Figure 2C, middle). If some more suction is applied, the seal is broken and electrical access to the cell interior is obtained (Figure 2C, right). The thus obtained recording configuration is known as the 'whole-cell patch clamp configuration'.

This configuration allows for continuous registration of the transmembrane potential. A serious disadvantage of the technique is that the cell interior is dialyzed with the pipette solution, so that cytosolic components regulating pacemaker activity may be diluted, resulting in 'rundown' (see below).

To prevent cell dialysis, 'perforated patch recording', first described in the 1980s, may be used. With this technique, electrical access to the cell interior is not obtained by rupturing the membrane under the pipette tip (Figure 2C, right), but by adding an antibiotic like nystatin or amphotericin B to the pipette solution that perforates the cell membrane with ion channels that are permeable to monovalent ions and small molecules only.

With both techniques, significant 'tip potentials', i.e. 'liquid junction potentials', may arise, which should be estimated and corrected for. Such liquid junction potential arises when two solutions of different composition, in terms of ion concentrations and ion diffusion speeds, are part of the same electrical circuit, like the bath solution and the pipette solution in a patch clamp experiment.

Also, errors in recording membrane potential may arise if the 'seal resistance' between the patch pipette and the cell membrane is not sufficiently high, resulting in a 'leakage current' to the bath solution.

This leakage current should not be confused with the membrane current of unknown nature that remains after resolving all time- and voltage-dependent or 'gated' ion currents, which is sometimes referred to as 'leakage current'.

Hodgkin and Huxley, e.g., separated the membrane current of the *Loligo* giant axon into the time- and voltage-dependent sodium and potassium currents that allow first sodium and then potassium to cross the membrane at a high rate when it is depolarized, thus producing an action potential, as well as a time-independent leakage current composed of sodium, potassium and chloride 'leakage' and "probably also ions transferred by metabolism against concentration gradients", thus referring to the at that time unknown net outward current resulting from the sodium-potassium pump.

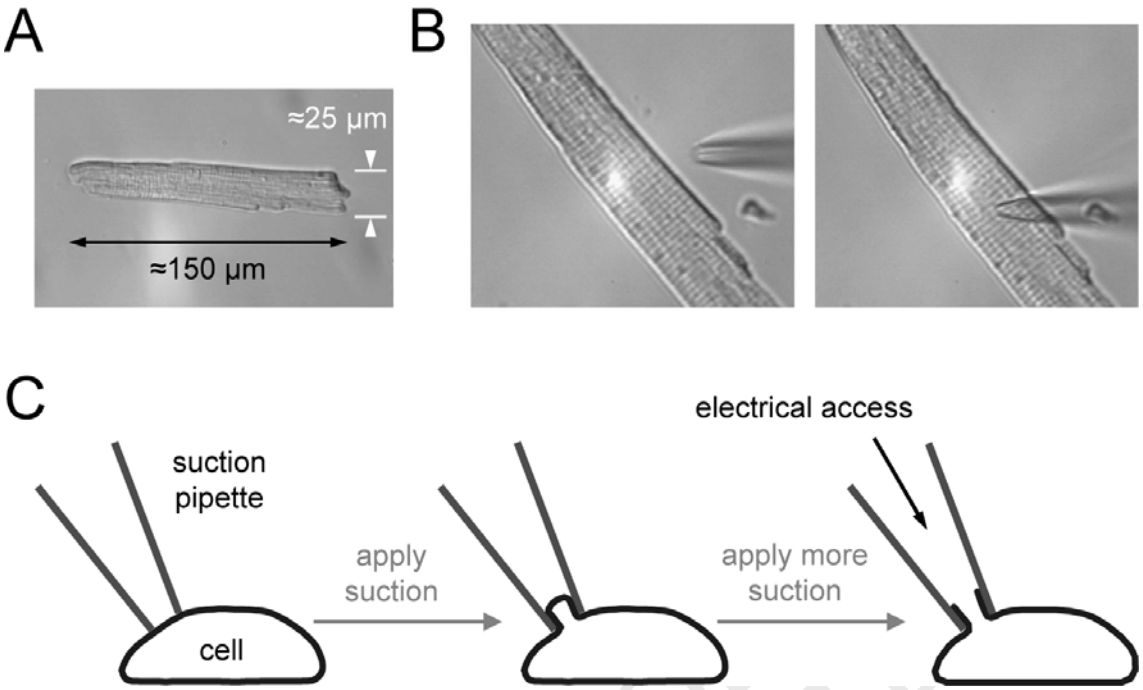


Figure 2. Recording electrical activity of single cardiac myocytes. (A) Enzymatically isolated single ventricular myocyte with typical dimensions of such cell. (B) Approaching the myocyte with a recording pipette. (C) Applying suction to obtain electrical access to the cell interior.

2.3. Current Clamp and Voltage Clamp

The 'whole-cell patch clamp configuration' of Figure 2C (right) can be used in different recording modes. The main recording modes are 'current clamp' (Figure 3A) and 'voltage clamp' (Figure 3B). In either case, the bath solution is grounded to earth, as indicated by '$V = 0$' in Figure 3A. In current clamp mode, the free-running membrane potential of the myocyte (V_m) is recorded.

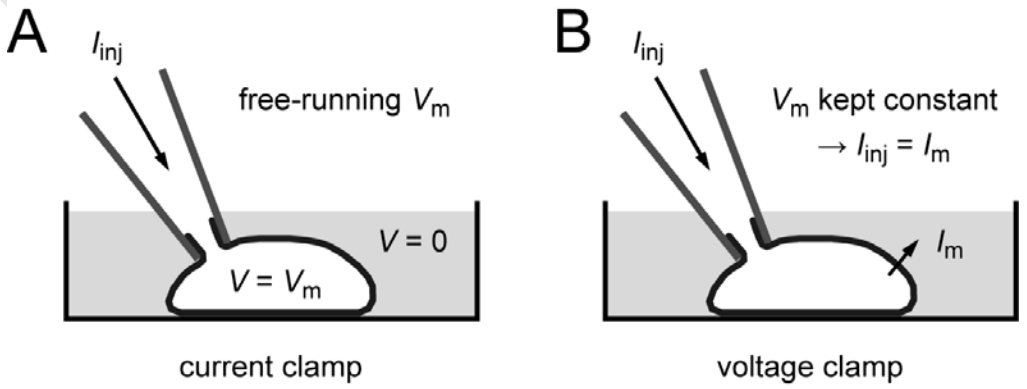
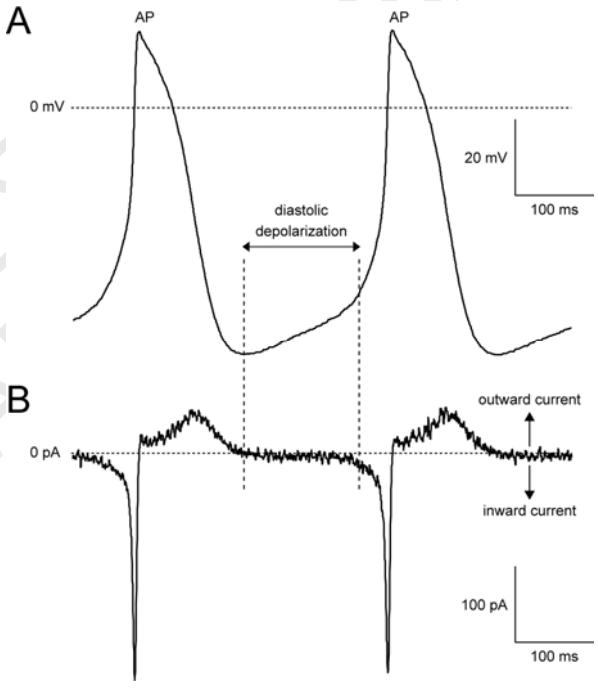


Figure 3. Different recording modes of the whole-cell patch clamp configuration. (A) Current clamp mode. A current (I_{inj}) can be injected into the myocyte through the

recording pipette, e.g. as a stimulus to elicit an action potential in a normally quiescent cell, and the free-running membrane potential of the myocyte (V_m) is recorded. (B) Voltage clamp mode. The membrane potential of the myocyte is held at a set level through a feedback circuit in the patch clamp amplifier. Consequently, the current that enters the cell through the recording pipette (I_{inj}) matches the current that leaves the cell through its membrane (I_m).

In the voltage clamp mode, the membrane potential of the myocyte is held at a set level through a feedback circuit in the patch clamp amplifier. Consequently, there is no (dis)charging of the cell membrane capacitance and the current that enters the cell through the recording pipette (I_{inj}) matches the current that leaves the cell through its membrane (I_m). This way, ion currents can be studied under carefully controlled conditions, applying dedicated 'voltage clamp protocols' (cf. Section 2.4).

When recording from pacemaker cells isolated from the sinoatrial (SA) node, spontaneous action potentials, as in Figure 4, can be acquired. When recording from intrinsically quiescent cells, like atrial or ventricular myocytes, a brief current pulse can be injected into the myocyte through the recording pipette as a stimulus to elicit an action potential (I_{inj}). Action potentials, whether spontaneous or elicited, are commonly characterized by 'action potential parameters' as diagrammed in Figure 5 for an SA nodal pacemaker cell.



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igure 4. Sinoatrial (SA) node action potentials (APs) and associated net membrane current. (A) Typical spontaneous APs of a pacemaker cell isolated from the rabbit SA node. (B) Associated net membrane current (I_{net}) calculated from $I_{net} = -C_m \times dV_m/dt$, where C_m and V_m denote membrane capacitance, which amounted to 40.4 pF for this

cell, and membrane potential, respectively (see Section 3.1 below). Note the small inward current underlying the slow spontaneous diastolic depolarization. By convention, an inward current, i.e. an inward flow of positively charged ions, is depicted as a negative current.

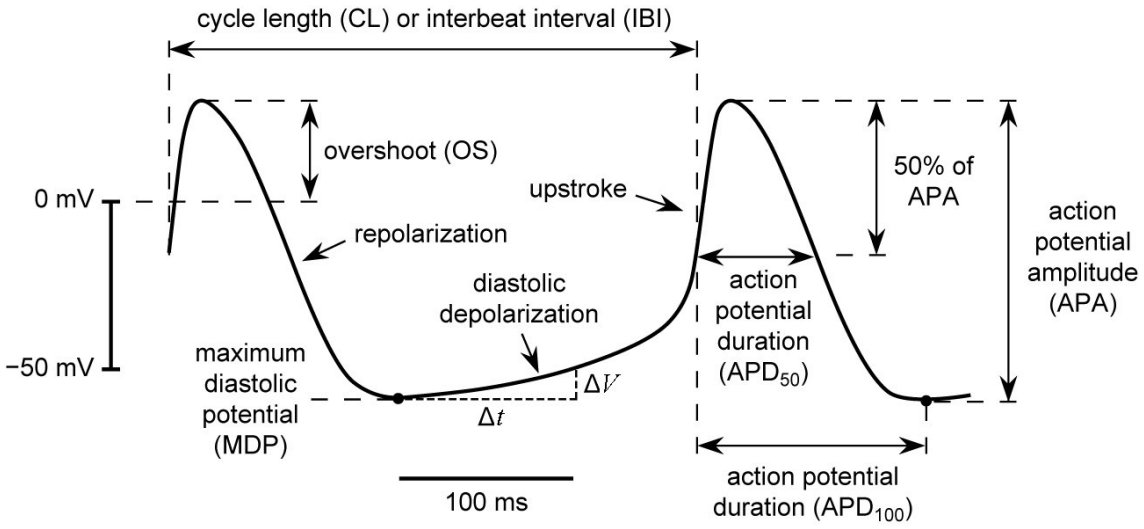

Figure 5. Outline of the electrophysiologist's nomenclature in characterizing action potentials. In addition to the indicated action potential duration at 50 and 100% repolarization (APD_{50} and APD_{100}, respectively), related measures like APD_{80} or APD_{90} are also widely used. Diastolic depolarization rate (DDR) is calculated from $DDR = \Delta V/\Delta t$, with ΔV and Δt as indicated. In this example, Δt is set to 100 ms, in which case DDR is sometimes denoted by DDR_{100}.

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Biographical Sketch

Ronald Wilders received his M.Sc. degree (cum laude) in applied mathematics, with physics as subsidiary, from the Faculty of Mathematics and Natural Sciences of the University of Amsterdam, the Netherlands, in 1985. He completed a research project on the electrical activity of sinoatrial nodal pacemaker cells with a Ph.D. degree (cum laude) from the Faculty of Medicine of the University of Amsterdam, the Netherlands, in 1993.As a student in mathematics, he worked as a part-time research assistant of the Professor of Mathematical Physics, Dr. Hans A. Lauwerier, for two years (1983–1985). During six years (1985–1991) he taught physics and mathematics in secondary school. In 1988, he joined the Department of Physiology of the Academic Medical Center (AMC) of the University of Amsterdam as a part-time pre-doctoral research fellow on a research project supervised by Dr. Habo J. Jongsma, Professor of Cell Physiology. Thereafter he worked with Dr. Jongsma on post-doctoral projects in the Department of Medical Physiology of the University Medical Center Utrecht, the Netherlands. He rejoined the AMC Department of Physiology as a faculty member in 2000. Currently, he is a Senior Scientist and Lecturer in the AMC Department of Anatomy, Embryology and Physiology. As a faculty member, he is responsible for a broad range of educational courses for students in medicine and related disciplines. He is involved in several research projects on cardiac cellular electrophysiology, which are carried out in the AMC Heart Failure Research Center and include *in silico* and *in vitro* experiments as well as combinations thereof. Dr. Wilders has contributed a large number of original articles, reviews and book chapters to the field of mathematical physiology. He is a member of the Dutch Society of Physiology, the Working Groups on Cardiac Cellular Electrophysiology and Computers in Cardiology of the European Society of Cardiology, and the IEEE Engineering in Medicine and Biology Society.