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ACETYLCHOLINE

AN APPROACH TO THE MOLECULAR MECHANISM OF ACTION

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An approach to the molecular mechanism of action

by

M. J. MICHELSON and E. V. ZEIMAL

The Sechenov Institute of Evolutionary Physiology and Biochemistry, Leningrad, U.S.S.R.

Translated from the Russian by

E. LESSER

Department of Pharmacology, Chelsea College, University of London

and

MIRA LESSER

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Foreword

AMONG the greatest achievements of modern science the establishment of the chemical nature of synaptic transmission of nervous excitation from the nerve ending to the innervated cell and the elucidation of the mechanism of this transmission occupy an important place. By selecting precisely a chemical mechanism, Nature solved several important problems at once, viz. the unidirectional character of conduction, the presence of a threshold, the capacity for summation and others. To do this, however, she had to work out not only special structures and mechanisms, but also new principles of biological action. The discovery of these principles by modern science has given an extraordinary impetus to the development of the physiology and biochemistry of the nervous system and, at the same time, enabled scientists to solve a series of important pharmacological problems in the search for new therapeutic agents.

Of the two most important features of the cholinergic nerve synapse—cholinoreceptors and cholinesterases—only the latter lend themselves to investigation by classical biochemical methods, viz. by means of the separation of the physiologically active structural unit, the establishment of its structure and the study of its properties. It is not possible to study the cholinoreceptive substance by such methods, for its very separation from the synaptic structure must, inevitably and in principle, be accompanied by the loss of its basic property, viz. having been acted upon by acetylcholine, to alter the ionic conductivity of the membrane. It is for this reason that the fundamental pathway of investigation of cholinoreceptors and the cholinoreceptive substance is chemical-pharmacological research in which, on the basis of a study of the responses of the whole structure to the action of natural or synthetic physiologically active substances, the chemical nature of the active centres of the structure under investigation can be determined. All the basic information on the structure and properties of cholinoreceptors has been obtained in precisely this way.

The chemical-pharmacological method of studying biological structures and functions, which first arose as a subsidiary method used in pharmacology to resolve problems in the search for new therapeutic substances, has now grown into an independent science—molecular pharmacology. This science approximates most closely to molecular biology, of which in a certain sense it is a component part, while in other respects it comes nearer to enzymology, the chemistry of physiologically active substances and biophysics.

Although the concept of the chemical nature of the transmission of nervous excitation originated 90 years ago, it is really only the last two decades that have brought the biggest advances, leading to a qualitative leap in this field. Other branches of molecular biology have developed in a very similar manner. It may be recalled that the nucleoproteins of the cell nucleus have been known since the end of the last century; but it is only in the last two decades, after the essential role of the nucleic acids as the matrix of the self-duplication and synthesis of protein in the living cell had been clarified, that a period of turbulent develop-

ment of the chemistry of the polynucleotides began. In exactly the same way, from the end of the forties and beginning of the fifties there began a vigorous and fruitful development of research into the physiology and chemistry of the nerve synapse. And it may be that today it is just this branch of molecular biology which, through molecular pharmacology, has yielded the greatest practical results for mankind.

The present work contains a detailed account of a large chapter in molecular pharmacology, concerned with the cholinergic nerve synapse, its structures and mechanisms and the pathways of the chemical (pharmacological) action on its function. The authors of the book—Professor M. J. Michelson and Dr. E. V. Zeimal—have by their research made a big contribution to the development of this chapter of molecular pharmacology and are recognized authorities in this field.

A special feature of this book is its wide use of modern concepts of electronic structure and conformation of molecules in relation to their reactive capacity. This feature is characteristic also of the personal research of the authors, conducted in close contact with the organic chemists A. L. Mndzhoyan, N. V. Khromov-Borisov, B. A. Porai-Koshits and the editor of this book and his collaborators, in the first place N. N. Godovikov. It distinguishes this book by Michelson and Zeimal to its advantage from the purely physiological and pharmacological reviews and monographs devoted to the nerve synapse.

Many of the problems treated in this book are, of course, still far from being resolved. There are still many contradictions among the theories, between fact and theory and, apparently, even contradictions among the facts. In such cases the authors, in giving preference to any particular one, have nevertheless tried as far as possible to set out objectively all the pros and cons of the explanations proffered in their book. Many of the matters in this book thus remain open. What of it? That is not a defect, but a merit of the book: it summons us to new efforts in research.

ACADEMICIAN M. I. KABACHNIK

Preface

ALTHOUGH it was not Claude Bernard himself who originated the idea of chemical transmission of nervous stimuli, it was his classical experiments with curare that created the basis for the birth of this idea. Bernard showed that curare evoked a paralysis of voluntary muscle without disrupting either transmission in the nerve or the capacity of the muscle to contract in response to direct stimulation. The site of action of curare, therefore, lay somewhere in the region of contact between nerve and muscle. This was the first demonstration of the peculiar chemical sensitivity of the neuromuscular junction, which differed from the chemical sensitivity of both nerve and muscle. This has been demonstrated also in the works of N. E. Vvedensky.

The idea of chemical transmission of nervous stimulation was first formulated in 1877 by Du Bois Reymond. "Von bekannten Naturprocessen, welche nun noch die Erregung vermitteln könnten, kommen, soviel ich sehe, in Frage nur zwei. Entweder müsste an der Grenze der contractilen Substanz eine reizende Secretion, in Gestalt etwa einer dünnen Schicht von Ammoniak oder Milchsäure oder einem anderen, der Muskel heftig erregenden Stoffe stattfinden. Oder die Wirkung müsste elektrisch sein."*

The theoretical proof of the need to postulate chemical transmission of nervous stimulation was provided by Langley (1878, 1905, 1906, 1907) on the basis of experiments with curare, nicotine, pilocarpine, atropine and other poisons carried out over many years.

By applying nicotine with a fine brush to the fibres of the sartorius muscle of the frog, Langley discovered that a shortening effect was observed only when the drop of poison hit the region of entry of the nerve into the muscle fibre. Application of the nicotine to other portions of the fibre caused no response. When curare was applied to the neural region of the muscle it blocked the responses to nerve stimulation and to nicotine, but it did not prevent contraction of the muscle to direct stimulation. Application of the poisons to the nerve trunk caused no effect. The effect of nicotine was retained even after degeneration of the nerve.

On the basis of these and other data obtained by him, Langley proposed that, in each cell (muscle, gland or nerve), two constituents, or two substances, must be distinguished. One, the chief substance, performs the main function of the cell (contraction, secretion, or generation of electric potentials), while the other, the accessory substance, has the task of receiving the action of the nerve and transmitting it to the chief substance. Langley called this accessory substance the receptive, or synaptic substance.

"Only in the light of modern data", wrote A. G. Ginetsinsky, "is it possible fully to appreciate at their true worth the thoroughness in observation and the profoundness of the

*"Of the natural processes known, that might evoke stimulation, only two are, in my opinion, worth talking about: either there exists at the boundary of the contractile substance a stimulatory secretion in the form of a thin layer of ammonia, lactic acid, or some other powerful stimulating substance; or the phenomenon is electrical in nature." E. Du Bois Reymond, *Gesammelte Abhandlung der allgemeinen Muskel- und Nervenphysik*, **2**, 700 (1877).

conclusions of Langley. It is hardly surprising that the further development of his thought led Langley to the hypothesis of chemical transmission of the nerve impulse . . . Langley's formulation of the hypothesis differs in no way from the modern one: 'The stimuli passing by the nerve cannot affect the contractile molecule, except by the radicle which combines with nicotine and curari. And this seems in its turn to require that the nervous impulse should not pass from nerve to muscle by an electric discharge, but by the secretion of a special substance at the end of the nerve'." (Langley, 1906, p. 183.)

"Physiological ideas, however", Ginetsinsky continued, "are only born as a result of logical reasoning. For them to live and influence the development of the science demands direct experiment."*

What experimental proof was required in order that the chemical hypothesis might "live and influence the development of science"?

In the first place it was necessary to show that, on stimulation of the nerve in the region of its endings, a biologically active substance is released, to isolate the substance in the pure state and to determine its chemical structure; secondly, to show that the adequate application of this substance to the synaptic region evokes the same effect as stimulation of the nerve; and thirdly, to be convinced that, taking the chemical hypothesis as the starting-point, the action of pharmacological agents on synaptic transmission can be satisfactorily explained. For a cholinergic synapse, for example, it was necessary to demonstrate that atropine or curare blocks both the effect of nerve stimulation and the action of acetylcholine, while eserine potentiates both effects.

Some of this evidence was either known before Langley's time or was produced by him, but in order to make it convincing, it had to be presented as a whole.

The requisite evidence was first obtained in the experiments of O. Loewi on the amphibian heart (1921–6). Loewi managed to identify the mediator of the parasympathetic nerves as acetylcholine, to discover an enzyme which hydrolysed this mediator (later called cholinesterase), and to demonstrate inhibition of this enzyme by eserine.

The publication of Loewi's results initiated a "chain reaction" which, in relation to the cholinergic synapse, was described by Ginetsinsky as "the triumphal march of acetylcholine". First, the principle of chemical transmission of the nerve impulse was extended to all peripheral synapses formed by parasympathetic nerves. This stage was completed in the main in the years immediately following Loewi's discovery. The second stage was the discovery (A. F. Samoilov's research and the work of Dale's school and Eccles) of the chemical link in the transmission of the stimulus from somatic nerves to skeletal muscle and from one neurone to another in autonomic ganglia. Loewi's work on the role of acetylcholine in the central nervous system should also be included here (Loewi, 1937; Loewi and Hellauer, 1938).† This stage was completed in the main in 1937.

*A. G. Ginetsinsky in *The Chemical Transmission of the Nerve Impulse and the Evolution of Muscle Function*, edited by N. A. Itina, Nauka, Leningrad, 1970. This brilliant book was begun in 1947, but Ginetsinsky was, unfortunately, unable to finish it. In 1950 his work on the "cholinoreceptive substance" was subjected to unjustified criticism (see *Stenographic Report of the Scientific Session of the Academy of Sciences and of the Academy of Medical Sciences of the USSR, devoted to Problems of the Teachings of I. P. Pavlov*). Ginetsinsky was compelled to stop work in the Pavlov Institute of Physiology and his research on this topic. He later became so deeply involved in his research on kidney function that he never returned to the problem of chemical transmission of the nerve impulse. After his death the manuscript of Ginetsinsky's book was prepared for publication by his research assistant, N. A. Itina, and appeared in 1970 to mark the seventy-fifth anniversary of his birth.

†This work of Loewi's was unfortunately cut short in 1938, when he was thrown into a concentration camp by the Nazis after the seizure of Austria. Loewi, who was a Nobel prizewinner in 1936, was later released, but did not in fact return to intensive experimental work.

Present-day research on this problem may be regarded as the third stage, and it is linked above all with the development of new methods of investigation, viz. the electron microscope, histochemistry, new biochemical micromethods and microelectrode techniques.

The first attempts to understand the molecular mechanism of action of the mediator had already been made by Langley. Starting from the general theory of immunity, propounded by Ehrlich, Langley proposed that "a receptive substance is a side chain molecule of the contractile substance" of the muscle fibre (Langley, 1905, pp. 399–400). But even Langley did not at that time see any advantage in attempting to consider the phenomenon at the molecular level.

A real approach to the molecular level of investigation of the action of mediators of nerve stimulation has become possible only in the present stage of scientific development. The study of molecular mechanisms, moreover, now became the principle task of the physiology, biochemistry and pharmacology of synaptic transmission. The study of the mechanism of interaction of the mediator with cholinoreceptors and cholinesterases, and the accumulation of information about the structure of cholinoreceptors and cholinesterases are central to this issue.

The methodological advances of recent years have made it possible to approach the study of these questions from various directions. The resolving power of the modern electron microscope approaches that of interatomic distances, and in some instances permits us even to see individual molecules. The development of histochemistry makes it possible to localize enzymes, and in particular cholinesterases in the synapse, using light and electron microscopes. Recording from microelectrodes and the micro-application of biologically active substances enable the reaction to excitation of a single cell to be studied. Changes in the membrane potential or membrane resistance of the cell have been used in this work as criteria, or as indicators that reflect changes in permeability to ions which arise as a result of a reaction of a substance with the receptor.

In the investigation of the active centres of cholinesterases and cholinoreceptors extensive use has been made of biochemical and chemical-pharmacological methods. This division of the methods used is, of course, to a certain extent artificial. In biochemical work, concerned with the elucidation of the active centres of enzymes, use is inevitably made of pharmacological agents capable of reacting with the active centres. Brilliant successes have been achieved in the study of the structure of the active centres of some enzymes, including the cholinesterases, thanks to a combination of biochemical and chemical-pharmacological methods.

The value of biochemical and preparative methods in the study of cholinoreceptors is more limited. Attempts to separate the cholinoreceptors of the postsynaptic membrane by biochemical methods, and to investigate their structure, come up against one principal obstacle, not to mention other difficulties. The basic function of the cholinoreceptor is to change the permeability of the postsynaptic membrane to ions. As soon as the receptor is separated from the membrane the possibility of identifying it by this function is lost, and consequently it is difficult to be convinced that it is indeed the cholinoreceptor that has been isolated.

In modern terms the chemical-pharmacological method of study of the structure of the active centres of the cholinoreceptors appears to offer the best prospect of advance. This method consists in the quantitative comparison of the effects of cholinergic substances with their chemical structure. This starts with the hypothesis that the presence and mutual disposition of chemical groups and bonds in the molecule of the substance must correspond

to complementary specific chemical groupings in the cholinoreceptor. By comparing the activity of substances that are closely related in structure it is possible to elucidate the significance of one or other atomic grouping for interaction with the receptor, and to construct a hypothesis concerning the presence of complementary groups in the receptor. Such hypotheses may be tested with the aid of specific syntheses, in which specific reactive groups are introduced into, or removed from, the molecule of the substance.

The chemical-pharmacological method is based upon the carrying over into pharmacology of the main idea of A. M. Butlerov's theory of structure, which is still valid, viz. that the chemical structure of any compound defines its properties, and consequently that by studying the reactive capacity of a substance, information may be obtained about its chemical structure.

Practically everything that is now known of the structure of cholinoreceptors has been obtained precisely by the chemical-pharmacological method. The material set out in this book might well be assigned to the field of molecular pharmacology (biochemical pharmacology)—a new biological discipline that has developed in the last decade in the areas of contact between physiology, biochemistry and pharmacology. The most important and as yet uncharted task of molecular pharmacology consists in the elucidation of the chemical and physico-chemical interactions of biologically active substances (of both endogenous and exogenous origin) with the corresponding receptors in the living organism. Most important in carrying out this task is, of course, the elucidation of the chemical structure of the biological receptors.

A great part of the evidence concerning the structure of cholinoreceptors, accumulated up to the present, relates to the receptors of the common laboratory animals, most of them higher vertebrates. The work in the Sechenov Institute of Evolutionary Physiology and Biochemistry has enabled our group to pay particular attention over the last 10–12 years to the comparative pharmacology of cholinergic synapses, and also to study changes in cholinoreception that occur in the process of individual development and after denervation, i.e. to make use of the basic methods of evolutionary physiology, developed in L. A. Orbeli's laboratories, in pharmacological research. These methods have been employed for a long time by Orbeli's students in the study of problems in comparative and evolutionary pharmacology, and in particular the pharmacology of cholinoreceptors (the work of Ginetsinsky and his co-workers, and of A. K. Voskrensenskaya). The use of these and other materials in the literature, and of some data obtained by our team, have enabled us to advance some hypotheses concerning changes in the molecular structure of cholinoreceptors that may have occurred in the process of evolutionary development.

Many of the suggestions made in this book, including those contributed by us, are for discussion. We have ourselves frequently modified them over recent years, and there is no reason to suppose that we shall not change them again in the future. We have at all events tried not to conceal, either from ourselves or from the reader, those facts that do not fit our hypotheses. It is worth recalling in this connection Claude Bernard's views on scientific theories: "Une théorie q.q. belle qu'elle soit, n'est jamais si belle que la vérité ou que le fait. Je crois qu'il n'y a pas, non seulement en physiologie mais en physique et chimie, une seule théorie actuelle vraie, absolue. Tout n'est que relatif. C'est donc une excellente chose d'avoir détruit une théorie. C'est un pas en avant, et il ne faut pas trembler qu'on vienne détruire une théorie, même sienne, il faut le rechercher, c'est une découverte qui est là-déssous, une révolution comme on dit, car la science est révolutionnaire et ne marche pas par additions successives comme on croit."*

This book gives an account of one aspect of the work, carried out over many years, by a large team of chemists, biochemists and pharmacologists, with whom we have been privileged to work. This team included the staffs of the chemical laboratories of Academician M. I. Kabachnik, N. V. Khromov-Borisov, corresponding member of the Academy of Medical Sciences of the USSR, A. L. Mndzhoyan, Academician of Armenian Academy of Sciences, and Professor B. A. Porai-Koshits; the biochemical laboratory, which worked successively under the direction of Professors V. A. Yakovlev and A. P. Brestkin; the toxicological laboratories of Professors R. S. Rybolovlev and N. V. Savateyev; B. N. Veprintsev's biophysical laboratory; and our pharmacological laboratory, the staff of which included I. B. Voronov, B. A. Ger, A. F. Danilov, I. V. Dardymov, Yu. Ya. Ivanov, I. L. Kratskin, V. V. Lavrentyeva, N. Ya. Lukomskaya, L. G. Magazanik, L. L. Protas, E. K. Rozhkova, Yu. F. Satrapinsky, N. K. Fruyentov, S. A. Shelkovnikov and the authors of this book.

All the original experimental data set out in this book are the work of this big team. The views put forward here were formulated by them.

In addition, many of our friends and working colleagues read particular chapters of this book and made valuable suggestions, including A. P. Brestkin, B. N. Veprintsev, R. I. Volkova, E. A. Vulfius, A. F. Danilov, N. A. Itina, A. N. Kachman, I. Ya. Kvitko, Yu. E. Mandelshtam, T. M. Turpaev and V. A. Yakovlev.

We ask them all to accept our sincere thanks.

We also wish to thank Dr. E. Lesser (Dept. of Pharmacology, Chelsea College, London) for an excellent translation of this work into English.

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* "Any theory, however beautiful, cannot compare in beauty with truth or with fact. I think that, not only in physiology but in physics and chemistry, too, there is not a single modern theory that is absolutely true. Everything is relative. It is thus an excellent thing to destroy a theory. It is a step forward and, far from being afraid to destroy a theory—even one's own—one should strive actively to do so. For beneath it a discovery lies hidden, a revolution one might say, for science is revolutionary and does not advance by the simple accretion of facts as is sometimes thought." Claude Bernard in *Introduction a l'étude de la médicine expérimentale*, quoted from L. N. Karlik in Claude Bernard, *Lectures in Experimental Pathology*, edited by L. N. Karlik, Biomedgiz, 1937.

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Fig. 37 from *Experimental Brain Research*, vol. 2, 1966a, p. 61, fig. 5. Williams & Wilkins Inc. for Fig. 34 from *Journal of Pharmacology*, vol. 85, 1945, p. 98, fig. 5 (first part); Fig. 8B from *Journal of Pharmacology*, vol. 120, 1957, p. 496, fig. 4; Fig. 8A from *Journal of Pharmacology*, vol. 126, 1959, p. 15, fig. 2; Figs. 40C, 41 and 49 from *Pharmacological Reviews*, vol. 18, N 3, 1966, p. 1073, fig. 9; p. 1059, fig. 3; p. 1077, fig. 10; and Fig. 23 from *Journal of Pharmacology*, vol. 158, 1967, p. 102, fig. 3.

M. J. M.
E. V. Z.

Contents

CHAPTER 1

The Function of the Cholinergic Synapse

". . . in all places where there is no union between the adjacent cells and where the process of excitation must pass from one cell to another, whether this be the synapse of Sherrington in the central nervous system, or the boundary between efferent nerve fibres and effector organs, we shall comprehend the peculiarities of transmission of the excitation, delay in time, the unidirectional character of the transmission, summation and so on only if we accept that, of the two adjacent cells, one has elaborated within it the capacity to liberate an excitatory substance, while the other has the capacity to react to this substance."

A. F. Samoilov, 1924

The chemical transmission of nervous excitation has been surveyed in recent years in a series of exhaustive monographs and reviews (Barlow, 1955a, 1964, 1968; Eccles, 1957, 1964, 1969; Feldberg, 1957; Nachmansohn, 1959, 1967; Katz, 1962, 1966, 1969; Turpaev, 1962, 1967a,b; Koelle, 1962, 1963a, 1972; McLennan, 1963; Kibyakov, 1964; De Robertis, 1964, 1971; Gill, 1965; Strumwasser, 1965; Shapovalov, 1966; Florey, 1967a,b, 1970; Koketsu, 1969; Ginetsinsky, 1970; Karczmar, 1970; Michelson and Zeimal, 1970; Porter and O'Connor, 1970; Bacq, 1971; Fischer, 1971; Shamarina, 1971; Michelson and Danilov, 1971). This permits us in this chapter to describe the function of the cholinergic synapse schematically, while dwelling in greater detail on those questions that are important to an understanding of the molecular mechanisms of action of acetylcholine.

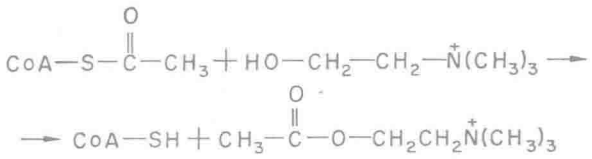
1.1. A General Scheme of Function of the Cholinergic Synapse

A diagrammatic representation of a synapse with chemical transmission of the nerve impulse is given in Fig. 1A. The presynaptic membrane (axon membrane) is separated from the postsynaptic (subsynaptic) membrane (membrane of the innervated cell) by a synaptic cleft 200–500 Å wide. Within the presynaptic ending, close to the presynaptic membrane, groups of synaptic vesicles, about 500 Å in diameter, are seen. The synaptic vesicles are thought to contain the mediator. Synapses in which acetylcholine (ACh) plays the role of mediator are called cholinergic.

Acetylcholine is synthesized in the nerve cell with the help of coenzyme A and the specific enzyme choline acetylase (choline acetyltransferase, E.C. 2.3.1.6)*, which catalyses the transport of the acetyl residue from coenzyme A to the choline (see Nachmansohn, 1963a).

Choline acetylase is dissolved in the cytoplasm. ACh is apparently synthesized in the cytoplasm and then concentrated and stored in the vesicles of the nerve ending, where its concentration corresponds to a solution of ACh isotonic with blood (about 0.15 M). Each vesicle contains several thousand molecules of ACh.

*Enzyme classification of the International Union of Biochemistry.



When the nerve impulse reaches the ending of the axon and depolarizes its membrane, a number of vesicles discharge their contents into the synaptic cleft (Fig. 1B). The molecules of ACh released rapidly reach the subsynaptic membrane.

The process of the passage of the mediator, liberated from the vesicle, across the synaptic cleft has not yet been studied. This may not be a simple process of diffusion through a homogeneous fluid, and for the present it is not known what the synaptic cleft contains. Under the electron microscope the synaptic cleft has a fibrillar structure, with the fibrils firmly joining the pre- and postsynaptic membranes. The synaptic structures are not usually destroyed by homogenization, but are merely torn away from the axon endings, while the postsynaptic membrane remains joined to the presynaptic membrane (Whittaker, 1966, 1967; Whittaker *et al.*, 1972).

Having reached the postsynaptic membrane, the molecules of ACh interact with the cholinoreceptors. What is usually understood by the term cholinoreceptor (ChR) is the part of the postsynaptic membrane (a molecule or complex of molecules) capable of reacting with ACh in such a way that, as a result of this interaction, events occur that lead to a sharp rise in the permeability of the membrane to ions. The flow of ions across the membrane in the direction of their electrochemical gradients increases by a factor of hundreds or thousands. One of the suggestions that explain this increase in permeability is that, on reacting with ACh, the conformation of the ChR molecules, and/or of other molecules of the cholinoreceptive membrane changes so that channels open in the postsynaptic membrane through which ions pass (Fig. 1B). The rise in permeability, under the influence of the mediator (ACh), occurs only in the subneural region, develops very rapidly, usually in the course of milliseconds, and lasts a short time, usually a few milliseconds. The termination of the action of the ACh liberated into the synaptic cleft is effected in vertebrates mainly by the enzyme acetylcholinesterase (AChE, acetylcholine acetylhydrolase, E.C. 3.1.1.7), which hydrolyses ACh to the physiologically relatively inactive choline and acetic acid. Apart from the specific AChE, there is also in the organism a less specific enzyme, pseudo cholinesterase (ChE, BuChE, acylcholine acylhydrolase, E.C. 3.1.1.8), which hydrolyses not only ACh but also a series of other esters of choline. It is AChE, however, that plays the principal role in the functioning of the cholinergic synapse.*

The enzymatic hydrolysis of ACh is not the only means by which its action is terminated. The action of ACh may be terminated as a result of its diffusion out of the synaptic cleft. It is suggested that this is the mechanism of termination of action of ACh in ganglionic synapses, where AChE is located mainly on the presynaptic membrane. The suggestion has also been made that, in white phasic muscle, diffusion into the secondary synaptic clefts is the first step in the termination of the action of ACh, which is followed by the destruction of ACh by acetylcholinesterase, located mainly in the secondary synaptic clefts (Ger *et al.*, 1972a, 1973a; Ger, 1973). It is possible that, in some cases, a reduction in the sensitivity of the receptors to ACh may play some part, arising as a result of a long-lasting action of ACh (desensitization, see Chapter 4), or as a result of the liberation of particular substances which act allosterically on the ChR (Turpaev's hypothesis, see Chapter 4).

The choline, which is formed as a result of the hydrolysis of ACh, is absorbed from the synaptic cleft by the nerve endings and is used there for the resynthesis of ACh (see Whittaker *et al.*, 1972).

*The symbol ChE will also be used when mention is made of both cholinesterases together.

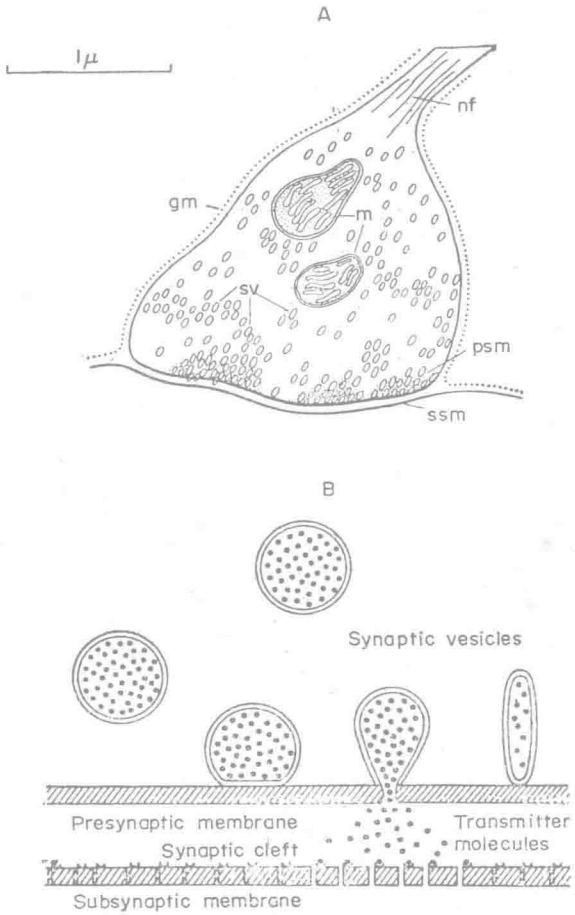
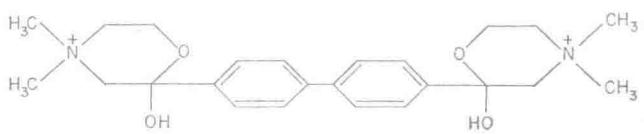


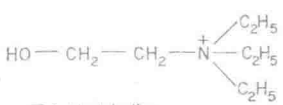
FIG. 1. Diagram of a synapse with chemical transmission. A, Electronmicrograph of the synapse (De Robertis, 1964). m, Mitochondria; nf, neurofibrils; sv, synaptic vesicles; psm, presynaptic membrane; ssm, subsynaptic membrane; gm, glial cell membrane (dotted line). B, Exit of molecules of mediator and their interaction with molecules of receptor (Eccles, 1965b).

The reabsorption of choline is inhibited in the presence of some quaternary ammonium compounds, and in particular of hemicholinium (I) (Schueler, 1960) and triethylcholine



(I)

Hemicholinium (HC-3)



(II)

Triethylcholine

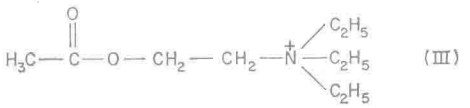
(II). Frequent stimulation results in exhaustion of the stores of ACh in the nerve endings, a diminution of the discharge of ACh in response to the nerve impulse and the disruption of synaptic transmission, which may be prevented by the administration of choline.

This was demonstrated for the cholinergic synapses of the superior cervical ganglion in experiments with hemicholinium (Birks and Macintosh, 1961). For the neuromuscular junction of skeletal muscle striking results were obtained with triethylcholine (Bowman and Rand, 1961; Bowman *et al.*, 1962; Bowman and Hemsworth, 1965). The injection of triethylcholine into a cat led to a gradual disruption of impulse transmission from motor nerve to muscle, when a frequency of stimulation of 1/sec and higher was used. With less frequent stimulation (1/10 sec) transmission was not affected. The injection of choline quickly restored the passage of frequent impulses (Fig. 2a). During the disruption of transmission of high-frequency impulses the response to the intra-arterial injection of ACh was maintained. This shows that the disruption of transmission, evoked by triethylcholine, is the result of a presynaptic action.

In experiments on the isolated rat diaphragm Bowman and Hemsworth (1965) showed that the disruption of transmission was in fact bound up with a reduction in ACh output in response to stimulation of the motor nerve (Fig. 2b), and that the addition of choline restores both the normal output of ACh and normal transmission. The effects of triethylcholine and choline are most pronounced in the presence of high frequencies of stimulation.

Triethylcholine, like hemicholinium, apparently blocks the reabsorption of choline, thus inhibiting the synthesis of ACh, and in the end leads to a reduction in the amount of ACh liberated by the nerve ending in response to the nerve impulse.

In the case of triethylcholine, as distinct from hemicholinium, a somewhat different mechanism of action may be involved. Being absorbed into the nerve ending in place of choline, triethylcholine is acetylated with the formation of acetyltriethylcholine(III), which is physiologically inactive (Bowman *et al.*, 1962). If, therefore, a part of the synthesized molecules of ACh is replaced by molecules of acetyltriethylcholine, this is equivalent to a reduction in the number of molecules of ACh. It has so far, however, not been possible to demonstrate the acetylation of triethylcholine by choline acetylase *in vitro* (Bowman *et al.*, 1968).

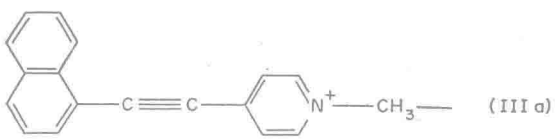


(III)

Acetyltriethylcholine

Triethylcholine also diminishes the transmission of high-frequency stimulation in other cholinergic synapses, e.g. in the wall of the guinea-pig intestine (Boullin, 1963), and in sympathetic ganglia (Matthews, 1965). Triethylcholine also diminishes the slowing action on the heart caused by stimulation of a branch of the vagus nerve (Bolton, 1967).

Some real choline acetylase inhibitors have recently been discovered. Substance (IIIa), for example, causes a 50% inhibition of choline acetylase in a concentration as low as 1×10^{-6} (Cavallito *et al.*, 1969; White and Cavallito, 1970).



(III a)

1.2. The Movement of Ions Across the Membrane

The concentrations of the main electrolytes within the nerve cell and in the surrounding medium are given in Table 1. Apart from these there are within the cell large organic anions that are incapable of passing through the outer membrane of the cell. These anions retain the free potassium ions inside the cell by electrostatic forces. The total concentration of anions inside the cell is greater than the concentration of cations. This is the cause of the considerable difference in potential on the two sides of the membrane, i.e. its polarization.