IONIC CHANNELS OF THE EXCITABLE MEMBRANE

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Summary

Structure and function of ionic channels is reviewed, and special attention is paid to probable physiological coding mechanisms of excitable membranes. The simplest models of molecular structure of sodium channels and their physiological roles in the nerve fiber membrane are discussed.

1. Introduction

The plasma membrane of all cells, including nerve cells, is about 8-nm thick and consists of a mosaic of lipids and proteins. Some of these proteins form membrane ionic channels. The cells use the cell membrane channels to regulate their physiological functions. With a progressive increase of the number of different cell types subjected to molecular physiological analysis, many instances of regulation by the cell membrane channels will undoubtedly be found. The sequence of events between membrane potential change and physiological response may be complicated, but it is expected to always result in movement, reorientation, or structural changes of the cell membrane of a voltage sensor, a molecular region that can respond to the cell membrane potential, as it has a large charge or dipole moment. The voltage sensor belongs to a *voltage-gated* ion channel molecule. In 1849, the German physiologist E. Dubois-Reymond was the first to describe generation of *action potentials* by nerve axons and muscle fibers. However, it was not until more than 100 years later that the underlying mechanism

could be explained in terms of properties of specific membrane proteins—the voltage-gated ion channels. In 1952, excitable membrane signaling was described by A.L. Hodgkin and A.F. Huxley. They analyzed kinetics of the ion conductance responsible for action potential generation in the axon membrane of the giant squid *Loligo forbesi*. Their model did not contain any specified conclusions on molecular mechanisms of this process and left open the question as to in which way the ions passed across the membrane, i.e. whether they did so through pores or by forming complexes with carriers. It was somewhat later that A.L. Hodgkin and R.D. Keynes (1955) put forward the concept of the "membrane ionic channels". In the giant squid axon, membrane depolarization that initiates action potential causes a transient change in the membrane that briefly switches its predominant permeability from sodium to potassium ions. The permeability changes occur due to opening of *voltage-gated* channels in the membrane, which allow sodium ions to move down its concentration gradient into the cell. It is the voltage-sensitive gating mechanism that controls open-closed transitions of the channel. Depolarization in the giant axon membrane opens *sodium channels* and allows increased sodium influx into the cell, thereby producing the rising phase of the action potential. The falling phase of the action potential is caused by the subsequent closing of the sodium channels due to the so-called inactivation gating process that reduces sodium influx and by the opening of voltage-gated potassium channels, which allows increased potassium efflux from the cell. Action potentials are all-or-none. Every action potential has the same shape, amplitude, and duration. These action potentials are conducted without fail along the entire length of the axon, which can be 1-2 meters. Therefore, the information in the signal continues to be coded by the frequency and the number of spikes (impulses). The higher the amplitude of the stimulus, the higher the frequency of the spikes. The longer the duration of the stimulus, the larger the burst of action potentials and, therefore, the higher the number of spikes.

2. Sodium Channel Protein

Our knowledge of ionic channels is now somewhat more detailed. A gene was cloned, which encodes the alpha subunit of the sodium channel. Analysis of the nucleotide sequence for the gene, as well as the amino acid sequence that it encodes, has revealed two fundamental structural features of the channel. First, the ion conductance of the channel is comprised of four internal repetitions, with only slight variations, of a basic amino acid sequence that is approximately 150 amino acids in length. Each repetition of this basic motif, when analyzed by a hydrophobicity plot, has been interpreted as having six membrane-spanning hydrophobic domains, each being likely to exist in the form of an alpha helix. The four repeated versions of the basic sequence are thought to be arranged roughly symmetrically, with the walls of the water filtering pore being formed by either one or two of the membrane-spanning helices repeated four times around the circumference of the pore. The second fundamental insight into the structural organization of the sodium channel is based on the observation that one of its putative membrane-spanning regions, called the S4 region, is highly conservative among sodium channels of different species. This region may transduce a change in membrane potential into a gating transition within the channel that opens the activating gate. A voltage sensitive ionic channel is a macromolecular complex, in which one part forms the so-called **gating mechanism** controlled by the transmembrane potential difference,

while the other part forms a **selectivity filter** controlling the passage of ions of a certain size across the membrane.

2.1. Selectivity Filter

To explain selectivity, the original pore theory was put forward first by L. Mullins and later by G. Eisenman and B. Hille who proposed that channels had a narrow region that acted as a molecular sieve. At this selectivity filter, an ion sheds its surrounding water molecules and provides an electrostatic interaction with charged or polar amino acid residues that line the walls of the channel. Permeant ions remain bound to the selectivity filter for a short time (less than 1 μs), after which the electrochemical gradient propels the ion across the channel. The ion channels in the nerve and muscle conduct ions across the cell membrane at extremely high rates of up to 100 000 000 ions per second.

2.2. Gating Mechanism

Ion channels are mainly allosteric proteins. Each channel has more than one conformational steady state. Each of these stable conformations represents a different functional state. The transition of a channel between closed and open states is called gating. Various authors have offered for voltage gating identical terms picturing permanent dipoles that flip-flop between two positions or charged particles that are trapped in the membrane and are redistributed between two sides. The first models assume that the gating system has only two states and that the dipole moment change is constant. In all these models the transition should obey the first-order kinetics, and asymmetric displacement currents should relax as single exponentials, whose time constant depend only on the membrane potential. While the two-state, constant dipole model provides a useful starting point for quantitative analysis, it probably does not apply to any of the electrically excitable membranes studied so far. When constructing the gating machinery models, it may ultimately seem preferable to present them in the form of what most likely happens at the molecular level: the dipole moment changes in a multi-state manner in a macromolecule, whose overall orientation in the membrane does not change. For simplicity we present below an illustration of the three-state model.

3. Operating of Voltage-Gated Channel

For these channels, such as a sodium channel, the opening and closing is associated with a movement of a charged region of the channel through the transmembrane electric field (see Figure 1). Changes in the membrane voltage tend to move this charged region back and forth through the electric field, and thus to drive the channel between the closed and open states. The rates, at which transitions occur between the open and closed states of channels, are steeply dependent on the membrane potential. Although these rates can vary from the microsecond to minute time scale, they tend to take, on average, a few milliseconds. The time scale of gating is much slower than the rate of ion permeation through an open channel, which occurs for less than a microsecond. Once a transition between the open and closed states begins, it proceeds virtually instantaneously, giving rise to abrupt, all-or-none, step-like changes in the single-channel current, as the channel is transformed from the fully closed to the fully open state.

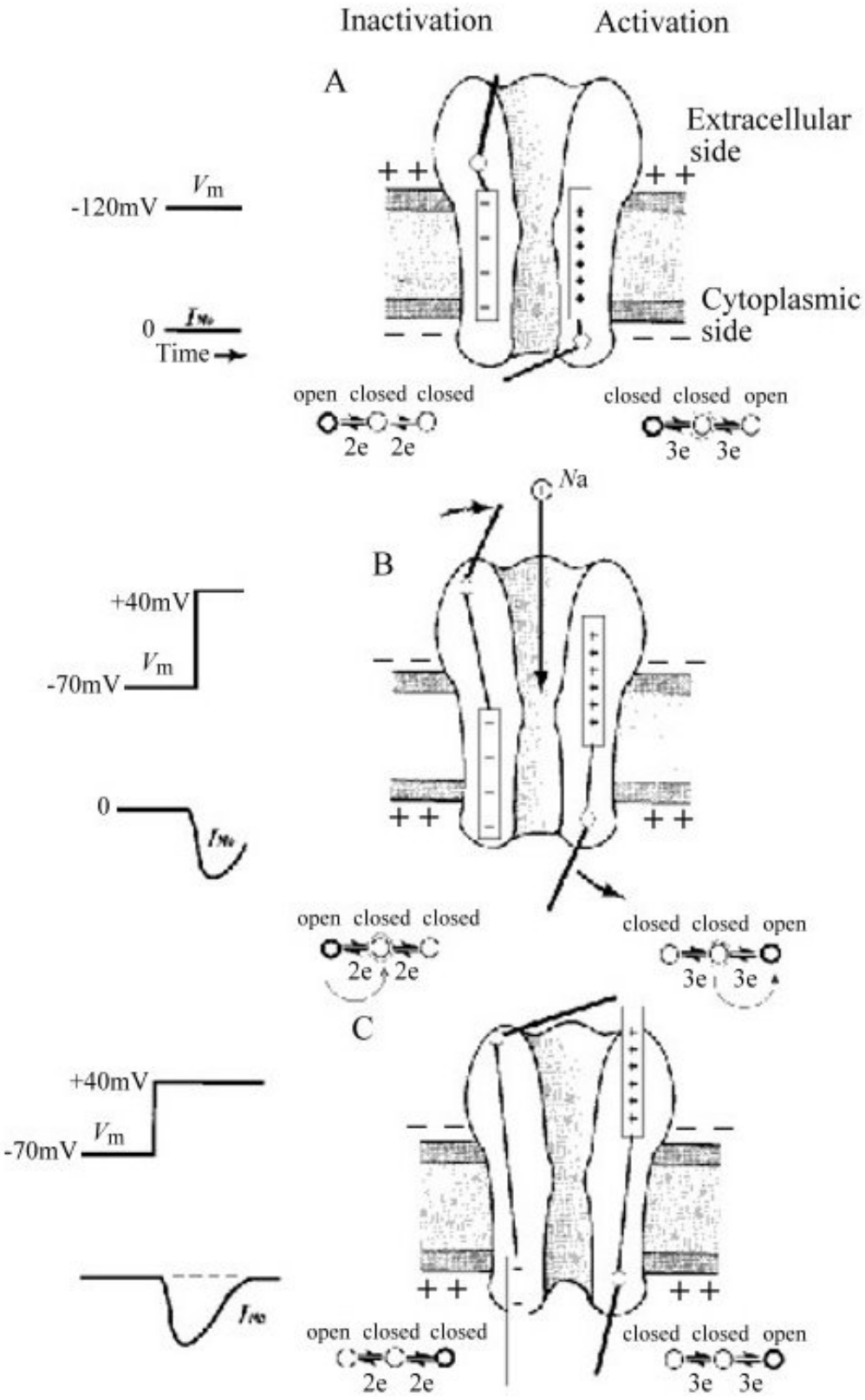

Figure 1. Hypothetical mechanism of sodium channel gating.

It is supposed that the sodium channel gating systems (activation and inactivation systems are not coupled) undergo sequences of transitions through three states. Each of the systems obeys the second-order kinetics. The charge transfer for opening the activation system is supposed to be 3e +3e = 6e (e is the electron charge value). In the inactivation system of our hypothetical sodium channel, which also obeys the second-order kinetics, effective charges are distributed as 2e +2e = 4e.

Hypothetical positively charged voltage sensors are presented inside the channel. Figure 1 displays a very simplified sequence of events in activation and inactivation gating systems after step change of membrane potential:

A. The cell membrane is hyperpolarized. The activation gate is closed, while the inactivation gate is open.
B. When the depolarizing voltage step is applied to the membrane, by a short period of time the channel becomes open due to opening of the activation gating system. The inactivation gate still remains open, but closing of the inactivation gate starts. In the two gating systems, charge transfers occur.
C. During maintained depolarization, the channel that was open begins to close because the inactivation gate is shut.

4. Ligand-Gated Channels

Some of the channels are regulated by a non-covalent binding of chemical ligands. These ligands may be neurotransmitters or hormones in the extracellular environment, which bind to the extracellular side of the channel, or they may be intracellular second messengers that are activated by the transmitters. The second messenger may act on the inside of the channel either directly, by binding to the channel, or indirectly, by initiating protein phosphorylation that is catalyzed by enzymes called protein kinases. This covalent modification (relatively long lasting) of the channel is reversed by dephosphorylation, a reaction catalyzed by protein phosphatases. The *ligand-gated channels* can enter the refractory state, when they are exposed to a high concentration of the ligand. This process is called desensitization.

5. Mechanically Activated Channels

The energy associated with membrane stretch is thought to be transferred to the channel through the cytoskeleton. Gating machinery of the *mechanically activated channels* is regulated by the mechanical stretch of membranes.

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Biographical Sketch

Dr. Boris Krylov graduated from St. Petersburg (Leningrad) State University in 1973. Since then he has worked at the I.P. Pavlov Institute of Physiology, Russian Academy of Sciences. He obtained his PhD and DSc in physiology at this Institute in 1979 and 1994, respectively. He was appointed professor of St. Petersburg State Medical Academy named after I.I. Mechnikov in 2000. Dr. Krylov is deputy director of the Pavlov Institute since 1997 and now he is a head of the laboratory of excitable membranes in this Institute. Since 1990 he has been a fellow of the Alexander von Humboldt Foundation (Germany), and he has worked for one year in Hamburg University Institute for Physiology. Dr. Krylov is co-author of one monograph published in “Springer” and approximately 100 publications in the field of sensory physiology, among them is one article published in “Nature”. He serves on the editorial board of a Russian journal “Sensory Systems”. Since 1998 he has been the president-elect of the St. Petersburg Club of the Alexander von Humboldt Foundation. Dr. Krylov and his co-workers have investigated a number of important membrane molecular mechanisms participating in sensory coding. His present research interests are in the elucidation of ionic channel physiological functions including investigations of ionic channel-membrane receptor coupling.