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[REVIEW]

Hundredth Anniversary of the "Synapse": I. A Short History of the Milestones in Synapse Research

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ABSTRACT—On the occasion of the hundredth anniversary of the term "synapse", gradual progress of the knowledges conducting to the notion of chemical transmission was related. It was particularly stressed how the synapse was understood by the major contributors in this field. The soundness of the choice of the term synapse, instead of syndesm that was proposed at first, was underlined by a comparison of the original meanings of the two words and confirmed by their use in a treatise of Aristotle. It appeared that the notion of humoral immunity influenced the birth of humoral theory of chemical transmission, which was illustrated in the textbook of M. Verworn.

INTRODUCTION

The year 1997 is the hundredth anniversary of the term "synapse", formulated by Sherrington during the redaction of a chapter dealing with central nervous system for a textbook of physiology edited by Foster in Cambridge in 1897. Sherrington expressed by the term synapse a particular property of the junctional zone between neurons or between neurons and effector cells. At that time the limited power of resolution of the light microscope did not allow indisputable elucidation of the mode of junction between neurons. Although the term synapse seems to have been used in England, as soon as it was proposed (see Elliott, 1904; Bayliss, 1924), it was far from being accepted so rapidly in the other countries. Indeed, though the mode of junction between neurons was discussed, the word synapse was not even mentioned, in the textbooks of physiology of Verworn (1909) and Gley (1928). Here, we try to outline a short history of the main discoveries giving rise to the twin notions of synapse and chemical neurotransmission.

EARLY HISTORY OF SYNAPSE

The question of transmission of excitation between nerve and end organs was not accurately addressed before the pioneering study of Du Bois-Reymond (1848), working in Berlin. He proposed that transmission at the junction between nerves and muscle might be either chemical or electrical. Afterwards, pharmacological agents were proposed as tools used to unravel the physiological properties of living organisms. This has been proved to be decisive in the elucidation of the mode of nerve transmission. The pioneering experiment in this field was made by C. Bernard (1856) in Paris. Bernard observed that after curare injection the motor nerves lost their ability to react on the muscles, whereas muscles retained excitability. (today, we know that curare blocks cholinergic transmission at neuromuscular junction by binding to nicotinic acetylcholine receptor). A few years later in Germany, Schmiedberg and Koppe (1869) found that muscarine (today known as cholinomimetic agent, reacting on muscarinic acetylcholine receptor) and vagus stimulation provoked similar depressing reaction on heart beats. Such an effect was blocked in both cases by atropine. Schmiedberg's interpretation was that atropine was able to paralyze the vagal terminals in frog heart (today we know that atropine blocks the muscarinic acetylcholine receptor of the effector cells), thus preventing any subsequent action of muscarine. These findings made Schmiedberg to be considered nowadays as the founder of modern pharmacology.

A further step was covered by the contribution of J. N. Langley on the autonomic nervous system, a term he introduced following his work in collaboration with W. L. Dickinson (1889) on the effect of nicotine on peripheral ganglia. These authors found that nicotine paralyzed cells of peripheral ganglia without affecting post-ganglionic fibers. Indeed, after application of nicotine on peripheral ganglia, stimulation of fibers entering the ganglia had no effect on innervated organs, whereas stimulation of fibers emerging from the ganglia produced normal response. Thus, this observation revealed indirectly the presence of connections between pre-ganglionic fibers and ganglionic cells. The structure of these connections remained unknown, which was expressed by Langley himself

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referring to "nerve fibers which end in the nerve cells". Nevertheless, these results led Langley to put forward the hypothesis that transmission from nerve endings was chemical (Langley, 1906).

During this period, anatomic investigations on the structure of nerve tissues were carried on with the aid of methods based on metallic impregnation. Despite the accuracy of such techniques, in particular Golgi's method, the resolution of the light microscope was insufficient to get definitive answer on the mode of connections between nerve cells. Thus, it is not very surprising that W. Waldeyer, who introduced the term neuron in 1891 (see Bayliss, 1924), had not yet a clear notion of cellular connection. Therefore, several interpretations on the organization of the nervous system remained a subject of debate between anatomists for a long time: theory of cellular anastomosis between processes of nerve cells proposed by Gerlach (see Freund and Berg, 1964), diffuse network of nerve fibrilles between nerve cells (Golgi, 1891), single and independent neurons forming the whole nervous system (Ramon y Cajal, 1890). A growing amount of data, in particular observation of outgrowth of axon from single cells, observed by His in 1886 (see Hamburger, 1981) and Ramon y Cajal (1890), seemed to agree with the neuron theory. However, the reticular theory still retained many adepts among the most eminent scientists for decades (Nissl, 1903; Bethe, 1903). A striking example is provided by Bethe (1952) still proposing anastomosed networks and synaptic discontinuity as two alternatives accounting for the organization of the nerve tissue. Finally, the definitive demonstration that nervous system was indeed composed of single cells was done by the first electron microscopic studies.

ADOPTION OF THE TERM SYNAPSE

The accumulation of data suggesting that chemical substances should be involved in nerve transmission led physiologists to suppose that connections between neurons formed a zone exerting a particular function. This idea was in agreement with the most substantiated anatomical theory which favored the existence of neurons, i.e. single cells with free endings. In the course of the redaction of a chapter intended for a textbook edited by M. Foster, C. S. Sherrington (1897) proposed a new term to describe the mode of connection between two nerve cells. The first proposition of Sherrington was syndesm. However, a friend of Foster, the hellenist Verrall, suggested to choose the word synapse, which has definitively been adopted afterwards. This modification has been proved to be very judicious. Indeed, the greek philosopher Aristotle already used the words syndesm and synapse in his treatise of anatomy, "Parts of animals", which is considered as the first textbook on animal physiology in the world's history. In this book, syndesm indicates the connection that spinal cord (referred erroneously as bone marrow) provides between segments of backbone (Parts of animals, II, 6). Thus, syndesm designates a continuous structure. On the contrary, synapse designates the junction between liver and the "great blood vessel" (dorsal aorta?) (Parts of animals, III, 4), in other words the zone of contact between two different tissues or organs. It is worth noting that the use of the two words in antique astronomy shows also clearly their different meanings. Indeed, syndesm referred as conjunction of two stars, while synapse as a group of stars. Then, it was fortunate that the term syndesm was replaced by synapse, since syndesm is related to the idea of fusion or continuity, which was apparently contrary to Sherrington's own thinking!

NOTION OF HUMORAL IMMUNE REACTION AND CHEMORECEPTORS

The discovery of specific immune substances (antitoxins) in the serum of animals infected by bacillus of diphteria or tetanus (Behring and Kitasato, 1890), performed under the direction of Robert Koch in Germany, raised the basis of the humoral theory of immunity. This finding was in contrast with the cellular theory of immunity, based on phagocytosis of toxins by macrophages, that was raised in Paris by Metchnikoff (1884). Humoral theory of immunity had a direct influence on the work of P. Ehrlich (1891) in Germany, who showed later that antitoxins were produced by a chemical cellular process. Ehrlich gave an interpretation of this process in the well-known side chain theory in which he introduced the notion of chemoreceptors, i.e. cell constituents on which specific drugs and toxins bind (Ehrlich, 1909). In addition, it is worth noting that Ehrlich knew the lock and key theory between enzyme and substrate, formulated by E. Fischer (1894).

The theory of humoral immune reaction and the notion of chemoreceptors which was developed by Ehrlich had a great influence on the physiology of cell excitability. Indeed, humoral excitation leading to formation of antitoxins (antibodies) and nerve excitation provoking muscle contraction were described in a same chapter dealing with cell stimulation and excitation in Verworn's textbook (Verworn, 1909). Thus, it is doubtless that humoral theory of immunity (excitation of blood cells by toxins resulting in production of antitoxins in the blood) provided a favourable medium for the idea of humoral theory of chemical nerve transmission.

SECRETIN AND NOTION OF HUMORAL HORMONE

W. M. Bayliss and E. H. Starling (1902) discovered a substance which was secreted from duodenal mucosa in blood circulation and provoked the release of pancreatic juice. They called it secretin, now known as a polypeptide of 27 amino-acid units responsible for the secretion of water and electrolytes from pancreatic exocrine cells. The term hormone (in Greek, hormao means to stimulate) was proposed by W. B. Hardy in the laboratory of Bayliss (see Bayliss, 1924) to designate molecules with the property to reach and stimulate target cells after being secreted in blood flow. Their finding was the first example of target cell excitation by chemical agents released in the blood. Then, it is very interesting to recall that O. Loewi (see below) studied in the laboratory of Starling in

1902.

ADRENALINE AND THE HYPOTHESIS OF CHEMICAL NEUROTRANSMISSION

The first experimental data suggesting that nerve transmission was mediated by chemical agents has been provided by T. R. Elliott (1904), a young student of Langley in Cambridge. Elliott found that adrenaline could mimick the effect of sympathetic nerve stimulation. The action of adrenaline did not exert on sympathetic ganglia but on nerve periphery, and remained after muscle denervation. Elliott put forward a clear-sighted hypothesis on nerve transmission that he summarized in these terms: "...The point at which the stimulus of the chemical excitant is received, and transformed into what may cause the change of tension of the muscle fibre, is perhaps a mechanism developed out of the muscle cell in response to its union with the synapsing sympathetic fibre, the function of which is to receive and transform the nervous impulse. Adrenalin might then be the chemical stimulant liberated on each occasion when the impulse arrives at the periphery.".

It is interesting to note that adrenaline was purified by J. Takamine in 1901 from adrenal glands, homologous tissue of sympathetic nerves (see Bayliss, 1924; Holmstedt and Liljestrand, 1981) and Elliott received a sample of Takamine's adrenaline. Today we know that the neurotransmitter released by sympathetic nerves is not adrenaline (except in few species, such as frog) but its precursor, noradrenaline (von Euler, 1946; Ostlund, 1954).

NEUROPHARMACOLOGY OF CHOLINE AND ACETYLCHOLINE

Choline esters entered the field of investigation with the work of R. Hunt, an American pharmacologist who received a decisive influence from P. Ehrlich when he was working in his laboratory in 1902-3. Hunt observed that extracts of suprarenal glands free from adrenaline could lower the blood pressure. In preliminary studies, he found that choline might be responsible for such effect. However, a further extensive analysis of a great number of choline derivatives led Hunt and Taveau (1906) to discover that acetylcholine had a lowering action on blood pressure hundred thousand times more potent than choline.

At the same time in Cambridge, W. E. Dixon (1907) studied the action of the vagus nerve upon the heart beats. Dixon showed that extract of heart stimulated by vagus nerve caused inhibition of heart contraction. This inhibitory effect was blocked by atropine and less pronounced when vagus stimulation prior to extraction was omitted. The interpretation of Dixon confirms Elliott's hypothesis in these terms: "I interpret these experiments to mean that some inhibitory substance is stored up in that portion of the heart to which we refer as a "nerve ending", that when the vagus is excited this inhibitory substance is set free, and by combining with a body in the cardiac muscle brings about inhibition". It is interesting to note that the thermal and alcoholic extract used by Dixon fortunately prevented acetylcholine (now known to be responsible for the inhibitory action on cardiac muscle) from being degradated by tissular acetylcholinesterase. The work of Dixon can be considered as a precursor of the experiment that O. Loewi performed in 1920.

The work of H. H. Dale, performed mainly in London, had a decisive influence on the pharmacology of acetylcholine. He showed that the effect of acetylcholine reproduced closely the effect of parasympathetic nerve stimulation (Dale, 1914). Thus Dale supposed that acetylcholine might be the substance released by the parasympathetic nerves. However at that time the presence of acetylcholine in the tissues was undetectable, owing probably to very quick in situ hydrolysis. Nevertheless, Dale had a clear view that the effect of adrenaline and acetylcholine paralleled the complementary and antagonistic action of the sympathetic and parasympathetic nerve supplies, respectively. This led to the notion called Dale's principle (see Eccles, 1986) that one neuron synthesizes one neurotransmitter.

CHEMICAL NEUROTRANSMISSION BY "VAGUSSTOFF" (ACETYLCHOLINE)

European science suffered from the consequence of the first World War. Therefore, the key experiment in the history of the research on chemical neurotransmission was performed only in 1920 by O. Loewi, working then at the university of Graz in Austria. This is not very surprising if we keep in mind that Loewi received the influence of Starling, Elliott and Dale.

Loewi reported that the idea of this experiment came in his mind during two successive dreams. Its principle was simple and sound. Loewi stimulated the vagus (parasympathetic) nerve of an isolated frog heart, thus provoking the stop of the heart beats. He collected physiological solution coming out from the inside of the heart and perfused a second isolated heart with the solution. Then the second heart stopped without any previous stimulation of vagus nerve. Loewi concluded that a substance, which he called "Vagusstoff", was released from the vagus nerve terminals innervating the heart (Loewi, 1921). This was the first demonstration of chemical neural transmission. Fifteen years later, Dale *et al.* (1936) proposed acetylcholine as transmitter of the neuromuscular junction. It is worth noting that Loewi used by chance Ringer solution instead of blood serum. Indeed, in that case, cholinesterases present in serum would have probably degradated the Vagusstoff (acetylcholine).

CELLULAR METABOLISM OF ACETYLCHOLINE

The importance of enzymatic degradation of neurotransmitters was pointed out by Loewi and Navratil (1926), who suggested that the short duration of the action of "Vagusstoff" on the heart was due to quick metabolic inactivation. They found that physostigmine increased the amplitude and duration of the effect of the Vagusstoff. They concluded that phy-

sostigmine might inhibit the enzymatic degradation of the neurotransmitter thus provoking heart sensitization. Afterwards, J. C. Eccles (1937) supposed that Vagusstoff (presumed as acetylcholine) should be destroyed by acetylcholinesterase in shorter time than refractory period of muscle contraction. At the same time A. Marnay and D. Nachmansohn (1937) and T. P. Feng and Y. C. Ting (1938) demonstrated successively that extract of innervated zone hydrolyzed acetylcholine more rapidly than that of non-innervated zone. However, the activity of acetylcholinesterase did not appear sufficiently important to account for the rapid destruction of acetylcholine as it had been proposed by Eccles (1937). Couteaux and Nachmansohn (1940) found that after muscle denervation, acetylcholinesterase activity was only partly decreased in the innervated zone. They concluded that most of acetylcholinesterase associated with the myoneural synapse was located not inside but outside of the nerve terminals (today we know that acetylcholinesterase is located in the synaptic space of neuromuscular junction, where it is highly concentrated). This indicated that acetylcholine was inactivated on the exterior of the nerve endings, thus fulfilling one important criterion to be considered as a neurotransmitter.

Besides, investigations were carried on to search the enzyme of acetylcholine synthesis (today called choline acetyltransferase). The first enzymatic acetylation of choline was done by Nachmansohn and Machado (1943). The mode of choline acetylation was elucidated after the discovery of Coenzyme A by Lipman and his group (1947) who described the structure of the acetylation agent.

The circumstances under which Nachmansohn pursued his work during these years, passing from Germany to France in 1937 and continuing his way until the U.S.A. in 1939 are a good illustration of the remarkable tenacity that many prominent scientists —in particular German Jewish researchers (Nachmansohn, 1979)— proved through the worldwide cataclysm raging in that period.

At the end of the war Couteaux (1947), using Janus green B staining, observed a continuous membrane on the surface of the muscle fibres, forming a border between muscle cells and nerve terminals. At the inner face of this membrane, stacks of rod-like lamellae, that Couteaux called subneural apparatus, were detected exclusively in the synaptic region. He showed furthermore that the sarcoplasmic membrane remained after denervation of the muscle fibers. These data strongly suggested the presence of a discontinuity between nerve terminals and muscle cells, and gave a few years before the first electron microscopic observations, a decisive credit to the neuron theory.

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