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Neurosciences

Some historical difficulties of the cholinergic transmission

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Abstract

For a very long time, arguments supporting the neuro-humoral theory, produced by the pharmacologist Henri Dale, did not convince one of the main supporters of the electrical theory, the neurophysiologist John C. Eccles. This article gives a reminder of certain technical elements of this debate between two giants, which remains one of the most symbolic arguments in the history of neurosciences. The difficulties of the cholinergic transmission often betrayed the scepticism concerning the possibility of a functional approach based on neurochemistry. ***To cite this article: J.-C. Dupont, C. R. Biologies ••• (••••).***

Résumé

?? Pendant très longtemps, les arguments en faveur de la théorie neuro-humorale avancés par le pharmacologue Henri Dale ne vont pas convaincre un des principaux partisans de la théorie électrique, le neurophysiologiste John C. Eccles. On propose ici de rappeler quelques éléments techniques de ce débat entre les deux géants qui demeure un des plus emblématiques de l'histoire des neurosciences. Les difficultés de la transmission cholinergique trahissaient souvent le scepticisme d'alors concernant la possibilité même de l'approche fonctionnelle en neurochimie. ***Pour citer cet article : J.-C. Dupont, C. R. Biologies ••• (••••).***

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1. Introduction: the historical question of neurotransmission

In his book of 1974, the pharmacologist Zenon Bacq rightly regretted the absence of systematic epistemological and historical studies concerning neurotransmission [1]. During nearly twenty-five years, the situation barely changed. Only scattered general reviews were available. This gap was surprising because, far from being an additional effect, the birth of the neurotransmitter concept was particularly important in the construction of biological and pharmacological thoughts in the 20th century. The concept was involved in the molecular foundations of neurochemistry and pharmacology. This story exceeds the understanding of simple elementary processes. It was also necessary to recognize the contribution of chemistry in the field of neurophysiol-

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ogy, and subsequently, to accept the idea that molecules could play a functional role. The fact that today the concept of the neurotransmitter represents a paradigm hides the difficulties of its origin. It is therefore necessary to reconstitute these early beginnings step by step. The immense interest of this reconstruction was perceived quite recently, and it is only at the turn of this century that specific studies appeared [2–5]. We shall try here to concentrate on the contents of debates on cholinergic transmission.

2. The beginning of the concept of the neurotransmitter

2.1. The problem of neurotransmission

Neurotransmission is an elaborate notion. Its origin is understandable only from concepts arising towards the end of the 19th century. It is only when neurotransmission was constructed from the concepts of the synapses and the action potential, in a still imperfectly confirmed notion, that the problem of neurotransmission could emerge. At that time, the histological debate was very active between advocates of reticular theory and those of the neurone theory. Beyond morphology, it was a question of generalizing cellular theory. In order to explain the reflex arc, Charles Scott Sherrington introduced the term 'synapse' and so advocated the neurone theory [6]. Another biological preoccupation was the ancient problem of animal electricity. If the nervous impulse was recognized as being a 'negative variation' and its physical shape determined by Julius Bernstein [7], one still ignored everything regarding the exact nature of the action potential (AP), even though its ionic nature was already suspected. The significance of the problem was beyond a simple elementary process. In other words, understanding the intimate physico-chemical mechanisms based on autonomic regulations could be only a step towards what Sherrington called 'nervous integration'.

Pharmacology, since Claude Bernard, faced the problem of the mechanism of action of neurotoxic substances such as curare [8]. In addition, since the work of the Cambridge School of Physiology, pharmacology was also confronted with the problem of neuromimetic substances as adrenaline [9]. These studies suggested the idea of chemical transmission. The concept of neurotransmitter constituted a plausible answer to the questions asked by physiology, histology and pharmacology at the end of 19th century. The chemical transmission hypothesis was formally expressed at the beginning of the 20th century by the Cambridge School of Physiology. The theory suggested that "*since there are chemical substances like adrenalin reproducing the effect of excitement (neuromimetism), these could represent the physiological agents of reexcitation*" [10]. In other words, the AP could cross the synapse through chemical substances, which took the name of chemical mediators. After the studies of John Langley and Thomas Elliott, pharmacological investigations developed and proposed several substances as candidates for neurotransmission. We will see that it is unmistakably Henry Dale's contribution that acted as a determining factor.

2.2. The electrical hypothesis

There was however another answer: the AP could be the direct agent of reexcitation. The French neurophysiologist Louis Lapicque named this thesis '*théorie chronaxique*'. Stemming from a criticism of Emil du Bois-Reymond, Lapicque showed that the excitability of the neurone depended upon two factors: the strength of the exciting current and its duration. He thus defined '*chronaxie*' as a time constant characterizing the excitability of tissues. By using a current shaped similarly to an AP as a stimulus, he proposed the idea that transmission was only possible if elements were isochronous, that is to say shared the same chronaxie value (law of isochronism). In this perspective, no chemical substance was needed [11]. The chronaxic theory also involved as a necessary condition, presynaptic and postsynaptic elements had similar time constants. However, the theory did not explain inhibitory impulses, and the synaptic polarization in the propagation of the impulse. Furthermore, it was more compatible than its chemical rival with the speed of transmission. The pharmacological data put forward by the Cambridge School (neuromimetism and modulation of effects of these neuromimetic agents by lytic agents, such as curare, nicotine, or atropine) could be interpreted within the framework of electrical theory. These agents were thus supposed to modify chronaxies of synaptic elements. Lapicque's views also included a global physical theory of the functioning of the nervous machine by the subordination of lower centres to superior ones. This functional generalization, which Jackson evoked, did not rely on any other data than that supplied by basic electrophysiological studies of the time from Adrian and Erlanger and Gasser's laboratories. Furthermore, Lapicque's ideas were perfectly compatible with reflexologic data of Sherrington and Pavlov. They therefore fitted the corpus of current neuroscientific knowledge and styles of thought when science was dominated by

the prestige of physics. It thus remained for long a plausible theory [12,13].

However, it was abandoned mainly because of a controversy raised by Cambridge physiologists (Lucas, Rushton), concerning the conditions of excitability, directly calling isochonism into question. Only after the Second World War did French neurophysiology recovered from the hegemony of Lapicque's dogmas, thanks to Alfred Fessard and his team.

3. Refinements of the chemical hypothesis

3.1. Dale's first pharmacological studies

Henri Dale began his astonishing career studying the pharmacology of ergot, from which alkaloids had not yet been previously isolated. From 1904 to 1914, he tried to isolate such compounds for a pharmaceutical firm. Dale had studied with the most talented physiologists from Cambridge and London (Gaskell, Langley, Bayliss, Starling). He knew very well the problems connected to adrenalin. He quickly showed ergot extracts opposed the hypertensive effects of adrenalin [14]. However, some of these extracts seemed contaminated with vasoactive substances. These contaminating substances, thereafter identified as tyramine, histamine and acetylcholine, became Dale's main interest for fifty years of research. He proposed the term 'sympathomimetic' to describe the effect of some of these substances [15]. Some ergot extracts also produced effects different from those of histamine and mimicked those of muscarine. This action could be due to the presence of an ester of choline. It is necessary to remember that chemists had succeeded in synthesizing acetylcholine (ACh) without being interested in its pharmacological properties. ACh was forgotten until Reid Hunt noticed a hypotensive substance in aqueous extracts of adrenal glands, which disappeared after hydrolytic treatment. Since the choline concentration increased upon time, Hunt suggested the extracts contained a choline precursor of the ester family [16]. A number of such esters were synthesized, among which ACh, a substance with a most remarkable effect. It was a shown to be a hundred thousand times more hypotensive than choline. Dale collected all these data in his 1914 publication, which corresponds to the appearance of ACh in the field of neurotransmission. He investigated the effects of injecting ACh and noted effects similar to the stimulation of autonomic nerves [17]. He clarified the complete pharmacology of ACh dissociating the muscarinic action obtained at a weak dose (a brief effect abolished by small doses of atropine), from the nicotinic action – at a strong dose (abolished by an excess of nicotine and reverting some of the previous effects). He acquired the conviction that ACh mimicked the action of parasympathic nerve impulses ('parasympathomimetic' effects), just as adrenalin mimicked the action of sympathic nerve impulses according to Langley. With Dale, chemical neurotransmission was susceptible to being connected with the two opposing constituents of the involuntary nervous system, for each of which was known a serious putative neurotransmitter.

These concepts opened considerable therapeutic perspectives and a path to the pharmacology of the autonomic nervous system based on the synthesis of lytic and mimetic substances. Nevertheless, the scientific problem of the nature of neurotransmission persisted. It was necessary to demonstrate experimentally that such substances were released at fibre endings. The First World War prolonged this enigma seven years long, when many pharmacologists were to dedicate themselves to 'more urgent' tasks, such as the elaboration of poisonous gas and their antidotes.

3.2. Transmission in the autonomic nervous system

The persistence of the electrical theory among neuroscientists did not just explain the extreme scepticism about what is often presented as the first direct experimental proof of the liberation of a chemical substance by nerve fibres, namely the crucial experiment of pharmacologist Otto Loewi on isolated frog's heart [18]. The lack of reproducibility of this experiment was rightly underlined and Loewi's interpretations were disputed. The debate lasted for several years. Nonetheless, due to the systematic use of eserine and the technical perfection of the experimental set up, it finally became possible to reproduce the 'Loewi effect', which was soon generalized to all nerve endings of the autonomic nervous system. Finally, Loewi himself finally identified the vagal substance as ACh.

Dale then appeared on the front scene again. During a study on histamine, Dale and Dudley found unexpectedly high concentrations of ACh in the ox and horse spleen, finally demonstrating ACh as an endogenous chemical of animal tissues [20]. ACh was shown to be a rather ubiquitous substance and was not only found in the nerve endings of parasympathetic system, but also in ganglia. Dale perceived the real biological importance of these findings. Loewi's works had succeeded to admit a chemical neurotransmission of nervous impulse at fibre endings from autonomic nervous system. From this point on, why not contemplate an extension to the whole peripheral nervous system and later to the central

nervous system itself? Sherrington considered neuromuscular connection could supply a model for central synapses. Nevertheless, one could not obviously be satisfied with such a simple analogical reasoning. It was necessary to investigate in each case the mechanism of neurotransmission separately. The central nervous system was almost fully inaccessible to any functional biochemical experiment, because of its anatomical complexity. Thus in 1933, Dale and his co-workers of the National Institute for Medical Research (NIMR), a brilliant team including Wilhelm Feldberg, John Gaddum, Marthe Vogt, Geoffrey Brown tackled the problem of synaptic transmission in other regions of the peripheral nervous system. In order to identify a possible neurotransmitter function of ACh, Dale and his co-workers had used since 1893 Anton Kibjakov's technique of superfused ganglia (Kazan). Kibjakov had succeeded to obtain the appearance after stimulation of a substance in the perfusate solution. This substance had the same effect as an electrical stimulation when injected into another ganglion [21]. In addition, he performed this experiment at the 1935 International Congress of Physiology held in Moscow–Leningrad. Carrying on with Kibjakov's work, Feldberg identified ACh in the perfusing fluid at every passage of the nerve impulse. He measured ACh and verified that the released quantity was sufficient to excite postganglionic fibres when injected into the perfusing fluid [22]. In addition, Feldberg investigated the hypertensive effects of ACh [23]. The intravenous injection of ACh elicited a normal hypotensive effect followed by a paradoxical hypertensive one. Since the injection of ACh provoked the secretion of adrenalin by adrenal glands innervated by the splanchnic (sympathetic) nerve, it was necessary to suppose the involvement of ACh in sympathetic nerve function. This role of ACh was confirmed by the potentialising effect of eserine, which increased the response to the stimulation of splanchnic nerve. The excitement of splanchnic nerve of adrenal glands perfused with eserine elicited the passage of ACh in the perfused liquid.

The splanchnic nerve contains preganglionic neurones, and therefore, the adrenal medullary cells are equivalent to ganglionic cells. ACh might thus be the transmitter that acted on medullary cells to evoke the release of adrenalin [24]. At this point, it was very possible to suppose that ACh was released at the synapse, and therefore a cholinergic general functioning of ganglionic synapses. If ACh were present at the same time in the parasympathetic system and in the ganglia, the purely anatomical classification of the nerve fibres, as belonging to one of these systems, became illogical. Some parasympathetic nerves were identified as adrenergic, while others were cholinergic. Dale called fibres 'cholinergic' and 'adrenergic' depending on whether they released ACh or adrenalin [25]. This chemical classification of nerve fibres was an essential step in the history the functional neurochemistry of nerves.

3.3. Neuromuscular transmission (striated muscle)

In 1934, Dale pursued his research in the direction of the extension of cholinergic transmission and tried to establish this concept for the mammalian striated muscle. The contractions of the striated muscles caused by ACh were well known and also classified as nicotinic effects because they were inhibited by an excess of nicotine and not inhibited by atropine. During observations of the nicotinic effects of ACh at the level of the *chorda tympani*, the contraction of the neighbouring voluntary denervated muscles was observed. This allowed the possibility of a cholinergic action occurring after diffusion through membranes. However, was there a link between this contraction and the transmission of the motor impulse? The experimental difficulties were greater than with ganglionic transmission. In the case of ganglion, ACh remained concentrated in the perfusate solution once released from the presynaptic fibre. With striated muscle, ACh diffused in the enormous muscular mass and was therefore much diluted in the perfusate solution. Dale and Feldberg overcame these difficulties by working on a pure motor nerve, the hypoglossal nerve of the cat (after degeneration of the sympathetic fibres contained in the hypoglossal nerve by destruction of the superior cervical ganglion). The excitation of the nerve thus prepared, elicited the release of ACh detectable in the perfusate solution of the muscle, through its action on the cat blood pressure and on the contraction of the parietal muscle of the leech, the most sensitive biological test known at that time [26]. Similar experiments were performed on other muscles after degeneration of a part of the sympathetic chain, such as the cat or dog gastrocnemius, the posterior leg muscle of the frog, and the dog quadriceps extensor of the thigh [27]. In all cases, ACh appeared in the perfusate solutions after stimulation of the motor nerve fibres, even in the presence of curare. Similar results were obtained by direct stimulation of the normal muscle, or of the muscle with autonomic innervation suppressed. On the other hand, when the muscle was completely denervated, there was no ACh release. The conduction of the impulses fell down by nervous fatigue after repeated stimulation and ACh was no longer released.

4. The difficulties of the chemical theory

Dale's experiments seemed technically almost irreproachable. Despite these data, some physiologists still did not accept the chemical theory and especially at the instigation of Eccles, contradictory debates started against Dale's school. In fact, although Loewi and Dale had obtained the Nobel Prize in medicine in 1936 "*for their discovery of the chemical transmission of impulse*", the intimate mechanisms of neurotransmission were unknown. Where and how was ACh released, how were the chemical mediators stored, synthesized and degraded and how it did act on the post-synaptic side? Data then accumulated which only partially helped to clarify the mechanisms in a way explaining the existence of important difficulties of the chemical theory, and opening the way for the development of electrical or 'mixed' theories. The last theory often represented very strong resistances against the concept of neurotransmitter. Let us consider some of these difficulties.

4.1. The release of ACh

One experimental fact advanced by Dale's school was that after stimulation of nerve fibres, ACh appeared in the perfusate solution near innervated structures, while there was no neurotransmitter release in the liquid along the nerve path. Thus, ACh was released exclusively at nerve endings. This was an important argument for its action as a neurotransmitter. Some observations reported, however, that when a nerve was sectioned, ACh was released in the fluid surrounding the sectioned surface, and this suggested a release of ACh all along the nerve [28,29].

On the other hand, ACh release could have a different meaning. In the vegetative system, an agreement between Lapicque's theory and the idea of a chemical mediator was possible. As we saw it, an experiment on the striated muscle was technically difficult. Fortunately, there was an organ similar to the muscle at the level of ganglia, represented by satellite cells in narrow contact with nerve endings. Could they represent the real place for ACh release? In 1938, at the Rockefeller Institute (New York), Rafael Lorente de Nó, a talented physiologist, tested this hypothesis and began a series of experiments on the release of ACh in the superior cervical and the nodal vagal ganglion of the cat. This was done using the technique of Kibjakov's perfusion, which was used in Dale's school. A release of ACh occurred spontaneously, without electrical stimulation. It was attributed to a tissue damage connected to the technique of perfusion. Through modifying the technique, no spontaneous release occurred when undamaged preparations were used. In addition, after stimulation, the release was very weak. Moreover, it arose not long after transmission stopped. Thus, if it was clear that there was a metabolism of ACh in ganglionic tissue. The technique of perfusion used by Dale did not determine whether it was really specific to synapses [32]. In the 1950s again, other results caste doubts on the release of neurotransmitter from nerve endings and disputed Dale's conclusions. According to Archibald McIntyre, even after degeneration of nerve endings, ACh appeared in the perfusate solution after direct stimulation of muscle. Dale's failure to find ACh in the perfusate solution of denervated muscles, a presentation by Dale as proof of the liberation of the ACh from nerve endings, was explained simply by the state of capillaries in muscles after denervation. He based this assertion, like Lorente de Nó, on histological observations. Furthermore, another source of error was the presence of eserine used by Dale, which elicited a release of adrenalin, noradrenalin and serotonin from the medulla of adrenals. Capillaries of denervated muscles were highly sensitive to such substances [33,34]. On the basis of these observations, while underlining the existence of a barrier of permeability by the axonal lipidic membrane to ammonium quaternary substance such as ACh, biochemist David Nachmansohn considered that its appearance in the perfusate solution of nerve endings and the absence of axonal leak were not an argument in favour of a limited role at nerve endings. Contrary to the neurohumoral theory, which attributed to ACh a role in the intercellular communication, Nachmansohn supported the idea of a purely intracellular role for ACh in nerve conduction [35]. Dale's experiments were far from having a masterful and definitive character presented by enthusiastic partisans. Even if Dale's results were admitted, they were not an irrefutable proof of the transmitter role of ACh. Other interpretations remained possible, especially when the mechanisms of release remained completely unknown.

4.2. Storage and formation of ACh

Depending on the method used, various quantities of ACh were found in tissues. The controversy concerning the techniques of extraction ended quickly with the problem of the physical or physico-chemical nature of ACh in the nervous system. This question was of a considerable physiological interest. It was necessary to know what was extracted and exactly measured by biological tests in order to explain how ACh was protected from the action of esterases, and how it was formed

and accumulated. Very early on, numerous authors had postulated, in tissue extracts, a bound form of ACh. Excitation of cholinergic nerves released it in an active form. This thesis was not fully supported. Some critics were in favour of a synthesis of ACh during stimulation of cholinergic fibres. One argument for this hypothesis stated that the prolonged excitation of cholinergic nerves increased ACh content chemically isolable in the innervated organ (heart for example). This fact later became controversial. For supporters of the hypothesis of preformed stocks, it was obviously necessary to admit that it was constantly affected by nervous excitation occurring at the same time as the synthesis of ACh. The problem remained unsolved of when and how a stock was reconstituted, and what was its importance.

John Eccles was one of Sherrington's pupils in Oxford after 1925. He signed with Sherrington the well known 1930 paper on the motor unit. Eccles was also interested in the temporal aspects of reflexes. He and Geoffrey Brown analysed the chronology of transmission in the autonomic nervous system. Studying the vagus, the summation of effects of two consecutive impulses separated by increasingly shorter intervals, Brown and Eccles thought it possible to estimate the time necessary for the synthesis of the mediator. By sending two stimuli at closer and closer times, they found that a second stimulus delivered 12 ms after the first one, could produce its maximal inhibitory effect. In other words, an impulse circulating in the postganglionic fibre 12 ms after the first could release the same quantity of ACh. Therefore, the formation time of the mediator was very short, which implied instead the existence of a preformed stock [36,37]. However, this short time contrasted with the even shorter time of crossing the synapses, which Eccles himself, through studying the latent periods of reflexes, estimated to 3 ms, and under certain conditions of stimulation at even lower values. For a long time, this reinforced his idea, shared by other opponents to the chemical theory that fast transmission, like that in ganglia or the neuro-muscular junction, could absolutely not be of a chemical nature.

4.3. Mode of action of ACh

According to the neuro-humoral theory, the mediator had to reproduce exactly a nervous excitation. This identity of action, if it were shown, would have been a very important argument for the neurotransmission hypothesis. However, the injection of ACh elicited only muscular spasms. A related problem was the mode of action of eserine. A condition for the possibility of a chemical transmission was the fast destruction of ACh by cholinesterase. If this enzyme were inhibited by eserine, a repetitive stimulation by ACh had to occur on the post-synaptic cell. But this answer was difficult to prove electrically. Furthermore, large doses of eserine did not modify ganglionic transmission of single excitations nor the corresponding potential. Finally, repetitive stimulation of the nictitating membrane of the cat produced identical contractions when stimulation was presynaptic or postsynaptic. However, a small delay in the decrease of effects in the case of the presynaptic stimulation was noted. After the action of eserine, this delay became much more important. Eccles thought that the accumulation of ACh was responsible for the prolonged depolarization of the muscular fibre and that slow contraction was identical to the contracture produced by arterial injection of ACh when reaching the ganglion. However, an initial excitation, not being prolonged by eserine, was due to an electrical transmission. Thus, according to Eccles, ACh could not be the only actor of neurotransmission: there would be a double process of transmission, one that was electrically fast and another, chemical and slower [38,39].

Following these studies, Dale and co-workers prepared experimental arguments against Eccles and the double theory. It was necessary to compare the action of a substance in situ and the action of the same substance added artificially. To get closer to the conditions of efficiency in which ACh exercised its action in physiological conditions, Dale and his colleagues made injections in the artery of a muscle, which was carefully vascularly isolated to avoid any decrease in ACh concentration. They noticed that during a muscular response provoked by excitation of the nerve, ACh administered as a fast intra-arterial injection provoked a contraction with all the characters of the neuro-muscular shock. If the experiment were performed again in the presence of eserine, the muscular response was intensified, provided that the stimulus was infrequent and of sufficient intensity to elicit a single depolarization wave on all fibres of the motor nerve. If a tetanus was provoked, contraction was not maintained under eserine. If single shocks were repeated, the response was decreased with regards to the one that was recorded before the tetanus. Thus, ACh, if not at once hydrolyzed by cholinesterase, remained at the level of the synapses where it caused its effect [40].

Brown's observations on the electrical expression of the acetylcholine shock could reinforce this point of view [41]. A muscular shock provoked by an electrical shock was expressed by a single electrical variation. However, acetylcholine shock provoked by the intra-arterial injection of ACh appeared as a periodic response. It would however be plausible that this dif-

ference, attached to the fact that the injection of ACh, affecting fibres by their capillary pole, did not achieve the simultaneity of action, which characterised the involvement of the motor units. The important detail was that electrical shocks also provoked a brief tetanus in the presence of eserine. Even in this case, ACh seemed to persist at the level of the motor endplate and excite it in a repetitive way. Gradually, Brown became a supporter of the chemical theory [42]. This was not the case for Eccles, according to whom eserine did not inhibit the cholinesterase, but acted directly on the motor endplate. Thus in 1937, he still remained a supporter of the mixed theory, suggesting that presynaptic AP was responsible for a brief initial excitation in the neuro-muscular junction and in the sympathetic ganglion, in addition to the fact that the neurotransmitter substance ACh was responsible for the prolonged residual depolarization [43].

5. New experimental validations

5.1. Electrogenic role of ACh

To eliminate ambiguities, it was necessary to show that a postsynaptic application exactly reproduced the postsynaptic electrical response obtained by the stimulation of the presynaptic neurone. The possibility of the electrogenic depolarizing action of ACh was envisaged previously. Furthermore, there was considerable literature on the anatomical characteristics and the properties of electrical organs of electric fish since the 18th century. Each electoplaques of an electrical organ develops a potential comparable to that found in nervous or muscular fibres. Their association in series (as in a voltaic battery) allows them to develop high voltages. They therefore constitute a material of choice for the study of nervous and muscular physiology. During studies on the "*rhythmic properties of the living substance*" and "*the rhythmogen excitability*", Alfred Fessard and David Auger used the electrical organ of torpedo (*Torpedo marmorata*) [44]. In 1937 Annette Marnay and David Nachmansohn showed the extraordinary high concentration of cholinesterase in this material [45]. Fessard and Auger would later test the effect of the eserine on the AP, to find that it was then considerably depressed and that the duration of this downward phase was much prolonged [46]. In view of these results, Fessard and Nachmansohn decided to verify whether or not ACh injected in the electroplaques would elicit a depolarization. For this experiment, they invited in August 1939 Feldberg to join them in Arcachon. The three scientists showed that injections of ACh in the organ could effectively generated potentials [47]. Nevertheless, if it confirmed that the nerve of the electrical organ was cholinergic and that ACh was electrogenic, it did not necessarily imply that this action was limited to the synaptic transmission. The interpretations of Nachmansohn and Feldberg were different. Nachmansohn thought ACh commanded the propagation of the impulse by intracellular action, while Feldberg admitted the role of an intercellular signal. It is significant to notice that the article was later published by Feldberg without mention of Nachmansohn's name [48].

5.2. Synaptic potentials

If the interest of all these experimental data was indisputable, it did not represent the irrefutable scientific proof of neurohumoral transmission. Finer information came from the progress of microphysiology. A first revolution in this domain had taken place in the 1940s, when using microdissection techniques. Small extracellular electrodes could be placed close to the synapse. First synaptic potentials were discovered by recording potential propagated along postsynaptic axons of sympathetic ganglia [49] and the ventral roots of spinal cord [50]. Eccles and other authors discovered a slower, flexible in amplitude, and not propagated potential at the level of the motor endplate. This synaptic potential was called endplate potential (EPP). Using microdissection, Stephen Kuffler succeeded in achieving a neuromuscular preparation limited to a single nervous fibre, ending on a single muscular fibre. On such curarized preparation, the excitation of nervous fibres did not induce any contraction, but only a weak and slow potential at the level of the motor endplate. However, as the effect of curare disappeared, the excitation gradually evoked a potential with the contraction of the muscular fibre. This potential proved to be the generator of muscular contraction after a synaptic delay. Its duration was incompatible with electrical transmission [51]. Furthermore, Eccles and co-workers had noted eserine increased the amplitude and duration of the potential originating in the motor endplate. Thus, the hypothesis of the inhibitory action of eserine on acetylcholinesterase, and the blockade of ACh receptors postulated by the chemical theory were strengthened [52]. Only chemical transmission could explain the action of curare and eserine, as well as the slowness of neuromuscular transmission. The cholinergic nature of ganglionic transmission was also confirmed. A similar search on sympathetic ganglia showed similar slow, localized potential [53]. Thereafter, the pharmacology of the striated muscle and ganglion were revised in the light of cholinergic transmission (Paton, Bovet).

6. New resistances to the cholinergic transmission and refinement of electrical theories

However, chemical conceptions still did not invalidate Eccles's mixed theory. He built a system on the hypothesis of a local post-synaptic response (theory of eddy currents), inspired by the model of artificial synapses proposed by Angélique Arvanitaki [54]. The artificial synapse model, which Arvanitaki named 'ephapses', had the interest to prove the possibility of an electrical transmission of excitation between nerves, without any chemical mediator. Various artificial synapses were tested. Arvanitaki's model was achieved using two giant cuttlefish axons attached on a small portion. This allowed a local analysis of the process of activation, which the second fibre underwent on the arrival of the AP of the first. It was possible to show experimentally that a propagated response was born when the local wave reached sufficient amplitude. From these experiments, Eccles imagined a succession of electrical events, which would take place at the junction of the post-synaptic membrane, close to a terminal button, and occurring according to the distribution of the current lines in time [55]. This purely electrical model considered synaptic potentials as intermediate in synaptic transmission. Therefore, it was still compatible with an electrical theory. In fact, it was necessary to be able to correctly place synaptic potentials within cellular structures. Some electrophysiologists placed synaptic potentials on the presynaptic side. Others doubted it was useful to appeal to an intermediate local response to explain transmission. Debate tended to stagnate until the beginning of the 1950s and the development of measurements of intracellular potentials with microelectrodes. The EPP was then located at the postsynaptic level and appeared as the necessary intermediate between the AP of the motor axon and the propagated potential eliciting the contraction of the striated muscular fibres. Eccles's resistances went on much longer for ganglion, which represented a neuroneuronal model closer to central synapses. Eccles's essential concern was not pharmacological. Rather, he was more fascinated by central transmission and neuronal circuits.

7. Inhibition, or the electrical proofs of cholinergic transmission

By 1950, even though ACh metabolism was clarified, especially after Nachmansohn's works [56], several problems on ACh's storage, liberation and action at the synaptic level remained. Adrenergic transmission was even less well known. Rosenblueth still defended his theory of the two sympathins [57]. In spite of the efforts of many pharmacologists, the synaptic action of drugs could only remain hypothetical. At this time, the concept of neurotransmitter did no fully belong to 'normal science', even though a sort of consensus emerged. At the Symposium held in Paris 1949 Eccles considered after Kuffler's works many doubts remained among electrophysiologists, particularly on central transmission. That same year however, he published a condensed form of his electrical hypothesis [58]. Eccles thus remained one of the most severe opponents to Dale's theory. Even after 1945, he would confess his electrical hypothesis was in very bad condition, although not wanting to admit it officially [59]. According to Bacq, it was at the end of the war that Eccles began his conversion to chemical neurotransmission: "*the observations which he harvested with his own techniques became less easily interpretable by the theory of eddy currents; one began to put in evidence, with electron microscopy, the particular vesicles in the axons endings, and histochemists demonstrated the exceptional concentration of cholinesterase in the postsynaptic membrane. Networks of proofs became more impressive everyday*." [60] Afterwards, he explained that his conversion to the chemical theory was linked to its meeting with the philosopher Karl Popper [61]. This reunification marked the end of his debate with Dale and aimed a fatal blow at the electrical theory. More than Popperian epistemology, technology and instrumentation had in this case a considerable heuristic value.

Rather paradoxically, a number of electrophysiological data concerning the temporal characteristics of transmission and especially inhibition have been considered as direct 'proofs' of the existence of chemical synapses. During the studies with Brown, Eccles had shown the speed of transmission seemed at first surprising within the hypothesis of chemical transmission, especially considering the time of diffusion of the substance through synaptic gap. In fact, the best images of synaptic buttons showed a cleft of a much overestimated dimension. Considering the phenomena of diffusion, it would have been expressed in the hypothesis of a chemical transmission by a delay of several ms, incompatible with a chemical transmission according to Eccles. In the mid-fifties, the appearance of electron microscopy, revealed the very narrow apposition of presynaptic and postsynaptic membranes. Chemical transmission became again a conceivable concept. Synaptic delay, being inflexible, became incompatible with a supposed and almost immediate electrical transmission. At first too short for chemical transmission, the delay had become too long for the electrical transmission. Elec-

tron microscopy later revealed the existence of an even narrower contact of membranes in electrical synapses. However, research on inhibition truly signed the definitive decline of the electrical theory.

Intracellular microelectrodes conceived by J. Graham, G. Ling and R.W. Gerard after World War II, were at first conceived for to measure propagated potentials. However, they could also be used to study synaptic potentials. First recordings of post-synaptic excitations were obtained by means of intracellular electrodes on neuromuscular junctions [62] and motoneurones [63]. These measures were confirmed in ganglia from mammals, squid, and aplysia. Microelectrodes were considered to measure synaptic delays with exactness, which was never previously obtained, and were allowed to clarify temporal characteristics of EPP and EPSP. The voltage clamp technique applied to neuromuscular junctions and to motoneurones further showed these potentials had a voltage sign strictly depending upon the level of polarization of the explored cells. This meant that depending on cases inward or outward currents crossed the neuronal membrane, and thus produced ionic flows. The variable polarization of the neuronal membrane and the recording with bielectrodes showed EPPs and EPSPs corresponded respectively to a transient modification of the membrane permeability to Na^+ and K^+ ions [64, 65]. Simultaneously a post synaptic potential able to hyperpolarize was discovered (IPSP) [66]. In 1951, because of the well-known stimulation on the nerve to the quadriceps muscle having a powerful direct inhibitory action on biceps-semitendinosus neurones, supposedly monosynaptic, Eccles inserted microelectrodes at this point. According to the electrical model, which he had proposed (Golgi cell theory of inhibition) [67], microelectrodes should have recorded a positive deflection. However, the opposite occurred. A negative deflection was recorded. In other words, a hyperpolarization of the membrane was seen. The PPSI could not be elicited with arrival of a continuously depolarizing AP. It was therefore necessary to bring in an inhibitory substance. It was evident that 'the mirror image' of the IPSP and the EPSP was also chemical. It is said that Eccles had been definitively converted to the chemical theory because of this 'crucial' experiment [68]. At the same time, Eccles and his co-workers performed a whole series of experiments to show the involvement of Cl^- and K^+ ions in IPSPs. These ionic flows and their kinetics were clarified in the sixties by microinjections of varied ions with iontophoresis. Eccles devoted his Nobel lecture to these questions [69]. He also studied the very fine interactions between EPSPs and IPSPs, as well as the organization and functioning of inhibitors' systems of the Vertebrates. Thus, in order to explain the bigger latency of the IPSP in the flexion reflex, he interposed an interneurone in the inhibitory pathway. To interpret Renshaw inhibition, he then introduced an intraspinal inhibitory circuit (negative feedback) [70]. Curiously, it was the study of this last system that allowed the identification of the first central synapses with an excitatory mediator. Eccles's school showed the first excitatory effects of ACh at the synapses of motoneurones in Renshaw cells with the help of microinjections techniques [71]. Afterward, the existence of other central inhibitory activities was demonstrated. It was now necessary to wait for the second microphysiological revolution of the fifties, in addition to measurements of postsynaptic potentials and the understanding of their ionic mechanism, in order for the electrical theory to be definitively worsened. These rapid progresses on the ionic bases of potentials resulting from initial works of the groups of Kenneth Cole, Alan Hodgkin, Bernard Katz, and John Eccles, brought an essential theoretical contribution to the humoral theory. According to Hodgkin and his school, the distribution of impulses was understandable on purely ionic basis and did not thus require the involvement of any chemical substance. The functional role of ACh was then limited to nerve endings, in accords with the humoral theory. When Eccles placed EPSPs on the post-synaptic side, and interpreted its ionic mechanisms, simultaneously the mode of action of the neurotransmitter on the membrane was clarified for partisans of the chemical theory. The permeability of the post-synaptic membrane to definite ions was modified. These ionic flows were difficult to explain within the framework of electrical transmission, unless ACh could play a role in conduction. This last concept always remained Nachmansohn's position. Afterwards, the complexity of cholinergic neurotransmission was revealed by the existence of inhibitory cholinergic synapses to ganglionic cells in Aplysia. Ladislav Tauc and Hersch Gerschenfeld demonstrated this inhibitory hyperpolarizing action with the help of iontophoretic techniques [72]. In fact, an interneuronal impulse could produce either an excitation on certain cells and an inhibition on the others, both of whose actions used ACh. By using the same mediator, a neurone could possess excitatory and inhibitory synapses. Therefore, the excitatory and inhibitory action of a mediator was connected to the properties of the receptive substance, and not to the mediator himself. Much later, immunological techniques succeeded in showing that certain neurones synthesize several different mediators. This definitively questioned the famous Dale principle (one neurone–one neurotransmitter), unless one considered this phenom-

enon of coexistence as being an extension of it. The isolation of the ACh receptor crowned the French contribution to cholinergic transmission [73].

8. Conclusion: towards the central neurotransmission

In the late 1950s, a rather complete molecular theory of the nervous signal existed. Moreover, after Eccles's conversion, discussions about neurotransmission became less polemical. According to the combined introduction of the electronics and the fine biochemical techniques in the laboratory of neurophysiology, the argumentation of the upholders of the electrical theory would weaken, whereas chemical theory would win in coherence and would extend. The subsequent discovery of electrical synapses, which possess a functional role, could not modify this historic situation.

However, because of the anatomical complexity, the extension of chemical theory to central levels was by far more difficult. In spite of Eccles' works on spinal neurones, and Marthe Vogt's studies [74], ACh did not reveal itself to play a pervasive role in central neurotransmission. Due to Eccles, but also to Rafael Lorente de Nó, Evarts Graham, David Llyod, and Herbert Gasser, there was already a powerful logic based on a strictly electrical determinism using neuronal circuits and excitability properties of the neurone to explain the characteristics of the reflex activity. Since the early 1950s, as much as instrumentation and techniques were pertinent, the renewal of the humoral context favoured this intellectual extension. This particular context is represented by the birth of neuroendocrinology (revealing the humoral nature of the hypothalamus-pituitary-adrenal axis) of new psychophysiological concepts (revealing the importance of humoral factors in the genesis of emotions), and by expectations from biological psychiatry. The emergence of psychotropes produced a decisive impulse. Chemical theory was thus able to propose a wealth of considerable interpretations concerning the modes of action of such substances. Psychotropes would, at the same time, become new research tools. One thus tried to extrapolate mechanisms of neurological (Parkinson disease, epilepsies) or even psychiatric diseases (depressions, psychoses). At this stage, the contributions of the Swedish schools were of major importance. In the 1960s, the discovery of new central substances as putative neurotransmitters (serotonin, dopamine, amino acids, and peptides) and the isolation of their receptors, widened the field of neurotransmission and developed the concept. Likewise, the progress of histochemistry allowed the blue print of the first chemical ways to superimpose itself more or less on the nerve ways. The central pathways thus realistically opened up for functional neurochemistry.

Uncited references

[19] [30] [31]

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