CHEMICAL AND MOLECULAR BASIS OF NERVE ACTIVITY

David Nachmansohn

Eberhard Neumann

Chemical and Molecular Basis of Nerve Activity

This page intentionally left blank

Chemical and Molecular Basis of Nerve Activity

DAVID NACHMANSOHN

WITH

SUPPLEMENT I

Properties and Function of the Proteins of the Acetylcholine Cycle in Excitable Membranes

DAVID NACHMANSOHN

Departments of Neurology and Biochemistry, College of Physicians and Surgeons, Columbia University, New York, New York

AND

SUPPLEMENT II

Toward a Molecular Model of Bioelectricity

EBERHARD NEUMANN

Max-Planck-Institut of Biophysical Chemistry Goettingen-Nikolausberg, Germany

1975

ACADEMIC PRESS New York San Francisco London

A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1

Library of Congress Cataloging in Publication Data

Nachmansohn, David, (date)
Chemical and molecular basis of nerve activity.

Bibliography: p.
Includes index.
PARTIAL CONTENTS: Supplement 1: Nachmansohn, D. Properties and function of the proteins of the acetylcholine cycle in excitable membranes.–Supplement 2: Neumann, E. Toward a molecular model of bioelectricity.
1. Neurochemistry. 2. Neural transmission. I. Nachmansohn, David (date) Properties and function of the proteins of the acetylcholine cycle in excitable membranes. 1975. II. Neumann, Eberhard. Towards a molecular model of bioelectricity. 1975. III. Title. [DNLM: 1. Neurochemistry. WL104 N122c]
QP356.3.N3 1975 612'.814 75-8750
ISBN 0-12-512757-X

PRINTED IN THE UNITED STATES OF AMERICA

Contents

Preface

Almost sixteen years have elapsed since this monograph was originally published. During this period many dramatic developments in the biological sciences have provided insights into the mechanisms of living cells on the molecular, subcellular, and cellular levels to a degree that only two decades ago seemed beyond the possibility of experimental testing. A few examples, selected at random, follow. The establishment of the tridimensional structure of biopolymers, nucleic acids, and proteins, including enzymes, by the use of X-ray diffraction methods created a new approach to analyzing their mechanisms of action. Electron microscopy, in combination with chemical and physicochemical analyses, provided a basis for the study of cell membranes. Since membranes were recognized as the site of the most vital of cellular functions, biomembranes have become one of the most actively explored fields in present-day biology. Moreover, the study of cellular behavior revealed striking differences among chemical reactions studied in solution and their modification due to membrane structure. Such spectacular progress has been made in genetics, evolution, cell differentiation, immunochemistry, among others, that they have become almost indispensable tools in biochemical studies for the understanding of cellular mechanisms.

Instrumental in all these developments has been the use of new and highly sophisticated physical methods such as fluorospectrometry, nuclear magnetic resonance, circular dichroism, and spin labeling in addition to the already mentioned use of X-ray diffraction and electron microscopy. The development of methods that permit precise measurements of formerly "unmeasurably fast" reactions, presented by Manfred Eigen in his Nobel Prize lecture, have played a special role because of their applicability to an extraordinarily wide range of physical, chemical, and biological problems. Relaxation spectrophotometric techniques have reached a level permitting measurements of reactions covering a time range from seconds to nanoseconds, an achievement thought impossible only fifteen years ago. The rapidity and revolutionary character of all these advances in biological sciences reminds one of the exciting period of the 1920's and 1930's during which atomic physics changed man's grasp of the physical world, and had an impact on the intellectual and philosophical level more profound than the entire preceding century.

As in all fields of biological science, these recent developments obviously have had profound influence on the problems of nerve impulse conduction, nerve excitability, and generation of bioelectricity. The vast number of pertinent and exciting advances in this particular field require a new monograph which is at present in preparation. The question may be raised whether it is useful under these circumstances to reprint a monograph written almost sixteen years ago. For several reasons an affirmative answer seems appropriate. The monograph summarizes the results of the first two decades of biochemical research in a field which until then was predominantly the domain of physiology and pharmacology. The investigations were based on the analysis of the properties and function of the specific proteins and enzymes associated with the action of acetylcholine initiated by the author almost four decades ago. This type of approach was essentially due to the background and training of the author. As outlined in his prefatory chapter Biochemistry as Part of My Life in the *Annual Review of Biochemistry* in 1972, the author had spent his most formative years at the Kaiser–Wilhelm Institutes in Berlin–Dahlem, which at that time comprised the most advanced center for the study of biochemistry. Also at the center, at that time, proteins and enzymes were stressed in research on cellular mechanisms.

When this monograph first appeared, few biologists interested in nerve activity believed that the analysis of proteins was a basic necessity for the understanding of molecular events underlying bioelectricity and nerve excitability. This situation has changed drastically. During the last decade the purely descriptive phenomenology of the events during electrical activity has come under vigorous criticism from many sources. It is now widely recognized that an understanding of nerve excitability requires knowledge of the molecular events within the excitable membrane responsible for this cellular function. Today, literally hundreds of investigators are working on the properties and function of proteins linked to the action of acetylcholine, two of which were already isolated and characterized by the author at the time this book was first published, and another two postulated. This change of attitude has revived the interest in the early phase of the biochemical approach presented in the monograph. There are many facts established in that early period which are still fully valid. Some of the incomplete information has been greatly enhanced by new advances. Some of the data obviously became questionable and some obsolete when tested with the new, more elaborate, and sophisticated techniques. However, much more pertinent is the fact that the fundamental concepts underlying the author's approach are still valid and are the basis of many advances of the last decade. Many proposals and postulates, some of them quite speculative, have been confirmed by recent experimental data. Although the basic concepts remain unchanged, many details have been modified to accommodate the ever-increasing amount of information. Einstein remarked once in a discussion with Werner Heisenberg, reported by the latter in a recent publication: "Whether you can observe a thing or not, depends on the theory you use. It is the theory which decides what can be observed."

It should be of interest to many to read about the origin of postulates confirmed experimentally many years later, such as the existence of an acetylcholine receptor protein, or the assumption that a conformational change of this protein may be induced by reaction with acetylcholine and may trigger the events leading to increased ion permeability, or the proposal of the cyclic nature of the reactions involving acetylcholine (see page 101). In some areas, e.g., those concerning the role of acetylcholine at synaptic junctions, many problems were at that time open to question. However, new data have drastically changed the situation. They have provided the basis for a much better understanding of the differences and similarities between conducting and synaptic parts of the excitable membrane and the specific function of the acetylcholine cycle in both parts.

Copies of this monograph have been unavailable for a decade. New developments have apparently revived interest in the early phase of investigations on the chemical basis of nerve activity. Knowledge of the initial efforts should be informative and useful to many investigators who at present work on the problems discussed in that first publication. Many readers, however, may not be aware of the striking new developments of the last decade supporting many views and postulates presented at that time; others may be interested in the present views of the author as to recent observations pertinent to his original concepts. Therefore two supplements in which recent advances are presented in condensed form have been added. The revised monograph now not only has historical value, but gives the reader an idea of the exciting progress made in this field, and thus acts as a bridge to the new monograph in preparation.

The first supplement concentrates on recent progress in the biochemistry of excitability, while in the second an *attempt* at an integral model of nerve excitability is described. This most exciting recent development was initiated by the late Aharon Katchalsky; a tentative version was published in 1973 (*Proc. Nat. Acad. Sci. U.S.*). The model is an attempt to integrate basic electrophysiological, biochemical, and physicochemical data on nerve excitability. During the last two years Dr. Neumann has greatly extended and broadened the theoretical basis of the model in physicochemical terms. The model, as is the nature of all models, is seen as a working hypothesis detailed to such an extent that it offers many challenges to the researcher.

Dr. Neumann kindly accepted an invitation to write the second supplement in which a presentation of his integral model in its present form is included. The author would like to express to him his thanks for this invaluable addition to the monograph. He also gratefully acknowledges Dr. Neumann's help in the formulation of many ideas which have greatly improved Supplement I.

David Nachmansohn

Preface to First Edition

The spectacular progress in biology and biochemistry during the last two decades has revolutionized our understanding of cellular mechanisms and has extended our knowledge of life processes far beyond the limits foreseeable only a generation ago. Many aspects of cellular function are today analyzed on molecular levels. The conduction of nerve impulses, the most important function of the nervous system and one of its most striking and remarkable features, is one of the fields in which much information has been obtained as to the underlying chemical and molecular forces. A comprehensive presentation of the development of this particular problem and an evaluation of its present state appears, therefore, desirable. To provide such a contribution is the aim of this monograph.

In the 1930's there were lively discussions at the meetings of the Physiological Society of England about the question of whether or not acetylcholine was a neurohumoral transmitter across myoneural and synaptic junctions. The interpretation of the role of acetylcholine was based essentially on pharmacological observations and was in contradiction to the conclusions based on electrophysiological evidence. Not the experimental data but interpretations of their meaning were hotly debated; even the most ardent proponents of the hypothesis of neurohumoral transmission admitted that there were many gaps and serious contradictions. The sharp conflict of views was a challenge to initiate an entirely new approach. The development of new chemical and biochemical methods and procedures, the rapid growth of protein and enzyme chemistry, and the notions and principles developed in the study of the chemical basis of other cellular functions, notably that of muscular contraction, seemed to offer great promise of providing a more satisfactory answer to the fascinating problem of nerve activity in general and in particular to that of the role of acetylcholine in this process.

Studies of the author and his associates, initiated about 24 years ago, have made it possible to establish the sequence of energy transformations associated with nerve activity and to integrate the formation and hydrolysis of acetylcholine into the metabolic pathways of the nerve cell. Proteins and enzymes of the acetylcholine system have been isolated, their properties, the molecular forces in their active sites, and their reaction mechanisms have been analyzed. Many relationships between chemical reactions and electrical events, between molecular forces active in the proteins in solutions and manifestations of the intact cell, or even in the intact animal, have been established. The results led very soon to a modification of the original hypothesis of neurohumoral transmission. They have shown that the action of acetylcholine is not an *inter-* but an *intra*-cellular, or rather an intra-membranous process essential for the generation of bioelectric potentials in all conducting membranes throughout the animal kingdom. The acetylcholine system is necessary for controlling the ion movements which form the basis of electrical manifestations in living cells.

As might have been expected, the new concept first proposed in 1940 initially met with much skepticism and frequently vigorous opposition and criticism. This is a natural and healthy reaction in all scientific fields. Some of the objections were helpful in that it became necessary to conduct crucial experiments to obtain an answer to justifiable doubts and questions. During the last two decades a considerable amount of experimental data has accumulated in support of the new concept. The idea has proved to be fruitful and has provoked investigations which have yielded information pertinent to the problem of cellular mechanisms in general.

ACKNOWLEDGMENTS

This monograph is dedicated to all those who have stimulated, encouraged, and helped the author in his work. He would like to express his gratitude to Dr. H. Houston Merritt, Professor of Neurology, Dean of the Medical School. Thanks to his efficient and unwavering support over many years it was possible to build up an active group of investigators and all the necessary facilities, thus providing the basis for the progress of the last decade.

Among the scientific colleagues and friends who stimulated his scientific thinking the author would like to mention in the first place Otto Meyerhof and the group of colleagues associated with him. The years spent in the unique atmosphere of the Kaiser Wilhelm Institutes in Berlin-Dahlem in the 1920's, at that time a center with many brilliant scientists, had a decisive impact on the author's scientific formation. The years at the Sorbonne initiated happy and fruitful associations with his French friends, among them René Wurmser, Edgar Lederer, and René Couteaux. The frequent discussion with Sir Frederick Gowland Hopkins, when the author worked in his laboratory, were invaluable.

Dr. John F. Fulton, in whose laboratory at Yale University the author spent three years, was the first neuro-physiologist to fully endorse the new theory and he has forcefully supported the new concept ever since.

The author would like to express his thanks to his many able collaborators who are at present and have been in the past in his laboratory. Without their help the work would never have advanced to the point which it has reached. It was particularly fortunate that Irwin B. Wilson, well trained in modern physical and physical organic chemistry in Columbia's Chemistry Department, joined the laboratory in 1949. The association with him over many years was extremely stimulating and pleasant and, it seems to the author, fruitful for the development of the field.

The generous financial support of several government agencies is gratefully acknowledged, in the first place of the United States Public Health Service, which supplied by far the greatest part of the funds. Essential additional support was received from the National Science Foundation, the Atomic Energy Commission, and the Surgeon General of the Army. Grants from private Foundations have been very helpful, in the earlier phase from the Dazian Foundation for Medical Research and the Josiah Macy, Jr., Foundation, at present from the Rachel Mellon Walton Foundation and from the Muscular Dystrophy Associations of America, Inc.

D. NACHMANSOHN

College of Physicians and Surgeons, New York
August, 1959

This page intentionally left blank

CHAPTER I

Physical Events during Nerve Activity

A. Electrical Manifestations

The primary function of nerve cells is that of receiving and carrying messages. They form the communication system between the outer world and the organism and between distant points of the body. The most important and vital functions of our organism are to a great extent controlled and regulated by the nervous system. The brain is the site of the human intellect, of memory, of men's creative forces. It is not surprising, in view of the paramount importance of the nervous system, that Galvani's observations on "animal electricity," published in 1791, and his suggestions about the role of electricity in nerve activity were received with great enthusiasm and passionate interest not only by scientists but by intellectuals all over the world. For the first time there seemed to be a ray of hope of penetrating into one of the great mysteries of the living world. Volta's criticism and his conclusions that Galvani's observations did not really prove his interpretation, were justified. Nevertheless, it turned out that Galvani's ideas were correct and that nerve activity is associated with electric currents. It took, however, half a century until the experiments of Matteucci from Pisa and DuBois-Reymond in Germany firmly established that a flow of current takes place in animal tissue. They discovered, with the aid of the newly developed galvanometers, that current flows through the instrument connected by electrodes with the longitudinal surface of the muscle fiber and the cut end. The former was electropositive, the latter electronegative, and in the external circuit the current flows toward the cut end. It was later recognized that this current flow is due to injury and it is, therefore, referred to as injury or demarcation current. DuBois-Reymond also noticed that the current flow diminished during activity. The observation indicates that the active point becomes less positive in relation to the cut end, i.e., negative to the resting surface. Bernstein later observed that this negativity was propagated like a wave with a velocity equal to that determined by Helmholtz for the rate of propagation of the nerve impulse. Since that time the conduction of the nerve impulse has been identified with the wave of negativity sweeping down the fibers. This flow of current is today usually referred to as action current, and the potential developed, as "spike" potential.

Following the early observations of Matteucci and DuBois-Reymond neurophysiology was for more than a century almost synonymous with electrophysiology. Progress in this field was greatly facilitated by the continuous improvement of highly sensitive recording instruments, culminating in the introduction of the cathode-ray oscillograph for electrophysiological measurements by Erlanger and Gasser more than thirty years ago. Electrophysiology has given us much important information about various properties of neurons in rest and during activity, about such features as facilitation, inhibition, and summation of impulses, about the time relations of the different parameters of the action potentials, and about the refractory period, the absolute and the relative one, during which the nerve excitability is either abolished or greatly decreased. Characteristics of excitability and stimulus strength, differences between axon and cell body, speed of propagation in various types of nerve fibers encountered in the animal kingdom, have been extensively studied. The localization of many specific functions and pathways of many neurons have been elucidated by electrophysiological techniques. Without attempting merely to enumerate all the aspects studied, it may be stated that the knowledge achieved as to the electrical properties of neurons and their electrical manifestations during activity form an integral part of the physiology of the nervous system.

B. The Membrane Theory

Neurophysiologists of the nineteenth century were well aware of the fact that knowledge of the electrical properties and the electrical manifestations of nerve activity are not sufficient for the understanding of the underlying mechanism. Whereas in the copper wire electron movements are responsible for the flow of current, the neuron, being a fluid system, can only be a second-degree conductor. The electric currents must in this case be carried by ions. This was clearly recognized by biologists of the last century. More detailed theories as to the mechanism of the generation of bioelectric currents began to be formulated in the latter part of the nineteenth century, when physicochemical studies revealed the great potential differences which may develop at semipermeable membranes. Notions and ideas proposed by Traube, Ostwald, Nernst, and other physical chemists led to the so-called membrane theory which is still the basis of most modern concepts concerning the mechanism of nerve conduction. The theory is best known through the formulation of Bernstein and Tschermak early in this century (Bernstein, 1902). According to this theory the nerve fiber is surrounded by a semipermeable membrane which has a positive charge on the outside and a negative one on the inside. It is selectively permeable for K^+. When a stimulus reaches a membrane, the permeability at the active site is greatly increased for all ions with a concomitant decrease in resistance. The active part becomes depolarized; thereby small electric currents are generated which stimulate the adjacent points and the same process takes place there. In this way successive parts of the membrane are activated and the impulse propagated along the axon.

It is a remarkable tribute to the ingenuity of the physical chemists of the last century that, in spite of all the great progress of methods and knowledge during the twentieth century, the membrane theory has remained the basis of our present concepts. One major modification has become necessary: at the activated point there is not merely a depolarization but a reversal of charge: the inside becomes positive and the outside becomes negative. This has been shown by Curtis and Cole (1942) and by Hodgkin and Huxley (1945) by insertion of microelectrodes into the interior of an axon. The material used in these experiments was the giant axon of squid (*Loligo pealii*), which has a diameter of about 400–800 μ, depending on the size of the specimen. The technique applied made it possible to measure directly the potential between the inside and the outside electrode. During the passage of the impulse the charge does not merely disappear, as was assumed in the original theory, but is reversed. By this "overshoot" the action potential becomes about twice as great as the resting potential.

An important advance was the experimental evidence by Cole and Curtis (1939) for a breakdown of resistance during activity. According to their measurements, which were carried out on the giant axon of squid, the resistance during activity drops from about 1000 to 40 ohms square centimeter.

C. Ion Movements

One of the characteristics of most living cells is the fact that the concentration of ions in the interior is quite different from that in the extracellular fluid. Sodium (Na) on the outside is usually about ten times as high as inside the cell, the reverse is true for potassium (K). In some types of tissue the differences are even higher. The conducting cell has developed the special ability to make use of the ionic concentration gradients resulting from the unequal distribution of ions for its special function, i.e., for the generation of small electric currents which conduct the impulse. Overton (1902) clearly recognized the specific function of Na for conduction. He found that a muscle kept in Na-free solution became inexcitable. No other ion except lithium was able to replace Na. He also suggested that conduction was associated with an exchange of extracellular Na with intracellular K.

The availability of radioactive ions after the Second World War made it possible to measure quantitatively ion movements in rest and during