International Series of Monographs on Pure and Applied Biology, Modern Trends in Physiological Sciences Division, Volume 20

Histophysiology of Synapses and Neurosecretion

E. de Robertis

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by

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PERGAMON PRESS

OXFORD · LONDON · EDINBURGH · NEW YORK
PARIS · FRANKFURT

1964

PERGAMON PRESS LTD.
Headington Hill Hall, Oxford
4 and 5 Fitzroy Square, London W.1

PERGAMON PRESS (SCOTLAND) LTD.
2 and 3 Teviot Place, Edinburgh 1

PERGAMON PRESS INC.
122 East 55th Street, New York 22, N.Y.

GAUTHIER-VILLARS ED.
55 Quai des Grands-Augustins, Paris, 6^e^

PERGAMON PRESS G.m.b.H.
Kaiserstrasse 75, Frankfurt am Main

Distributed in the Western Hemisphere by
THE MACMILLAN COMPANY · NEW YORK
pursuant to a special arrangement with
Pergamon Press Limited

Library of Congress Catalog Card Number 62–12347

MADE IN GREAT BRITAIN

INTERNATIONAL SERIES OF MONOGRAPHS ON PURE AND APPLIED BIOLOGY

Division: MODERN TRENDS IN PHYSIOLOGICAL SCIENCES

General Editors: P. ALEXANDER and Z. M. BACQ

Volume 20

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INTRODUCTION

SOME of the most important developments of modern biology have originated from the use of physical and chemical techniques at a subcellular level of structure and function. In several cases, e.g. muscle, action of genes, sickle-cell anemia and so forth, this approach has reached the molecular realm and has permitted the interpretation of biological phenomena as resulting from the properties of the participating molecules. This refined field—now called molecular biology—is the desideratum of Histophysiology since at this level both from and function represent only different aspects of a single entity.

In the case of the nervous tissue, advances are not as important as in other cell territories, but in recent years subcellular analysis has also been reached. With the use of fine microelectrodes, intracellular recordings of the electrophysiological events accompanying nervous activity were obtained in single cells and even from special regions of a cell. This study has led to the detection of a wide variety of local or propagated potentials which include dendritic, generator, receptor potentials, excitatory and inhibitory synaptic potentials, pacemaker and spike potentials, all of which indicate the existence of specialized loci of activity in the nerve cell. Minute amounts of drugs can now be injected electrophoretically, mimicking or interfering with the physiological activity. By means of radioisotopes the ionic mechanisms involved in membrane potentials may be studied and at certain synapses the sites of receptor proteins to some transmitters can be marked.

Until recent years this wide variety of activities of the nerve cell was correlated mainly with the structure revealed by the light microscope. This showed the complexities of the cellular processes and intracellular connections, but did not give any information of the subcellular components involved in the above mechanisms. Only by means of the electron microscope, with its much higher resolving power that reaches the level of macromolecules, has it been possible to uncover some of the subcellular structures that are at the bases of nerve activity.

The idea of writing this monograph was started by a kind suggestion of Prof. Z. M. Bacq from the University of Liège after his attendance to the XXI International Physiological Congress in Buenos Aires. Without it and the help of the Pergamon Press, Inc., it would never have been born. Now that it has been completed, the author realizes how difficult it will be for it to survive the great advances that day after day are being made in this field. This is the shortcoming of all attempts to explore new domains with an interdisciplinary approach that cuts across well defined boundaries.

This book is mainly based on original researches started in 1953 which led to the finding, with Prof. H. S. Bennett, of the *synaptic vesicles* as the main component of synapses, and which have been continued along different aspects of the ultrastructure, chemistry and function of synaptic junctions. In the course of these investigations it was realized that at most synapses there was an active process of synthesis and release of material that essentially corresponded to a localized neurosecretion. This led to an enlargement of viewpoints and to an electron-microscopic analysis of other nerve structures that for a long time had been recognized as neurosecretory—such as the hypothalamic–neurohypophyseal system—and also of other tissues of nervous origin such as the pineal and the adrenal medullary gland. This analysis permitted the recognition of basic similarities in the mechanisms of formation, storage and release of these different neurosecretory processes and led to a unitary concept of neurosecretion, which is the main thesis that will be maintained here.

This book can thus be considered as an attempt to correlate subcellular structure and function in synapses and to demonstrate that a phenomenon of neurosecretion takes place in these important areas of the central and peripheral nervous system. In the treatment of the different subjects, attempts are made to integrate the study of synapses and neurosecretion from several methodological angles. In view of this goal the literature does not need to be complete, covering only the points of interest that are being studied at present or should be investigated.

The material is divided into two parts and twelve chapters. The first part is dedicated to *The Synapse* and comprises the study of the ultrastructure, chemical composition and function of these important areas of the nervous system, in which nerve impulses are transmitted and where other unknown but no less important functions probably take place. This first part is divided into eight chapters. The *initial one* on general concepts on synaptic transmission starts with a brief account of the historical developments both from the morphological and physiological viewpoints and is followed by an elementary treatment of the synaptic mechanisms that are suggested by microphysiological studies. The *second chapter* is a brief review of the morphological aspects of synapses as were revealed by the light microscope. The *third* is a general study of the ultrastructure of the synaptic region in the central nervous system, in which the main characteristic of the synaptic vesicles and the complex organization of the synaptic membranes are described. New structures such as the intersynaptic filaments and subsynaptic web are mentioned. The *fourth chapter* deals with the ultrastructure of special synapses such as: the myoneural junction, synapses of the autonomic system, of electroplates of electric fishes, the synapses of invertebrates in general, and the so-called electrical synapses. The general problem of the localization and significance of synaptic vesicles and the possible dual role of acetylcholine at pre- and postsynaptic sites are mentioned. In the *fifth chapter* some of morphophysiological correlations in certain synapses are presented. Changes of synaptic vesicles with electrical stimulation are observed and related to the rapid formation of the vesicles at the ending and with its mobilization and release at the junction. The physiological evidence to consider the synaptic vesicles as quantal units of transmitters is discussed and the essential similarities between the structure and the functional operation of synapses is stressed. *Chapter six* deals with changes in synaptic vesicles, and of the junction in general, with nerve degeneration. In *chapter seven* the structure of synapses of the retina is analyzed. *Chapter eight* deals with recent studies of our laboratory on the isolation of nerve endings and synaptic vesicles from the CNS This has led to the isolating of a population of cholinergic nerve endings from a larger one of non-cholinergic endings. The significance of synaptic vesicles as units of transmitter substances has now been proven.

The *second part* dealing with *Neurosecretion* starts with *chapter nine*, in which the unitary concept of neurohumoral mechanisms is defined and the hypothalamic–neurohypophyseal system is studied in its ultrastructure and function. The concept of axoplasmic flow of neurosecretion is supplemented with that of progressive synthesis and storage along the axon until the quantal size of the neurosecretory unit is reached. The possible role of synaptic vesicles at the neurosecretory endings is discussed. In *chapter ten* the adrenomedullary cells are studied in their ultrastructure and function. The synthesis and secretion of catecholamines is followed under the electron microscope. *Chapter eleven* deals with the nerve endings in the pineal gland in which submicroscopic studies have revealed an active process of secretion. The so-called plurivesicular material is seen to vary with factors that change the metabolism of biogenic amines. The final *chapter twelve* on the secretion of adrenergic nerve and endings in a certain way bridges the gap between synaptic secretion and neurosecretion in the classical sense. The plurivesicular material which is characteristic of adrenergic nerves, is described and related to the storage of the adrenergic transmitter. These findings open the possibility of a histophysiological study of the mechanism of storage and release of the adrenergic transmitter with the electron microscope.

A book like this could not be written without the unselfish collaboration of many. Firstly I would like to express my gratitude to Dr. Amanda Pellegrino de Iraldi, who has collaborated directly with me in the later studies on the structure of central synapses, the plurivesicular components of adrenergic nerves, and the ultrastructure and function of the pineal gland. To Prof. C. J. Gómez, Dr. Georgina Rodríguez de Lorez Arnaiz and Dr. Leon Salganicoff for their collaboration on the isolation and chemical analysis of synaptic endings and synaptic vesicles.

I would like to acknowledge Dr. H. M. Gerschenfeld for his collaboration on the concepts of synaptic barrier, for critical reading of the whole manuscript, for contributing with Dr. Tauc some of the beautiful intracellular recordings in *Aplysia* and together with Dr. J. M. Tramezzani for the work on the hypothalamic–hypophysial system.

I wish to express my gratitude also to Dr. A. Lasansky for reading and improving the chapter of retinal synapses. To Dr. Flora Wald for her contribution in the work on glial cells and retina. To Dr. D. D. Sabatini for his collaboration on the studies of the adrenal medulla. To Miss Lina Levi for her excellent collaboration in all technical matters related to electron microscopy. To Mr. Walter Ludwig and Mrs. Wilma Hubscher for their work of typing and tidying the manuscript.

Contributions to the better illustration of this book have kindly been provided by: Profs. R. J. Birks, B. B. Boycott, Sir Lindor Brown, D. R. Curtis, Sir John Eccles, R. W Guillery, E. G. Gray, H. Huxley, H. Hyden, B. Katz, A. Lasansky R. Miledi, W. K. Noell, G. L. Rasmussen, L. Tauc and J. Taxi.

Finally I want to express my most sincere thanks to Prof. Z. M. Bacq for starting the whole thing, to Dr P. Alexander for correcting the manuscript and to all members of Pergamon Press who have edited and produced this monograph.

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PART I

The Synapse

CHAPTER 1

GENERAL CONCEPTS ON SYNAPTIC TRANSMISSION

THE early development of ideas on *synapses* or *synaptic junctions* is intimately related to the discoveries made at the end of the nineteenth and beginning of the twentieth century on the morphological and physiological organization of the nervous system.

From the structural viewpoint, the concept of synapse was a direct consequence of the establishment of the neuron doctrine as opposed to the older reticular theory. Early neurohistologists such as Gerlach (1871) and Golgi (1885) and others, impressed by the complex and apparent net-like structure of the nervous system, thought that this constituted a reticulum of anastomosing branches forming a network of dendrites or axons or of both, with the cells lying at nodal points.

His (1886, 1889), Forel (1887) and independently Cajal (1888, 1890, *a, b, c*) opposed the reticular theory and affirmed that each nerve cell—*the neuron* (Waldeyer, 1891)—is an independent unit whose branches are not in continuity but may be in close contact. These conclusions were reached by the study of more simple systems, such as the observation of individual neuroblasts during embryonic development or by the use of techniques, such as that of the Golgi silver chromate method, that may stain some independent units among the complex pattern of cells and fibres of the nervous system.

MORPHOLOGICAL CONCEPT OF THE SYNAPSE

A direct consequence of the neuron theory was the assumption that the relationships between nerve cells were not through continuity, but by continuity or *functional contact.* After the discovery of the independence of the neuron with all its profuse branching, Cajal thoroughly and systematically studied the connexions between neurons showing the existence of definite and specific contacts between the axons or their collaterals with the dendrites and somata ot other neurons. He described the profuse branching of nerve terminals around the nerve cells forming the so-called baskets *(corbeilles)* and terminal bushes, and the climbing fibres around the dendrites of the Purkinje cells (Cajal, 1890, *a*). On the other hand, Held (1891) described the "*Fasern-knorbe*" in the trapezoid body. At first, the typical nerve endings were not well demonstrated because of the ineffectiveness of the techniques in staining the ultimate terminals, but finally Held (1897), Auerbach (1898), Cajal (1903) and others, by the use of neurofibrillary methods, were able to demonstrate the characteristic *boutons* and other types of synaptic knobs that will be described in Chapter 2.

The neuron theory was only accepted after long controversy. Golgi (1890, 1891) and later Held wrote as recently as 1929 in defence of the reticular theory. Held (1905) thought that the continuity between neurons could be effected by fine neurofibrils, and Boeke (1940) and Stöhr (1957) described a periterminal reticulum establishing the continuity across the contacting elements. Cajal (1934) had to write his memorable last work critically examining the whole controversy; since then, it may be said that the neuronal theory has not been seriously challenged, at least, for the vertebrate nervous system (Eccles, 1959).

PHYSIOLOGICAL CONCEPT OF THE SYNAPSE

While this wealth of morphological information was establishing some definite order in the complex organization of the nervous tissue, the functional concept of the synapse was established. As a direct consequence of the neuron theory, Sherrington, in 1897, explained the special properties of the reflex arc as depending on the junctional region of contact between the two neurons involved. He coined the name of synapse for this special locus of contact. By that time, Cajal (1895) had already expressed this concept of dynamical polarization of the neuron, by which the dendrites and the somata conduct toward the axon, and had postulated that this phenomenon could be related to the intercellular connexions. However, it was Sherrington (1900), who clearly attributed to the *valve-like* action of synapses the one-way conduction within the neuron.

In his studies on the reflex transmission, Sherrington discovered some of the other fundamental properties of synapses, such as: the additional delay that the impulse has in traversing the junction, the