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On the quantal mechanism of neural transmitter release

Nobel Lecture, December 12, 1970

I have been asked on more than one occasion to explain the common denominator between the three of us who are sharing this year's award in physiology or medicine. I think the answer is quite simple: the work of all three has a single source, namely the "discoveries relating to chemica1 transmission of nerve impulses" for which Henry Dale and Otto Loewi received a previous award in 1936. Dale and his colleagues, W. Feldberg, Marthe Vogt and G. L. Brown, had shown that in spite of the rapid and unfailing nature of neuromuscular transmission, the motor nerve impulse is not simply passed on to the muscle fibre, by a continuous process of electric excitation, but that there is intervention of a chemical mediator, involving the release from the nerve and the subsequent action on the muscle, of a specific transmitter substance, acetylcholine. This concept is summarized in the following scheme

$$\overset{\text{I}}{\text{N}} \rightarrow \text{ACh} \overset{\text{II}}{\rightarrow} \text{M}$$

It was only to be expected that on closer examination this intermediate process would resolve itself into a sequence of reactions made up of a number of discrete steps, each of which calls for experimental study. What I should like to do in this lecture is to deal briefly with certain advances that have been made during the last 20 years in the investigation of the first stage of the transmission process, namely the mechanism by which arrival of an impulse enables the motor nerve ending to release the transmitter substance. I shall concentrate on studies made by a micro-electrophysiological approach and shall refer in particular to the work carried out together with my colleagues Paul Fatt, José del Castillo and Ricardo Miledi with all of whom I had the privilege to collaborate (for refs. see refs. 1-4).

It had been known for many years[5,6] that the end-plate region of the muscle fibre, that is the surface area contacted by the motor nerve, serves as a sensitive chemo-detector for a variety of cholinesters and especially for acetylcholine. When acetylcholine is applied in small amounts to the outer surface, it opens

up ionic channels in the membrane through which ambient cations can pass[7]. This allows sufficient current flow to produce a measurable discharge, or local "depolarization" *(i.e.* lowering of the normal membrane potential) of the muscle fibre. The end-plate surface of the muscle acts, in effect, as a chemo-electric transducer which enables us to register impacts of small quantities of acetylcholine in the form of local potential changes.

Normally, the never impulse liberates an amount of acetylcholine which is sufficient to produce a very large local depolarization, often of more than 50 mV, the so-called end-plate potential. This rises quickly above the <firing threshold> of the muscle fibre and so initiates a new propagating wave of membrane excitation.

Some 20 years ago, using the method of intracellular recording, Paul Fatt and I came across something quite unexpected. In the absence of any form of stimulation, the end-plate region of the muscle fibre is not completely at rest, but displays electric activity in the form of discrete, randomly recurring <miniature> end-plate potentials. Each is only of the order of 0.5 mV in amplitude, but in other respects resembles the much larger end-plate potential evoked by the nerve impulse: it shows the same sharp rise and slow decay, and has the character of a discrete all-or-none phenomenon though on a much smaller amplitude scale (see Fig. 1).

Numerous experiments have shown that each miniature end-plate potential arises from the synchronous impact of a large multi-molecular quantum of acetylcholine spontaneously discharged by the adjacent nerve terminal. Each event is highly localized and involves only a very small portion of the synaptic axon surface; successive discharges form a random sequence in temporal as well as in spatial distribution along the motor nerve ending.

One of the unanswered questions concerns the precise number of acetylcholine molecules which make up each quanta1 unit of discharge. This is still uncertain; on present estimates one may assume that at least a thousand molecules are contained within an elementary packet, possibly many more. As an upper limit R. Miledi[8] gave a figure of 10^5 molecules; this was based on the minimum quantity of applied acetylcholine which was needed to produce an equivalent effect.

One of the difficulties in answering this question is that neither the chemosensitivity of the end-plate nor the resolving power of our recording system is high enough to enable us to detect the effects of individual or of pauci-molecular reactions of acetylcholine directly. An indirect approach was chosen quite recently by R. Miledi and me[9]. We found that a steady dose of acetyl-

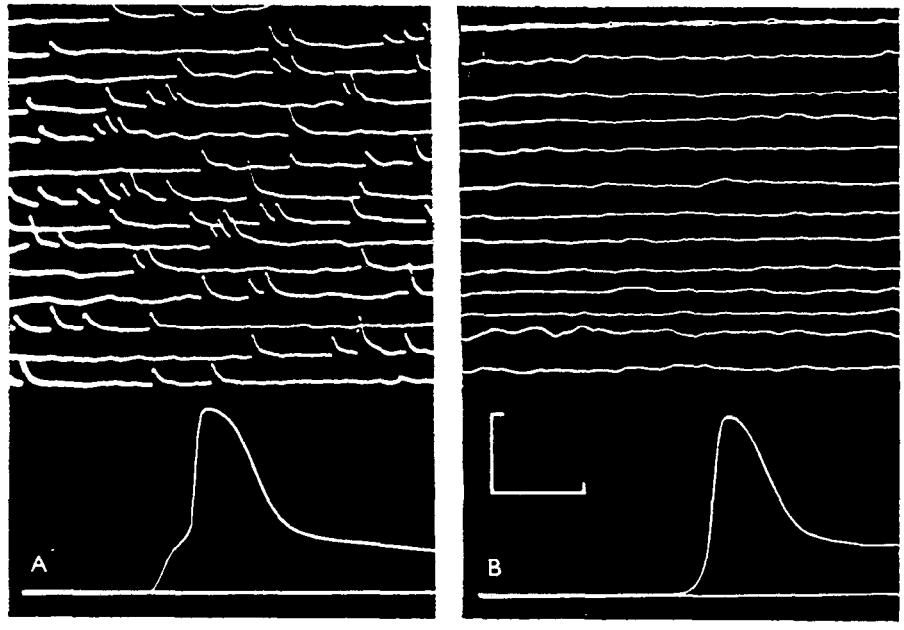

Fig. 1. Spontaneous "miniature end-plate potentials". (From ref. 1) A: intracellular recording at an end-plate. B: recorded 2 mm away in same muscle fibre. Upper portions were recorded at low speed and high amplification (calibrations 3.6 mV and 46 msec): they show the localized spontaneous activity at the junctional region. Lower records show the electric response to a nerve impulse, taken at high speed and lower gain (calibrations 50 mV and 2 msec). The stimulus was applied to the nerve at the beginning of the trace; response A shows step-like end-plate potential leading to a propagating muscle spike; in B, the spike alone is recorded after additional delay due to conduction along 2 mm of muscle fibre. In all figures, unless otherwise stated, upward deflexion means positive-going potential change at the micro-electrode.

choline produces not only a measurable depolarization, but also a measurable excess of voltage noise in the end-plate zone. These experiments are still in progress; a preliminary analysis indicates that the elementary "shot effects" which underlie, and statistically build up, the steady acetylcholine-potential amount to a fraction of a microvolt in amplitude. This elementary voltage change is presumably due to the transient opening of an ionic channel in the muscle membrane, by the action of one or several acetylcholine molecules. The miniature potential is a thousand times larger and would thus require the opening of one or a few thousand "ionic gates", but this figure still leaves us in doubt about the number of acetylcholine molecules which are discharged in a single quanta1 unit from the nerve terminal.

The discovery that acetylcholine is spontaneously released from nerve endings in the form of large multi-molecular packets acquired further significance as a result of the following findings.

(i) The presence of miniature potentials is not peculiar to the neuromuscular junction nor is it confined to cholinergic systems, but has been found to occur at diverse kind of synapses, in the peripheral and central nervous system, and throughout the animal kingdom. It appears to be a characteristic property of many, maybe of all, those neuronal and neuro-effector junctions at which chemical mediation occurs.

(ii) An important structural correlate has been found by De Robertis and Bennett[10] and by Palade and Palay[11] in the synaptic vesicles which form a distinct population of intra-axonal organelles clustered together near the presynaptic release sites. Recent biochemical studies, especially on the electric organ of Torpedo[12] have shown that the major part of the acetylcholine stores of a cholinergic axon is parcelled up within these vesicular organelles. Electron-microscopic studies on the same tissue, using the freeze-etching technique[13] have revealed many instances in which such vesicles are found to be attached to the presynaptic axon membrane and to have opened into the synaptic cleft.

(iii) One of the most important findings was that the frequency of the miniature end-plate potentials, that is the rate of acetylcholine secretion, is controlled by the membrane potential of the axon terminals: depolarization of the presynaptic membrane causes the rate of the discharge to increase in a graded manner without change in the size of the individual <blips> (ref. 14, see Fig. 2). This is, in fact, what occurs after the arrival of an action potential : after a short delay the frequency of miniature end-plate potentials rises by several orders of magnitude for a brief period and then rapidly falls again towards the resting level[15]. The result is a very large synchronous end-plate potential which exceeds the <firing threshold> of the muscle fibre.

It has been well establishedthat the normal end-plate potential is made up of a statistical fusion of quantal components which are identical with the spontaneously occurring units. In effect, the nerve impulse does not start up a new secretory process, but it facilitates or, in statistical terms, raises the probability of events that occur all the time at a low rate, so that - instead of an average of one packet per second - we obtain a few hundred packets of trans--mitter substance released within a millisecond.

How does the nerve impulse, or more generally, how does a depolarization raise the probability of occurrence of this quanta1 event? The membrane po-

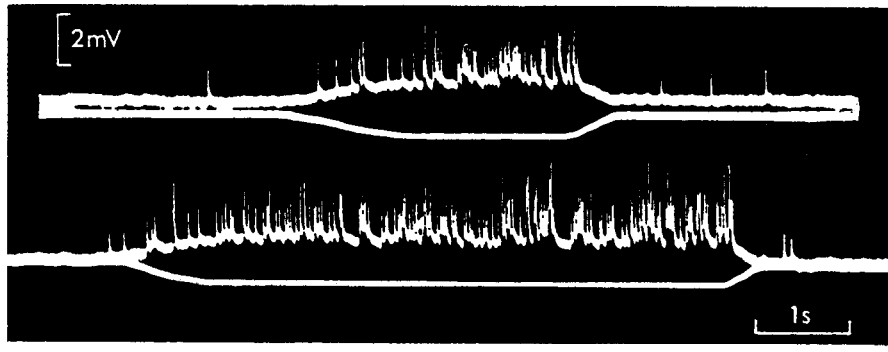

Fig. 2. Electrical control of the frequency of min.e.p.p.'s (cf. ref. 14). In each pair of traces, the upper shows miniature potentials, the lower indicatescurrent flowing through the terminal part of the motor axon. The cathode was placed near the junction so as to depolarize the nerve endings. This caused the frequency of the discharge to increase dramatically.

tential change alone is not sufficient. The presence of calcium ions in the external medium is required to make depolarization effective (see Fig. 3). If one progressively reduces the calcium concentration, or adds a <competitive> ion such as magnesium or manganese in increasing amounts, depolarization becomes less and less capable of accelerating the discharge rate of miniature potentials above their resting frequency. Over the last six years, experiments by Miledi and myself have led us to conclude that external calcium is the only immediate ionic requirement for depolarization to evoke transmitter release[16-18].

On our present evidence, the sequence of events may be described as follows: depolarization opens specific <calcium gates> in the-terminal axon membrane-this leads to an influx of calcium ions (provided the membrane potential has not been displaced excessively, *i.e.* to or beyond the calcium equilibrium level, see ref. 19). Having reached the internal surface of the axon membrane, calcium ions then initiate the "quanta1 release reaction". Up to this point of the argument we are on reasonably firm ground established by electrophysiological experiments. Beyond this point, I must draw on converging observations from ultrastructural and biochemical studies, all of which go to make up a plausible and, I think, very strong hypothesis, namely that the quanta of transmitter molecules are enclosed within synaptic vesicles which undergo frequent transient collisions with the axon membrane, that calcium brings about attachment and local fusion between vesicular and axon membranes, and that this is followed by all-or-none discharge of the vesicu-

lar content into the synaptic cleft. To students of neurosecretory process the postulate of <evacuation> of vesicles or of dense-cored granules from the cell

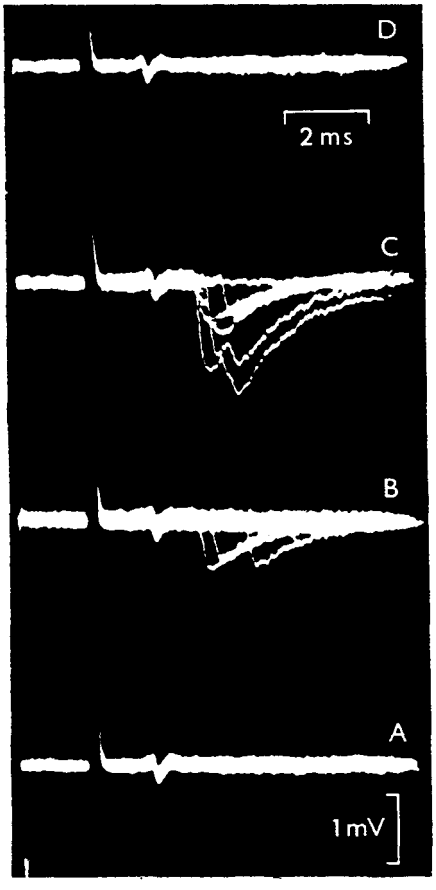

Fig. 3. Exploration of the nerve-muscle junction with a "calcium micropipette". (From ref. 29.) A frog sartorius was immersed in Ca-free solution containing 0.84 mM Mg. A micropipette filled with 0.5 *M* $CaCl_2$ was used to record focal external potentials from a localized junctional spot. Efflux of Ca was controlled electrophoretically. In the 4 records, A to D (each obtained by superimposing six traces), Ca-efflux was stopped initially by applying sufficient negative voltage to the pipette (in A); the bias was then reduced in two steps (in B and C) allowing increasing amounts of Ca to escape; finally the negative bias was re-applied (in D) to stop Ca-efflux again. In A (no Ca), each stimulus is followed at constant interval by a small spike in the nerve terminal, but there is no transmitter release. In B (little Ca), single transmitter quanta were released, after variable "synaptic delay", in 3 out of the 6 trials. In C (more Ca), single or multiple transmitter quanta were now released in 5 out of 6 trials, while in D (no Ca) transmitter release was stopped again.

surface is a familiar and well- documented event; in present- day terminology it is usually described as <exocytosis>.

If I am asked how all this research, which is developing rapidly along with biochemical, physiological and cytological micro-techniques, is going to influence our understanding of the operation of the central nervous system and of its functional defects, I am on much more tenuous ground. There is some evidence that in certain peripheral neuromuscular syndromes the quantal release efficacy of the motor impulse is impaired[20], resembling somewhat the experimental condition of low Ca/high Mg, while in other forms of myoneural disease a <packaging failure>, *i.e.* insufficient accumulation of transmitter by individual vesicles, has been suggested[21]. It would clearly be of interest to pursue this line and to find out whether there is similarly localized involvement in some central-nervous lesions.

Finally, there is the more general question, whether the statistical fluctuations and <uncertainties> which are inevitably associated with the quantal nature of transmitter release, play any recognizable role in the organized function of the nervous system. That there are large quanta1 fluctuations in the response of unitary synapses in the central nervous system, has been shown very clearly by Kuno[22] and others. In many instances, the number of packets released by an impulse impinging on a spinal motoneurone was found to be small, and the predictability of the synaptic response appeared to follow the statistical rules of Poisson's law. In other instances, the fluctuations were much smaller, indicating a greater efficacy of the afferent impulse, so that either a large average number ofpackets was being discharged from the terminal[23-25], or a small number was being released with high probability. One would presume that in a <fully trained> neuronal pathway, quantal fluctuations become unimportant because of simultaneous involvement of a large population of synaptic transfer sites. The larger the average number, the smoother and more predictable becomes the synaptic performance. However, large numbers and smooth performance may not be the rule at *all* times and in *all* pathways. Experiments on the neuromuscular junction have shown that certain processes of synaptic modification during and after prolonged activity, are associated with quanta1 recruitment, that is with an increase in number of packages delivered per impulse[26-28]. Similar changes would be expected to occur, and make synaptic performance more predictable, during development and <training>, while the opposite trend might underlie some forms of pathological and degenerative impairment.

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