Ionic Channels of Excitable Membranes

BERTIL HILLE

Ionic Channels of Excitable Membranes

BERTIL HILLE
UNIVERSITY OF WASHINGTON

SINAUER ASSOCIATES INC. • *Publishers*
Sunderland, Massachusetts

Ionic Channels of Excitable Membranes

Library of Congress Cataloging in Publication Data

Hille, Bertil, 1940–
Ionic channels of excitable membranes.

Includes bibliographies and index.
1. Ion channels. 2. Ion-permeable membranes.
3. Neurons. 4. Excitation (Physiology) I. Title.
[DNLM: 1. Cell Membrane—physiology. 2. Ion Channels—physiology. QH 601 H651i]
QH601.H55 1984 574.87'5 84-10587
ISBN 0-87893-322-0

Printed in U.S.A.

10 9 8 7 6 5 4 3 2

To my parents
and to Merrill, Erik, and Trygve
who have consistently supported scientific inquiry

PREFACE

Ionic channels are elementary excitable elements in the cell membranes of nerve, muscle, and other tissues. They produce and transduce electrical signals in living cells. Recently with the welcome infusion of new techniques of biochemistry, pharmacology, and membrane biophysics, ionic channels have become easier to study, and we can now recognize an increasingly wide role for them in non-nervous cells. Sperm, white blood cells, and endocrine glands all require channels to act. The number of kinds of known channels has grown as well. A single excitable cell membrane may contain five to ten kinds and our genome probably codes for more than 50.

Some textbooks of physiology give an excellent introduction to the excitability of nerve and muscle. Their orientation is however more toward explaining signaling in specific tissues than toward the channels per se. On the other hand, most of the original papers in the field use biophysical methods, particularly the voltage clamp. For many biologists these papers are difficult to read because of an emphasis on electronic methods, circuit theory, and kinetic modeling. As more scientists enter the field, there is need for a systematic introduction and summary that deals with the important issues without requiring that the reader already have the mathematical and physical training typical of biophysicists.

This book is meant to be accessible to graduate students, research workers, and teachers in biology, biochemistry, biophysics, pharmacology, physiology, and other disciplines who are interested in excitable cells. Throughout the emphasis is on channels rather than on the physiology of specific cell types. I have tried to introduce all the major ideas that a graduate student in the area would be expected to know—with the exception of questions of technique and electronics. Biological, chemical, and physical questions are discussed, often showing that our ideas have strong roots in the past.

The book has two major parts. The first introduces the known channels and their classical, diagnostic properties. It is descriptive and introduces theory gradually. The second part is more analytical and more difficult. It inquires into the underlying mechanisms and shows how physical theory can be applied. It ends with the chemistry of channel molecules and ideas about their biological evolution. Throughout the emphasis is conceptual. Each chapter may be read by itself as an essay—with a personal bias. Many subtle points and areas of contention had to be left out. Major classical references are given, but the 900 references used are less than 10 percent of the important work in the area. Hence I must apologize in advance to my many colleagues whose relevant work is not quoted directly.

Scientific concepts have a long history and are refined through repeated usage and debate. Many investigators contribute to the test and development of ideas. This book too owes much to those who have come before and is the result of years of discussions with teachers, students, and colleagues. Parts of the text borrow heavily from reviews I have written before. Every chapter has been read in manuscript by generous colleagues and students at several universities. I am deeply grateful for their insightful suggestions which have much improved the text. I owe particular debt to the following experts for extensive help: W. Almers, W.A. Catterall, C. Edwards, R.S. Eisenberg, G. Eisenman, A. Finkelstein, K. Graubard, K. Hille, R.W. Tsien, and W. Ulbricht. I would also like to thank Lea Miller for invaluable help in the preparation of the manuscript. She typed many drafts of the text and assembled bibliographic materials with precision and a strong eye for form and style. Patrick Roberts kindly prepared more than 100 photographs for the figures. The thinking, writing, and original research in this volume were supported for the last 16 years by my research grant from the National Institutes of Health.

BERTIL HILLE
Seattle, Washington
March 1984

CONTENTS

PART II

Chapter 1

INTRODUCTION

Ionic channels are pores

Ionic channels are macromolecular pores in cell membranes. When they evolved and what role they may have played in the earliest forms of life we do not know, but today ionic channels are most obvious as the fundamental excitable elements in the membranes of excitable cells. These channels bear the same relation to electrical signaling in nerve, muscle, and synapse as enzymes bear to metabolism. Although their diversity is less broad than the diversity of enzymes, there are still many types of channels working in concert, opening and closing to shape the signals and responses of the nervous system. As sensitive but potent amplifiers, they detect the sounds of chamber music, guide the artist's paintbrush, yet generate the violent electric discharges of the electric eel or the electric ray. They tell the *Paramecium* to swim backward after a gentle collision, and they propagate the leaf-closing response of the *Mimosa* plant.

More than three billion years ago, primitive replicating forms became enveloped in a lipid film, a bimolecular diffusion barrier that separated the living cell from its environment. Although a lipid membrane had the advantage of retaining vital cell components, it would also prevent access to necessary ionized substrates and the loss of ionized waste products. Thus new transport mechanisms had to be developed hand in hand with the appearance of the membrane. One general solution would have been to make pores big enough to pass all small metabolites and small enough to retain macromolecules. Indeed, the *outer* membranes of gram-negative bacteria and of mitochondria are built on this plan. However, the cytoplasmic membranes of all contemporary organisms follow a more elaborate design with many, more selective transport devices handling different jobs, often under separate physiological control.

How do these devices work? Most of what we know about them comes from physiological flux measurements. Physiologists traditionally divided transport mechanisms into two classes, carriers and pores, largely on the basis of kinetic

criteria. For example, the early literature tries to distinguish carrier from pore on the basis of molecular selectivity, saturating concentration dependence of fluxes, or stoichiometric coupling of the number of molecules transported. A carrier was viewed as a ferryboat diffusing back and forth across the membrane while carrying small molecules that could bind to stereospecific binding sites, and a pore was viewed as a narrow, water-filled tunnel, permeable to the few ions and molecules that would fit through the hole. The moving-ferryboat view of a carrier is now no longer considered valid because the several carrier devices that have been solubilized and purified from membranes are quite large proteins—too large to diffuse or spin around at the rate needed to account for the fluxes they catalyze. Furthermore, the transport protein already extends fully across the membrane. The newer view of carrier transport is that much smaller motions in the protein might leave the macromolecule fixed in the membrane while still exposing the transport binding site(s) alternately to the intracellular and extracellular media. It is not difficult to imagine various ways to do this, but we must develop new experimental insights before such ideas can be tested. Thus the specific mechanism of transport by such important carrier devices as the Na^+–K^+ pump, the Ca^{2+} pump, Na^+–Ca^{2+} exchange, Cl^-–HCO_3^- exchange, glucose transport, the Na^+-coupled co- and countertransporters, and so on, remains unknown.

On the other hand, the water-filled pore view for the other class of transport mechanisms has now been firmly established for ionic channels of excitable membranes. In the period since 1965 a valuable interplay between studies of excitable membrane and studies on model pores, such as the gramicidin channel in lipid bilayers, has accelerated the pace of research and greatly sharpened our understanding of the transport mechanism. The biggest technical advance of this period was the development of methods to resolve the activity of single, channel molecules. As we will consider much more extensively in later chapters, this led to the discovery that the rate of passage of ions through one open channel—often more than 10^6 ions per second—is far too high for any mechanism other than a pore. The criteria of selectivity, saturation, and stoichiometry are no longer the best for distinguishing pore and carrier.

Channels and ions are needed for excitation

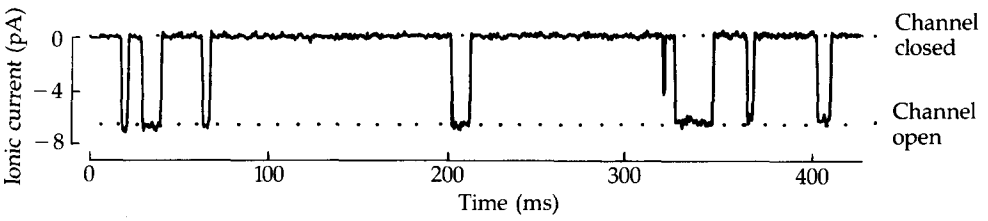
Physiologists have long known that ions play a central role in the excitability of nerve and muscle. In an important series of papers from 1881 to 1887, Sidney Ringer showed that the solution perfusing a frog heart must contain salts of sodium, potassium, and calcium mixed in a definite proportion if the heart is to continue beating long. Nernst's (1888) work with electrical potentials arising from the diffusion of electrolytes in solution inspired numerous speculations on an ionic origin of bioelectric potentials. For example, some suggested that the cell is more negative than the surrounding medium because metabolizing tissue makes acids, and the resulting protons (positive charge) can diffuse away from the cell more easily than the larger organic anions. Soon, Julius Bernstein (1902,

1912) correctly proposed that excitable cells are surrounded by a membrane selectively permeable to K^+ ions at rest and that during excitation the membrane permeability to other ions increases. His "membrane hypothesis" explained the resting potential of nerve and muscle as a diffusion potential set up by the tendency of positively charged ions to diffuse from their high concentration in cytoplasm to their low concentration in the extracellular solution. During excitation the internal negativity would be lost transiently as other ions are allowed to diffuse across the membrane, effectively short circuiting the K^+ diffusion potential. In the English-language literature, the words "membrane breakdown" were used to describe Bernstein's view of excitation.

During the twentieth century, major cellular roles have been discovered for each of the cations of Ringer's solution: Na^+, K^+, Ca^{2+}, as well as for most of the other inorganic ions of body fluids: H^+, Mg^{2+}, Cl^-, HCO_3^-, and PO_4^{2-}. The rate of discovery of new roles for ions in cell physiology has been accelerating rather than slowing, so the list of ions and their uses will continue to lengthen. Evidently, no major ion has been overlooked in evolution. Each has been assigned at least one special regulatory or metabolic task. None is purely passively distributed across the cell membrane. Each has at least one carrier-like transport device coupling its movement to the movement of another ion. Both Na^+ and H^+ ions have transport devices coupling their "downhill" movements to the "uphill" movements of organic molecules. At least Na^+, K^+, H^+, and Ca^{2+} ions are pumped uphill by ATP-driven pumps. Protons are pumped across some membranes by electron transport chains, and their subsequent downhill flow can drive the phosphorylation of ADP to make ATP. Proton movements, through their effects on intracellular pH, will also influence the relative rates of virtually every enzymatic reaction. All of the ionic movements listed above are considered to be mediated by the carrier class of transport devices, and although they establish the ionic gradients needed for excitation, they are not themselves part of the excitation process. Readers interested in the details of ion pumps or coupled cotransport and exchange devices should consult other books on cell physiology.

Excitation and electrical signaling in the nervous system involve the movement of ions through ionic channels. The Na^+, K^+, Ca^{2+}, and Cl^- ions seem to be responsible for almost all of the action. Each channel may be regarded as an excitable molecule as it is specifically responsive to some stimulus: a membrane potential change, a neurotransmitter or other chemical stimulus, a mechanical deformation, and so on. The channel's response, called GATING, is apparently a simple opening or closing of the pore. The open pore has the important property of SELECTIVE PERMEABILITY, allowing some restricted class of small ions to flow passively down their electrochemical activity gradients at a rate that is very high ($>10^6$ ions per second) when considered from a molecular viewpoint. We consider the high throughput rate as a diagnostic feature distinguishing ionic channel mechanisms from those of other ion transport devices such as the Na^+–K^+ pump. An additional major feature is a restriction to downhill fluxes not coupled stoichiometrically to the immediate injection of metabolic energy.

These concepts can be illustrated using the neurotransmitter-sensitive chan-



1 OPEN–SHUT GATING OF AN IONIC CHANNEL

Ionic current flowing across a tiny patch of excitable membrane showing eight brief openings (downward current deflections) of single ionic channels. The membrane patch has been excised from a cultured rat myotube and is bathed on both sides by Na salt solutions. Approximately 300 nM of the neurotransmitter, acetylcholine, applied to the extracellular membrane face is causing channels to open occasionally. At the −140-mV applied membrane potential, one open channel passes −6.6 pA, corresponding to a prodigious flow of 4.1×10^7 ions per second through a single pore. T = 23°C. [Courtesy of D. Siemen, unpublished.]

nels of muscle fibers. At the neuromuscular junction or endplate region of vertebrate skeletal muscle, the nerve axon has the job of instructing the muscle fiber when it is time to contract. Pulse-like electrical messages called ACTION POTENTIALS are sent down the motor nerve from the central nervous system. When they reach the nerve terminal, action potentials evoke the release of a chemical signal, the neurotransmitter acetylcholine, which in turn diffuses to the nearby muscle surface and causes acetylcholine-sensitive channels to open there. Figure 1 shows an electrical recording from a tiny patch of muscle membrane. The cell is actually an embryonic muscle in tissue culture without nerves, but it still has neurotransmitter-sensitive channels that can be opened by applying a low concentration of acetylcholine. In this experiment ionic fluxes in the channels are detected as electric current flow in the recording circuit, and since the recording sensitivity is very high, the opening and closing of one channel appears as clear step changes in the record. Each elementary current step corresponds to over 10^7 ions flowing per second in the open channel. Gating keeps the channel open for a few milliseconds. Other experiments with substitutions of ions in the bathing medium show that this type of channel readily passes monovalent cations with diameters up to 6.5 Å but does not pass anions.

How do gated ionic fluxes through pores make a useful signal for the nervous system? For the electrophysiologist the answer is clear. Ionic fluxes are electric currents across the membrane and therefore they have an immediate effect on membrane potentials. Other voltage-sensitive channels in the membrane detect the change in membrane potential, and they in turn become excited. In this way the electrical response is made regenerative and self-propagating. This explanation does describe how most signals are propagated, but it is circular. Is the ultimate purpose of excitation to make electricity so that other channels will be excited and make electricity? Clearly not, except in the case of an electric organ.

Electricity is the means to carry the signal to the point where a nonelectrical response is generated. As far as is known, this final transduction always starts through a single common pathway: A membrane potential change opens or closes a Ca^{2+}-permeable channel, either on the surface membrane or on an internal membrane, and a Ca^{2+} flux into the cytoplasm is altered, causing a change in the internal free Ca^{2+} concentration. The ultimate response is then triggered by the internal Ca^{2+} ions. This is how the nervous system controls the contraction of a muscle fiber or the secretion of neurotransmitters, neurohormones, digestive enzymes, and so on. Internal free Ca^{2+} also controls the gating of some channels and the activities of many enzymes.

Ionic channels are undoubtedly found in the membranes of all cells. Their known functions include establishing a resting membrane potential, shaping electrical signals, gating the flow of messenger Ca^{2+} ions, controlling cell volume, and regulating the net flow of ions across epithelial cells of secretory and resorptive tissues. The emphasis in this book is on well-known channels underlying the action potentials and synaptic potentials of nerve and muscle cells. These have long been the focus of traditional membrane biophysics. As the biophysical methods eventually were applied to study fertilization of eggs, swimming of protozoa, glucose-controlled secretion of insulin by pancreatic beta cells, or acetylcholine-induced secretion of epinephrine from chromaffin cells, similar channels were found to play central roles. We must now consider that nerve, muscle, endocrine and secretory glands, white blood cells, mast cells, platelets, gametes, and protists all share common membrane mechanisms in their responsiveness to stimuli. Similarly, as biophysical methods were applied to transporting epithelia, ionic channels were found. They too are ion-selective, gated pores, controlled by hormonal influences.

Nomenclature of channels

The naming of ionic channels has not been systematic. In most cases, the biophysicist first attempts to distinguish different components of membrane permeability by their kinetics, pharmacology, and response to ionic substitution. Then a kinetic model is often made expressing each of the apparent components mathematically. Finally, it is tacitly assumed that each component of the model corresponds to a type of channel, and the putative channels are given the same names as the permeability components in the original analysis. Thus in their classical analysis of ionic currents in the squid giant axon, Hodgkin and Huxley (1952d) recognized three different components of current, which they called sodium, potassium, and leakage. Today the names NA CHANNEL and K CHANNEL are universally accepted for the corresponding ionic channels in axons. The name LEAKAGE CHANNEL is also used, although there is no experimental evidence regarding the ions or transport mechanism involved.

Naming a channel after the most important permeant ion seems rational but fails when the ions involved are not adequately known, or when no ion is the *major* ion, or when several different kinetic components are all clearly carried

by one type of ion. Such problems have led to such "names" as A, B, C, and so on, for permeability components in molluscan ganglion cells (Adams, Smith and Thompson, 1980) or qr, si, and x_1 in cardiac Purkinje fibers (McAllister et al., 1975). Other approaches are simply descriptive: Channels have been named after anatomical regions, as in endplate channel or rod outer segment Na channel; after inhibitors, as in amiloride-sensitive Na channel; or after neurotransmitters, as in glutamate channels of crustacean muscle. Eventually, this loose nomenclature will be confusing, and perhaps a systematic approach analogous to that taken by the Enzyme Commission will be needed. However, such a revision ought to wait until the diversity of channels is better understood and the reality of many putative channels is well established. By that time some clear chemical and evolutionary relationships may form the basis for a natural classification.

Ohm's law is central

The study of ionic channels illustrates more than most areas of biology how much can be learned by applying simple laws of physics. Most of what we know about ionic channels was deduced from electrical measurements. Therefore, it is essential to remember rules of electricity before discussing experiments. The remainder of this chapter is a digression on the necessary rules of physics. To do biophysical experiments well, one must often make sophisticated use of electrical ideas; however, as this book is concerned with channels and not with techniques of measurement, the essential principles are few. The most important is Ohm's law, a relation between current, voltage, and conductance, which we now review.

All matter is made up of charged particles. They are normally present in equal numbers, so most bodies are electrically neutral. A mole of hydrogen atoms contains Avogadro's number ($N = 6.02 \times 10^{23}$) of protons and the same number of electrons. Quantity of charge is measured in coulombs (abbreviated C), where the charge of a proton is $e = 1.6 \times 10^{-19}$ C. Avogadro's number of elementary charges is called the FARADAY CONSTANT: $F = Ne = 6 \times 10^{23} \times 1.6 \times 10^{-19} \simeq 10^5$ C/mol. This is thus the charge on a mole of protons or on a mole of Na^+, K^+, or any other monovalent cation. The charge on a mole of Ca^{2+}, Mg^{2+}, or other divalent cations is $2F$ and the charge on a mole of Cl^- ions or other monovalent anions is $-F$.

Electrical phenomena arise whenever charges of opposite sign are separated or can move independently. Any net flow of charges is called a CURRENT. Current is measured in amperes (abbreviated A), where one ampere corresponds to a steady flow of one coulomb per second. By the convention of Benjamin Franklin, positive current flows in the direction of movement of positive charges. Hence if positive and negative electrodes are placed in Ringer's solution, Na^+, K^+, and Ca^{2+} ions will start to move toward the negative pole, Cl^- ions will move toward the positive pole, and an electric current is said to flow through the solution from positive to negative pole. Michael Faraday named the positive electrode the ANODE and the negative, the CATHODE. In his terminology, anions flow to