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[REVIEW]

Hundredth Anniversary of the "Synapse": II. Study of the Cholinergic Synapse

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ABSTRACT—Major contributions to the research on the cholinergic synapse in the last five decades was described. The original notion of active zone, composed of a presynaptic dense projection and associated synaptic vesicles, was analyzed in order to underline its functional meaning in synaptic transmission. An overview was done on the findings made on acetylcholine release (ultrastructural aspect, cytochemistry, non-vesicular hypothesis and mediatophore), acetylcholinesterase (ultrastructural localization, discovery of molecular polymorphism) and acetylcholine receptor (characterization, purification). The structural base of the smallest functional unit in a synapse was attributed to a single synaptic vesicle of active zone and corresponding area of synaptic cleft and postsynaptic membrane interacting with the neurotransmitter released from the vesicle.

INTRODUCTION

After the war, which devastated Europe and Far East, the scientific leadership came to the U.S.A. Nevertheless, European scientists had already developed original fields of research and number of their works were pursued fruitfully in their own countries. Research on the morphology and function of synapse, through the study of the cholinergic synapse, is certainly a characteristic example of this phenomenon. In the second part of this review we will outline how the advance in this field has contributed to the present knowledge of the synapse.

HISTOCHEMISTRY OF ACETYLCHOLINESTERASE

A short time after Couteaux (1947) put forward a hypothesis on a high concentration of acetycholinesterase in the subneural apparatus, Koelle and Friedenwald (1949) in the U.S.A. proposed a histochemical method for localization of acetylcholinesterase activity by means of acetylthiocholine, an artificial substrate closely related to the natural substrate concerning the pharmacological action.

Couteaux and Taxi (1952) performed methodological research in order to avoid important diffusion artifact inherent in the original Koelle's method. Thus, they succeeded in localizing the activity of acetylcholinesterase on the subneural apparatus at optical microscopic level. The image they obtained with the modified histochemical method underlined the folding of the sarcolemma below the nerve terminal and was absolutely superposable with the histological image obtained by Janus green staining (Fig. 1). These data confirmed biochemical findings that acetylcholinesterase activity was indeed outside of the nerve endings (Couteaux and Nachmansohn, 1940).

SYNAPTIC ULTRASTRUCTURE

The first electron microscopic descriptions of synapses were done in the U.S.A. by Palade and Palay (1954), De Robertis and Bennet (1955) and Robertson (1956) in various regions of the nervous system, such as cerebellar cortex, sympathetic ganglia and motor endplate. These observations demonstrated the presence of distinct pre- and postsynaptic membranes separated by a synaptic space. These authors observed synaptic vesicles, i.e. vesicles of around 30 to 50 nm located within the nerve terminals, close to the presynaptic membrane. The ultrastructural observations of the motor endplate demonstrated the discontinuity between nerve and muscle cells separated each other by a space of about 50 nm. The electron microscopic pictures revealed that the lamellae of the subneural apparatus of Couteaux corresponded in fact to folds of the sarcoplasmic membrane.

QUANTAL RELEASE OF ACETYLCHOLINE

Spontaneous discharge of miniature endplate potentials of a few millivolts was detected at the resting neuromuscular junction by Fatt and Katz (1952) in England. The discontinu-

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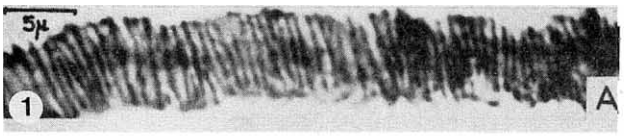
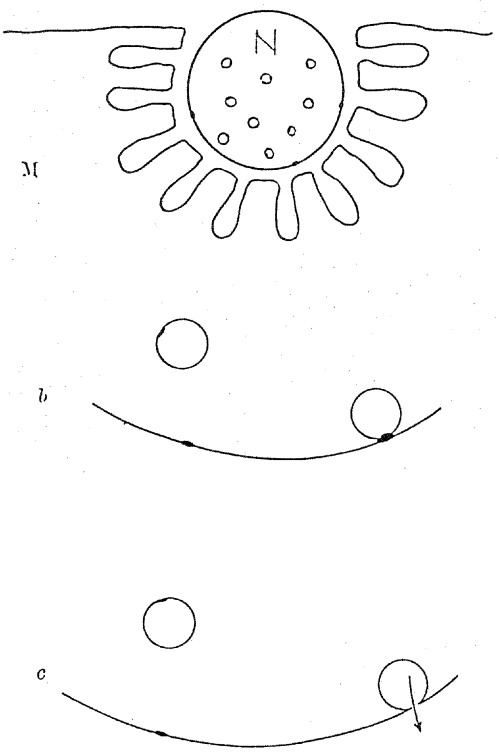
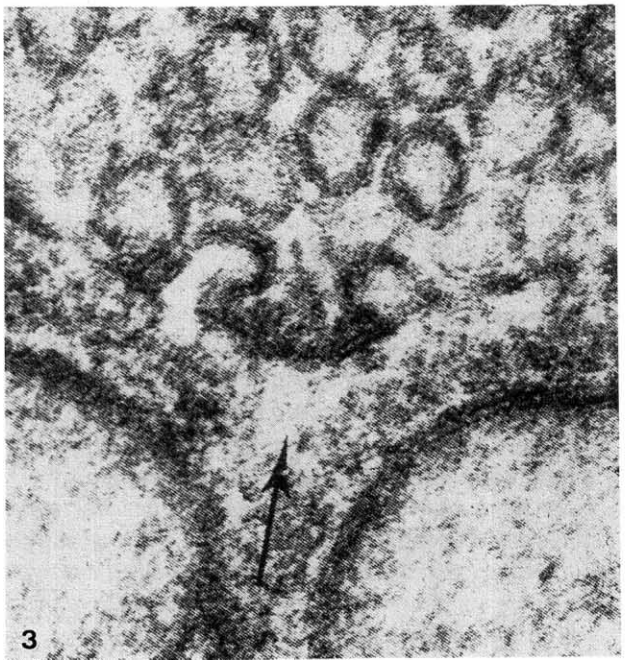
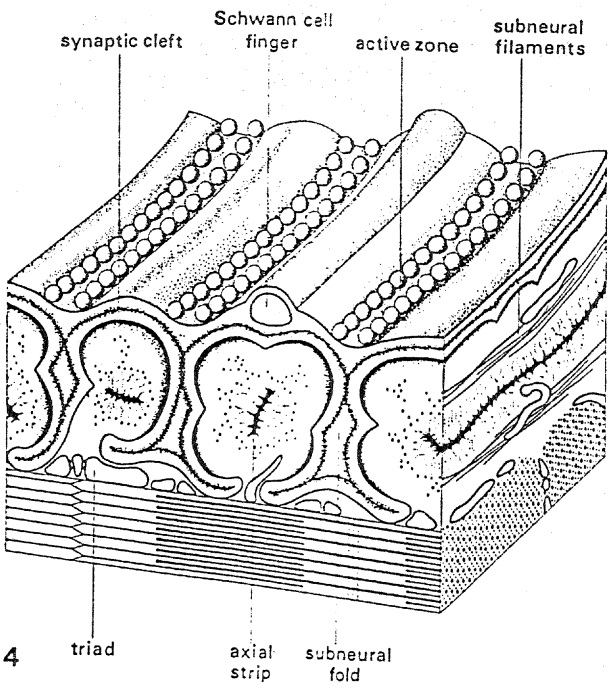








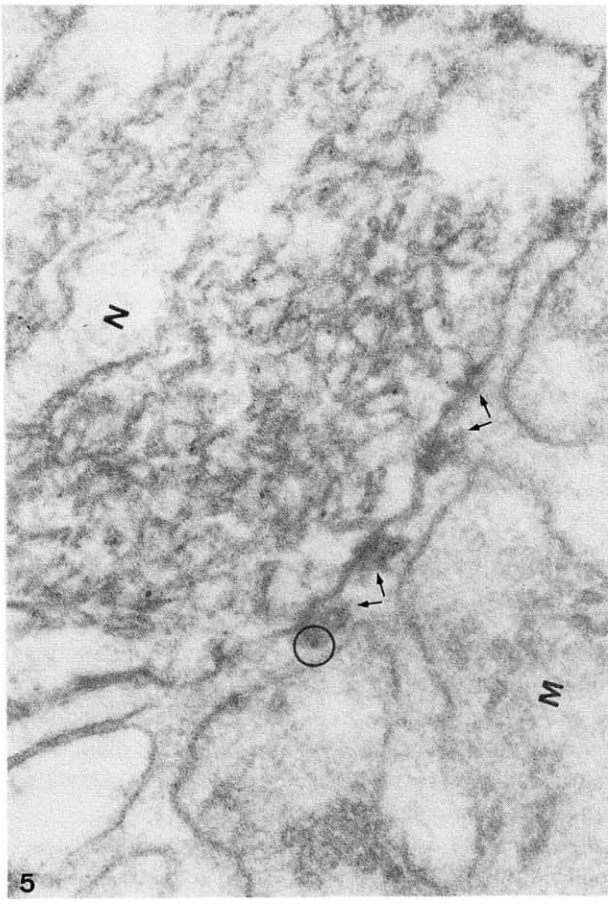


Fig. 1. Histochemical localization of acetylcholinesterase activity in the subneural apparatus of frog neuromuscular junction (Couteaux and Taxi, 1952). The striated structure corresponds to the junctional fold observed later in electron microscopy (Robertson, 1956). Couteaux R, Taxi J (1952) Arch Anat Micr 41: 352-392, Masson, Paris.

Fig. 2. Schematic representation of quantal discharge of acetylcholine from the motor nerve terminal (N). M: muscle cell with junctional fold (see Robertson, 1956). It is presumed that transmitter is preformed in the intracellular microsomes which, after critical collision with the nerve

ous values of their amplitude intrigued the electrophysiologists. The miniature endplate potentials were interpreted as the consequence of the release of multiples of a single quantum of acetylcholine.

The synaptic vesicles were thought to be the morphological base of the quantal miniature endplate potentials, thus representing the site of quantal storage and release of the neurotransmitter. The first idea of vesicular quantal theory was presented in 1955 in a symposium in Gif sur Yvette (near Paris) by Del Castillo and Katz from England. With a simple design, the authors explained an exocytotic vesicular discharge of the neurotransmitter. This ingenious idea marked the last thirty years and is still considered as a basic notion of neurobiology (Fig. 2).

NOTION OF "ACTIVE ZONE"

Subregions of the synapses characterized by a cluster of synaptic vesicles associated with a dense part of pre-synaptic membrane and a corresponding thickening of post-synaptic membrane were observed in the central nervous system. They were named synaptic complexes and presumed to represent the sites of synaptic transmission (Palay, 1956, 1958). According to Eccles (1964), these structures were called active zones by Couteaux who considered them as putative sites of exocytosis of the synaptic vesicles. Here, we wish to precise under what circumstances was proposed the term active zone. Indeed, Eccles, in his book "The Physiology of Synapses" (1964) used the word "active zone" referring to Couteaux (1961). In fact, the term active zone has been used by Couteaux in a personal communication addressed to Eccles in 1961, and not, as cited erroneously in "The physiology of Synapses", in a report published in 1961. This is the reason why the notion of active zone had already been used several years before its well-known description on the neuromuscular junction (Couteaux and Pécot-Dechavassine, 1970, 1974). Indeed, Taxi (1965) and Sotelo (1971), both belonging to the Parisian school of neurobiology, used the term "active zone" to designate structures equivalent to the synaptic complexes of Palay (1958), including the thickening of the postsynaptic membrane, in the sympathetic ganglion and the central nervous system, respectively. The term active zone was published at last by its author in a report showing images of exocytosis of synaptic vesicles, which gave full sense to this notion.(Couteaux and Pécot-Dechavassine, 1970, 1974) (Fig. 3).

In the frog neuromuscular junction, each active zone is composed of a double row of synaptic vesicles arranged along a bar of dense material. The double rows of vesicles in close contact with the presynaptic membrane face the subneural folds of the postsynaptic membrane (Fig. 4). The active zone is a well defined structure (presynaptic dense bar and associated synaptic vesicles). This contrasts with diffuse acetylcholinesterase activity in the synaptic cleft (Couteaux, 1963) and large surface of postsynaptic membrane thickenings. Thus, Couteaux preferred to limit the use of "active zone" only to the presynaptic side. Similarly, in the central nervous system, synaptic vesicles were observed in close association with the presynaptic dense projections, described previously by Gray (1963) in England. These structures called presynaptic vesicular grids by Akert *et al.* (1972) in Switzerland were naturally compared to the active zone of the frog neuromuscular junction.

Since the presynaptic active zone defined by Couteaux and Pécot-Dechavassine (1970, 1974) probably sustains a major role in the function of the synapse, this notion may lead in future to important developments comparable to those brought by the synapse at the present time. Here, it is worth noting that cytochemical ionic fixation of acetylcholine-like cations by means of silicotungstic acid (see below: Cytochemical localization of acetylcholine) brought a further suggestion on the prominent role of active zone. Indeed, after cytochemical reaction performed at low temperature, pairs of spots of diffuse precipitates were observed in the synaptic cleft, only in the part adjacent to active zones (Tsuji, 1985) (Fig. 5). Although such assumption needs additional confirmation, active zones would indeed be the sites where most (if not all) of acetylcholine is released after nerve stimulation.

In active zones, two paired synaptic vesicles did not always display simultaneous exocytosis (Couteaux and Pécot-Dechavassine, 1970). Thus, one synaptic vesicle of a presynaptic active zone might be the base of the ultimate unit of synaptic transmission. Indeed, a single synaptic vesicle and the part of synaptic cleft (basal lamina bearing acetylcholinest-

membrane, release their acetylcholine content in the intersynaptic space. This phenomenon is illustrated in b and c and one can suppose that it occurs when certain reactive molecules (illustrated by points) of the two surfaces match. Original french text translated in English. Del Castillo J, Katz B (1955) Colloque Internat, n°67, CNRS, Paris.

Fig. 3. "Active zone" of frog neuromuscular junction with exocytosis of synaptic vesicles. The arrow is placed at the entrance of the junctional fold and directed toward the presynaptic dense projection. Couteaux R, Pécot-Dechavassine M (1970) C R Acad Sc 271: 2346–2349, with kind permission from Elsevier, Paris.

Fig. 4. Schematic representation of frog neuromuscular junction including "active zone" with two rows of synaptic vesicles along central presynaptic dense projection, synaptic cleft bearing barbed wire-like basal lamina, postsynaptic membrane with dense thickening, subsynaptic interfold sarcoplasm with axial strip, and myofilaments. Couteaux R (1981) J Neurocytol 10: 947–962, with kind permission from Chapman and Hall, London.

Fig. 5. Cytochemical localization of acetylcholine-like cation by silicotungstic acid in the synaptic cleft of frog neuromuscular junction. Pairs of diffuse precipitates (arrows) are observed beneath active zones. The cytochemical precipitates were only visible when the cytochemistry was performed at low temperature, which reduced diffusion of acetylcholine-like cation in the synaptic cleft. Within the circle, a point-like precipitate is observed, which is a characteristic precipitate obtained normally in the synaptic vesicles after cytochemical reaction at room temperature (see Fig. 6). M: muscle cell, N: nerve terminal. Tsuji S (1985) Histochemistry 83: 213–219, with kind permission from Springer, Heidelberg.

erase) and postsynaptic membrane (bearing acetylcholine receptor) interacting with the neurotransmitter released from the vesicle may be considered as a functional entity.

The ultrastructural detail of the active zone was studied by freeze-fracture of the synaptic membranes in Switzerland and the U.S.A. By this method, opening of synaptic vesicles was observed at the level of active zones. Rows of intramembrane particles (channels?) were detected along the bars of active zones, confirming the specialization of the presynaptic membrane in these regions. Moreover, particles corresponding probably to the acetylcholine receptors were found in the postsynaptic membrane (Pfenninger *et al.*, 1972; Dreyer *et al.*, 1973; Heuser *et al.*, 1974).

ACETYLCHOLINESTERASE IN THE SYNAPTIC CLEFT

At the same time, the ultrastructural localization of acetylcholinesterase activity was made in the synapses of peripheral and central nervous system. The methods of Koelle and Friedenwald (1949) and Karnovsky and Roots (1964) were invented in the U.S.A. and widely used in cytochemistry of acetylcholinesterase. Mechanisms of chemical reactions were analyzed in order to adapt these methods for electron microscopic observation. In spite of methodological limit due to diffusion of the precipitates, acetylcholinestease activity was localized in the synaptic cleft of the neuromuscular junction (Tsuji, 1974). At the same moment, a fine localization of synaptic acetylcholinesterase of the electroplax was obtained by Gautron (1974) using thio-acetic acid, as substrate of the enzyme.

NON VESICULAR HYPOTHESIS OF ACETYLCHOLINE RELEASE

In the study of the synaptic mechanisms, French neurobiologists benefited by early introduction of the electric organ of Torpedo electric fish, a modified motor end-plate, containing a huge amount of acetylcholine. Already in 1940 at the Marine Biological Station in Arcachon, the electric organ was studied by Fessard, Feldberg and Nachmansohn. The chapter on the electric organ written by Fessard (1958) remains, even today, an excellent introduction to the electric organs and electromotor neurons.

A friendship originating in the east front of France during the War between late R. Garcin (a professor of clinical medecine) and R. Couteaux (a professor of faculty of science, who received medical and biological formation) resulted in the foundation of an original biomedical laboratory at the beginning of the fifties, in the Salpêtrière Hospital in Paris. In this laboratory, Fardeau worked on human neuromuscular junction and demonstrated the presence of a highly anastomosed subneural apparatus (Fardeau, 1973). In the same laboratory, Israël worked on electric organ of Torpedo fish, after coming back from Whittaker's laboratory in Cambridge, where a pioneer work was performed on the isolation of synaptic vesicles from brain (Whittaker, 1959). The fraction of cholinergic vesicles of electric organ isolated by Israël's group, however, contained thousand times more acetylcholine (Israël *et al.*, 1968) than the fraction of synaptic vesicles previously obtained from the brain. The separation of synaptic vesicles made possible an efficient measurement of vesicle-bound and cytoplasmic acetylcholine in the course of nerve stimulation. A surprising result showed that acetylcholine newly synthesized in cytoplasm is released before vesicule-bound acetylcholine (Dunant *et al.*, 1972). The non-vesicular hypothesis of acetylcholine release was founded on this result (Israël *et al.*, 1979; Dunant and Israël, 1985). The same group of Israël transferred to Gif near Paris isolated synaptosome from the electric organ (presynaptic membranes containing synaptic vesicles). By addition of ionophores, artificial channels were formed on the synaptosome membranes, rendering possible analysis of acetylcholine release by Ca^{++}-dependent process (Morel *et al.*, 1978). Using this excitable cholinergic synaptosome, Israël and Lesbats (1981) invented a highly sensitive chemiluminescent method for detection of released acetylcholine. A quantal release of acetylcholine, similar to that occurring in physiological conditions, was observed from the synaptosomes put in contact with *Xenopus* myocytes (Dunant and Israël, 1993).

L. Tauc, in Gif near Paris, injected acetylcholinesterase in a giant neuron of Aplysia and observed a blockade of acetylcholine release from the nerve terminal (Tauc *et al.*, 1974). Thus, Tauc (1982) proposed also non-vesicular theory for acetycholine release.

A protein (mediatophore) responsible for non-vesicular release of acetylcholine was purified from presynaptic membrane extracts (Israël *et al.*, 1986). By means of immunogold technique, the presence of mediatophore was demonstrated at the presynaptic membrane especially in the active zone (Brochier *et al.*, 1993). After injection of mRNAs extracted from Torpedo electric lobe (electromotorneurons) in *Xenopus* oocyte, Cavalli *et al.* (1991) observed the expression of the activity of mediatophore. Moreover, rapid-freezing technique performed in Geneva showed increase of particles considered as mediatophores in the presynaptic membrane at the moment of presynaptic excitation (Dunant and Israël, 1993). Finally, after transfection of a plasmid encoding mediatophore in release-deficient neural cell lines, Falk-Vairant *et al.* (1996) obtained quantal release of acetylcholine.

SUBUNITS OF MINIATURE ENDPLATE POTENTIAL

As described above, in the vesicular theory of neurotransmitter release, a single synaptic vesicle is considered to liberate an unitary quantity (quantum) of the neurotransmitter (acetylcholine), which provokes in postsynaptic side one miniature endplate potential. However, subminiature potentials (about one tenth of single miniature potential) were discovered by Kriebel *et al.* (1976). This led Wernig and Stirner (1977) to bring up the question of the morphological base of miniature potential. Motelica-Heino with Wernig demonstrated the presynaptic nature of the quantal subunits (Wernig and

Motelica-Heino, 1978) and put forward the hypothesis of a single quantum corresponding to activation of a single active zone (Motelica-Heino and Wernig, 1978). A few years later, Motelica-Heino with Kriebel in the U.S.A. studied distribution of subminiature endplate potentials (Kriebel and Motelica-Heino, 1987). At that time an interesting hypothesis was put forward to question whether one subminiature potential corresponded to acetylcholine content of a single vesicle, a miniature potential to one active zone, multiple miniature potentials to several active zones and endplate potential to most, if not all, of the active zones. The frog neuromuscular junction provides attractive ultrastructural basis for the study of this hypothesis.

It should be also mentioned another proposition according to which one subminiature potential would be generated by one molecule of mediatophore, one quantum corresponding then to acetylcholine released by synchronous opening of a group of mediatophores. (Israël and Dunant, 1993)

CYTOCHEMICAL LOCALIZATION OF ACETYLCHOLINE

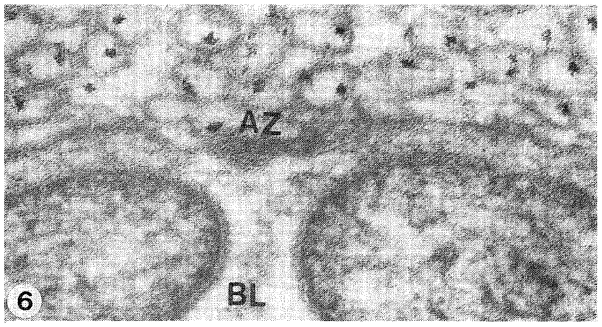
Since acetylcholine is highly soluble, its immunocytochemical and cytochemical localization is quite difficult. Until now, studies performed by group of M. Geffard in Bordeaux in order to transform, in situ, acetylcholine into an immunogenic molecule (Geffard *et al.*, 1985) did not allow an easily reproducible localization. An attempt of ultrastructural localization of vesicular acetylcholine was carried out by Tsuji *et al.* (1983), by means of rapid fixation of fresh nervous tissues by silicotungstic acid. Recently Tsuji and Motelica-Heino (1993) obtained fine electron dense precipitates in the synaptic vesicles (Fig. 6). Anglade *et al.* (1995b) in Salpêtrière Hospital in Paris used the same method in the substantia nigra from postmortem human brain and observed cytochemical precipitates in the synaptic vesicles of the putative cholinergic nerve terminals (Fig. 7). Though this method seems relatively specific for acetylcholine compared to the other neurotransmitters (Ohoka and Tsuji, 1988), the presence of acetylcholine in the electron dense precipitate formed in the synaptic vesicles remains to be proved. However, the same fixation method of acetylcholine was used successfully for localization of [^{3}H]-choline by electron microscopic radioautography in both peripheral and central nervous system. (Tsuji, 1984; Tsuji *et al.*, 1992).

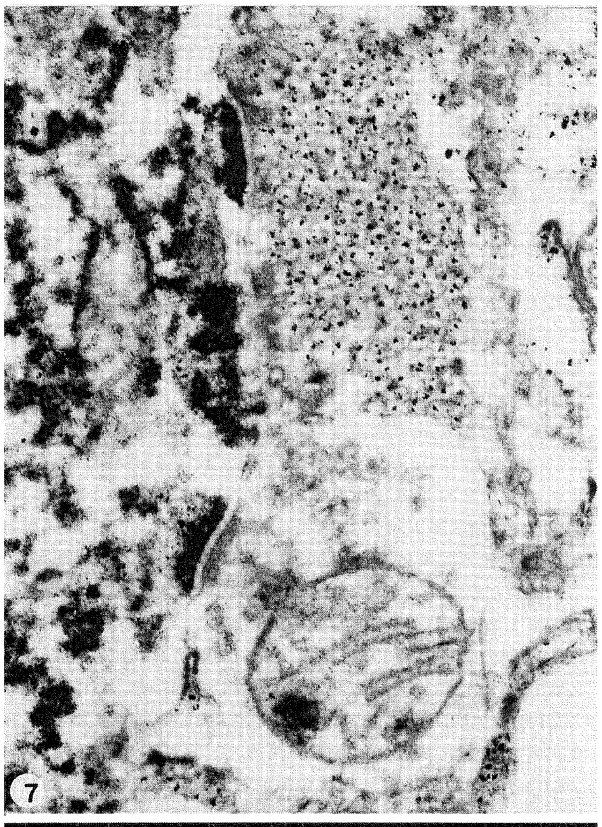
SUBSYNAPTIC SARCOPLASM

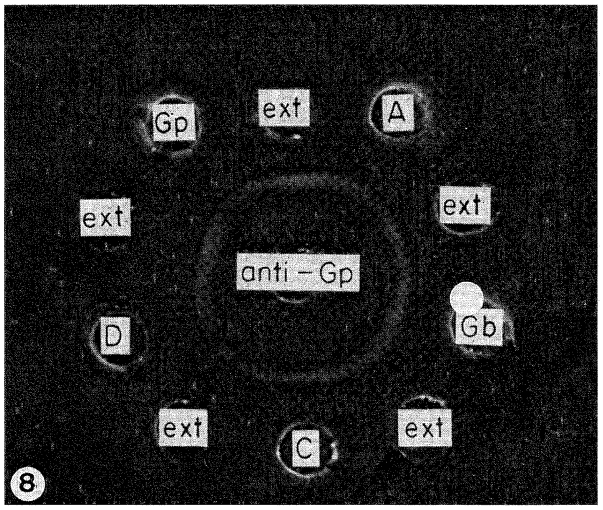
Electron microscopy revealed the complex organization of the interfold sarcoplasm of the neuromuscular junction. Dense thickenings are associated with the postsynaptic membrane of the interfold sarcoplasm bordering the primary synaptic cleft (adjacent to presynaptic membrane). Radioautographic studies made by Fertuck and Salpeter (1974) in the U.S.A. indicated that acetylcholine receptors were located in the part of the postsynaptic membrane associated with the dense thickenings. Inside the interfold sarcoplasm, axial strips surrounded by bundles of subneural filaments run along the great axis of the interfolds (Couteaux, 1981) (Fig. 4). By means of cytochemical method for detection of acetylcholine-like cations, Anglade *et al.* (1995a) observed a heavy staining of the interfold sarcoplasm after stimulation of the motor nerve and subsequent muscular contraction. It was supposed that choline, resulting from hydrolysis of acetylcholine in the synaptic cleft, penetrated through the postsynaptic membrane into the sarcoplasm. Indeed, Dwyer *et al.* (1988) in the U.S.A. showed that choline could penetrate through nicotinic acetylcholine receptor of the endplate. Thus, the subsynaptic sarcoplasm might take part in the metabolism of choline.

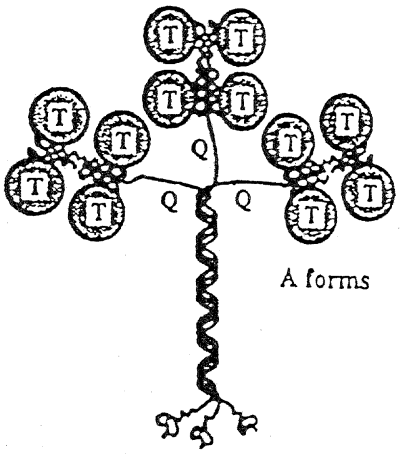
MODERN BIOCHEMISTRY OF ACETYLCHOLINESTERASE

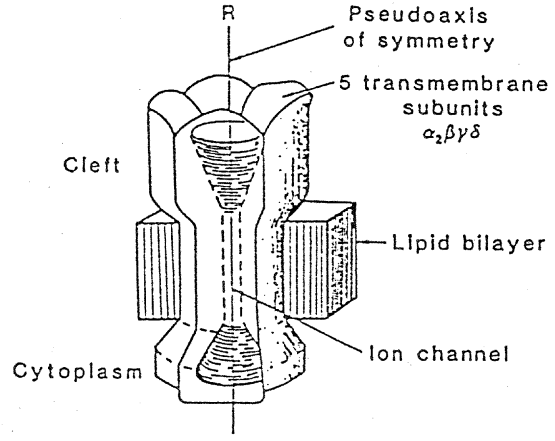
The fish electric organs, that contain a gigantic amount of cholinergic nerve terminals, have facilitated molecular biological studies of both acetylcholinesterase and acetylcholine receptor. The acetylcholinesterase of electric eel was purified and crystalized in the U.S.A. (Leuzinger *et al.*, 1968). In the laboratory of Changeux in the Pasteur Institute, a rabbit immune serum was raised against this purified enzyme and the first immunofluorescent localization of acetylcholinesterase was performed on eel electroplaque (Benda *et al.*, 1970). At the same time Massoulié and his collaborators in the "Institut de Biologie Physico-chimique" in Paris discovered the polymorphism of acetylcholinesterase of the electric organs (enzymatically active molecules with different sedimentation constants) (Massoulié and Rieger, 1969; Massoulié *et al.*, 1971). An antiserum directed against a commercially available acetylcholinesterase of the electric eel, showed immunoreactivity with the differents forms of acetylcholinesterase (common epitopes) (Tsuji *et al.*, 1972) (Fig. 8). Furthermore in the same study, it was shown that these different forms of acetylcholinesterase of the electric organ, were also present in the muscle and in the central nervous system of the fish. The purified different forms of the acetylcholinesterase were observed under electron microscopy by Cartaud of Molecular Biology Institute in Paris in collaboration with Massoulié's group (Rieger *et al.*, 1973; Cartaud *et al.*, 1975). Since the polymorphism of acetylcholinesterase is supposed to be derived from aggregation of monomeric form (Bon and Massoulié, 1976), physicochemical conditions for aggregation were studied (Bon and Massoulié, 1980). As expected, the polymorphism of acetylcholinesterase of the electric fishes was also found in the mammals (Rieger and Vigny, 1976). Immunological properties of acetylcholinesterases of rat and other vertebrates were studied by Grassi's group in Saclay near Paris (Marsh *et al.*, 1984). Recently, detailed aspect of the polymorphism was elucidated and classified in two main groups, the globular and asymmetric forms. The asymmetric form is composed of the globular form to which collagen-like tails are added. The globular and asymmetric forms are subdivided according to the degree of polymerisation. The largest form is composed of three catalytic tetramers with triple helical tail (Fig. 9). Synthe-











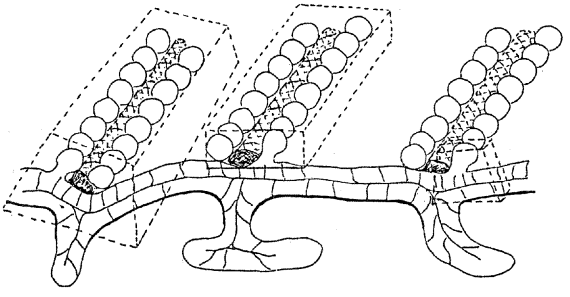


Fig. 6. Cytochemical localization of acetylcholine-like cations in the synaptic vesicles of frog neuromuscular junction. Point-like precipitates are visible in the vesicles. Paired diffuse precipitates beneath active zones (see fig. 5) were not visible in this cytochemical condition (room temperature). AZ: Active zone, BL: Basal lamina. Tsuji S, Motelica-Heino I (1993) Acta Histochem Cytochem 26: 577–582, with kind permission from Jap Soc Histochem Cytochem, Kyoto.

sis, transport and location of different forms of acetylcholinesterase in the neuromuscular junction were reviewed (Massoulié *et al.*, 1993).

MODERN BIOCHEMISTRY OF ACETYLCHOLINE RECEPTOR

Changeux, the author of "L'homme neuronal" (1983) translated in several languages, made important contribution to neurobiology of acetylcholine receptor in the last three decades. He learned neurobiology in the laboratory of D. Nachmansohn in the U.S.A. and participated to the foundation of allosteric theory of protein activity in the laboratory of J. Monod in the Pasteur Institute in Paris (Monod *et al.*, 1963). Changeux decided to study conformational change of proteins in excitable membranes. It is worth noting that structural analogy between acetylcholinesterase and acetylcholine-macromolecular receptor was discussed at first, but their molecular identity was not recognized (Changeux *et al.*, 1969). At that time, M. Kasai (Osaka) in Changeux's laboratory isolated membrane fractions of the innervated side of the electroplax (postsynaptic membrane) and obtained closed vesicles. Excitability of these membrane fragments to cholinergic agonists was demonstrated (Kasai and Changeux, 1970). The laboratory of Changeux was ready for the first characterization of cholinergic receptor protein. The chance was given by a visit of. C. Y. Lee of Taiwan University who purified the snake polypeptide neurotoxin, α-bungarotoxin, which revealed to be an irrevesible blocking agent of acetylcholine receptor protein (Changeux *et al.*, 1970). By means of snake α-toxin and immune serum directed against the toxin, cholinergic receptor protein was localized in the innervated side of the electroplax (Bourgeois *et al.*, 1971). As expected, it was revealed that an immune serum directed against the cholinergic receptor protein blocked the excitation of isolated electroplax membrane by cholinergic agonists (Sugiyama *et al.*, 1973). Afterwards, it was shown that large scale purification of acetylcholine receptor could be done without affinity chromatography (Sobel *et al.*, 1977), facilitating other researches in vitro of the receptor protein. Electron microscopic observation of acetylcholine receptor of postsynaptic membrane of Torpedo electric organ revealed pentameric structure with central hole (Cartaud *et al.*, 1981). Today acetylcholine receptor is regarded as an ion channel (Changeux *et al.*, 1984) (Fig. 10). The group of late Numa in Kyoto, Japan, succeeded by molecular biological technique in revealing the primary structure of acetylcholine receptor (Noda *et al.*, 1984). Recently, desensitization of acetylcholine receptor by calcitonin gene-related peptide was studied by Changeux's group (Mulle *et al.*, 1988). A summary of these data on acetylcholine receptor was presented by Changeux (1990).

PERSPECTIVES

Although chemical transmission is now a well established fact, major aspects of the cholinergic synapse functioning remain to be elucidated. Here, we wish to draw particular attention to one of these questions, the pathway followed by acetylcholine from its release at the presynaptic membrane to binding to postsynaptic receptors. Passage of acetylcholine through the synaptic cleft is probably closely related to space distribution of acetylcholinesterase and acetylcholine receptor. Acetylcholinesterase is bound to basal lamina (McMahan *et al.*, 1978), and acetylcholine receptor to postsynaptic membrane (Cartaud *et al.*, 1981). The ultrastructure of basal lamina was observed by quick-freezing and deep-etching and revealed "barbed wire" structure (Hirokawa and Heuser, 1982). The barbes are directed toward the pre- and postsynaptic membranes. In our opinion, it is highly interesting to note that the density of the barbes is the highest in the region of active zone. It is possible that the barbes in this region play a role in the guidance of acetylcholine to postsynaptic membrane (acetylcholine must go through the zone of basal lamina occupied by acetylcholinesterase to reach acetylcholine receptor). Then, several questions may come in mind. Is there a safety factor protecting acetylcholine from hydrolysis, giving rise to an ad-

Fig. 7. Cytochemical localization of acetylcholine-like cations in substantia nigra from postmortem human brain. One nerve terminal is labeled by point-like precipitates in the synaptic vesicles (putatively cholinergic), while another terminal is devoid of precipitates (putatively non-cholinergic). Both nerve terminals make synaptic contacts with a dendrite of a dopaminergic neuron marked by tyrosine hydroxylase immunoreactivity.

Fig. 8. A double diffusion immunochemical test of different forms of acetylcholinesterase (A, C, D, Gb, Gp) with anti-Gp-acetylcholinesterase immune serum. The immune serum recognized all molecular forms of acetylcholinesterase. An extract of the electric organ (a mixture of the different forms of acetylcholinesterase) provided also a continuous immunoprecipitation line with the different molecular forms. Tsuji *et al.* (1972) J Neurochem 19: 989–997, with kind permission from Lippincott-Raven, U.S.A.

Fig. 9. Quaternary structure of the major oligomeric forms of acetylcholinesterases in vertebrates. For simplification, only tetrameric globular (soluble G4) form and largest asymmetric form, composed of three catalytic tetramers and collagenic triple helical tail (A forms) are shown. Massoulié *et al.* (1993) Prog Neurobiol 41: 31–91, with kind permission from Pergamon, Oxford.

Fig. 10. Schematic representation of nicotinic acetylcholine receptor, composed of pentameric subunits ($\alpha_2\beta\gamma\delta$). Acetylcholine molecules bind to α subunits of ion channel, causing a conformational allosteric change and permeability to ions. Changeux *et al.* (1984) Science 225: 1335–1345.

Fig. 11. Schematic drawing of "synaptic complex" (left), active zone (middle) and "microsynapse" (right). A synaptic complex of the central nervous system is composed of clusters of synaptic vesicles attached to the presynaptic membrane, synaptic cleft and thickening of the postsynaptic membrane (Palay, 1956, 1958). Here, synaptic complex is transposed to the neuromuscular junction. One active zone is made of two rows of synaptic vesicles attached to the dense projection of the presynaptic membrane (Couteaux and Pécot-Dechavassine, 1970, 1974). A microsynape corresponds to a single synaptic vesicle and area of synaptic cleft (basal lamina bearing acetylcholinesterase) and postsynaptic membrane (acetylcholine receptor) interacting with the neurotransmitter released from the synaptic vesicle. A microsynapse is presumed to correspond to subunit of quantal release (subminiature potential).

equate amount delivered to the receptors? Is there a kind of "channel" of acetylcholine in the basal lamina? All these questions evidence a link between the synaptic structures encountered by acetylcholine from vesicular release to receptor binding. Therefore, we ask whether the smallest functional unit in a synapse might be composed of one synaptic vesicle of active zone and its corresponding area of synaptic cleft (basal lamina bearing acetylcholinesterase) and postsynaptic membrane (acetylcholine receptors) interacting with molecules of neurotransmitter released by the vesicle. This ultimate functional unit tentatively considered as a "microsynapse" may represent a morphological base for subminiature endplate potential (Fig. 11).

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