# ELECTROPHYSIOLOGICAL EVALUATION OF TRANSMITTER SECRETION AND RESERVES AT A SINGLE RELEASE POINT OF THE FROG NEUROMUSCULAR SYNAPSE

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Transmitter release in the neuromuscular synapse takes place in definite portions or quanta [6]. In the nerve ending, transmitter exists in the form of several pools. Rhythmic activity of the neuromuscular synapse leads initially to utilization of the transmitter reserves available for release, which are replenished from the mobilization reserves [3, 7]. Electrophysiological evaluation of these transmitter fractions in the nerve ending gave the following values: reserves of accessible transmitter about 1000 quanta, mobilization reserves 50,000 quanta [4].

Electrophysiological and morphological studies of synapses suggest that transmitter is not secreted over the whole surface of the nerve ending, but only at specialized sites (release points), located some distance apart [11].

Transmitter secretion from a nerve ending is known to be well described by binomial statistics [2, 8]. The number of quanta of acetylcholine released in response to the first impulse depends on the parameters n and P, where n is the number of release points on the presynaptic membrane and P the probability of transmitter secretion in a release point. Most of the data on transmitter secretion and storage in the neuromuscular synapse have been obtained by intracellular recording, when activity of the whole synapse is recorded.

This paper gives the results of an electrophysiological study of secretion and turnover of transmitters in a single release point.

## EXPERIMENTAL METHOD

Experiments were carried out on neuromuscular preparations of the sciatic nerve and sartorius muscle of *Rana ridibunda*. During the experiment the preparation was placed in a bath with continuously flowing Ringer's solution. Activity of a region of the nerve ending was recorded extracellularly by means of high-impedance (10-20 MΩ) glass microelectrodes filled with 0.5 M $CaCl_2$ solution. A battery, from which a steady potential could be applied to the microelectrode, thereby enabling controllable outflow of $Ca^{++}$ from the microelectrode, was included in the circuit of the recording electrode. In that case the electrode served simultaneously for recording biopotentials and for controllable iontophoresis of $Ca^{++}$ to a region of the nerve ending [9]. Endplate potentials (EPPs) arising in response to stimulation of the motor nerve, and miniature EPPs (MEPPs) and action potentials of the nerve endings were recorded extracellularly. All experiments with extracellular recording were performed in calcium-free Ringer's solution with very low (under 0.3) quantal abundance (m) of EPP.

The motor nerve was stimulated electrically by square pulses 0.3-0.5 msec in duration. Postsynaptic potentials were recorded from the oscilloscope screen, using an FOR-2 camera attachment.

At the beginning of the experiment the motor nerve was stimulated at a frequency of 0.1-0.5 Hz for 5-20 min; 100-300 EPPs were recorded and the initial level of secretion determined from these results. After a pause of 5-10 min the nerve was stimulated with a frequency of 5-10 Hz for 5 min and postsynaptic responses were recorded continuously during the first 3 min and for 30 sec every subsequent minute. The number of quanta of mediator released in response to every 50 stimulations of the motor nerve throughout the period of stimulation was determined

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by analysis of these traces. The binomial parameters m, P, and n were determined by known equations [3, 8]. The experimental results were subjected to statistical analysis.

## EXPERIMENTAL RESULTS

Depending on the diameter of the microelectrode tip (judged from the impedance of the electrode) two types of responses could be observed. If the impedance of the electrode was 0.5-3 MΩ (tip of large diameter) the distribution of amplitudes of single-quantum EPPs was asymmetrical, with a shift of the mode of the distribution toward low values (Fig. 1A). Usually two populations of signals (of low and high amplitude), forming two independent peaks, could be seen to the side of the distribution histogram. Calculation of the binomial parameters of transmitter secretion under these conditions gave values of the parameter n from 1.5 to 4.0. In this case, EPPs of high amplitude may be considered to reflect transmitter secretion at the release point beneath the recording electrode, whereas EPPs of low amplitude reflect secretion at points distant from the electrode [1]. Consequently, under these conditions activity of a considerable area of the nerve ending, including several release points, was recorded. This conclusion was confirmed by values of the binomial parameter n, which were considerably higher than 1.

When high-impedance electrodes (10-20 MΩ; tip of small diameter) were used the histograms of amplitudes of single-quantum EPPs had as a rule only one peak (Fig. 1B), i.e., they consisted of one population of high-amplitude signals. In this case values of the binomial parameter n varied from 1.0 to 1.5. All this indicates that when high-impedance microelectrodes were used activity of a single release point, located immediately beneath the recording electrode, was recorded [1].

This paper describes experimental results obtained only by the use of high-impedance extracellular electrodes. Experiments in which a polymodal distribution of EPP amplitudes was observed and in which values of the parameter n exceeded 1.5 were disregarded.

Stimulation of the motor nerve with a frequency of 5-10 Hz led to an initial increase and subsequent decrease in transmitter secretion. At very low initial values of m (under 0.1) stimulation was accompanied by a slow increase in transmitter release for 1-2 min. Transmitter secretion then fell steadily (Fig. 2A). With higher initial levels of secretion (m 0.2-0.3) there was a sharp rise (in the course of 10-30 sec) in release followed by a quite rapid decrease. After 2-3 min transmitter release became stabilized (the "plateau" state) at the level of 0.2-1.0 quantum/sec (curve 2B). The decline of transmitter release during repetitive stimulation was accompanied by a decrease in the binomial parameter P, with no significant changes in the parameter n. After stimulation had ceased, slow recovery of transmitter secretion to the initial level (in the course of 10-20 min) was observed.

Hemicholinium-3 (a specific blocker of acetylcholine synthesis) [10], in a concentration of $(1\text{-}2)\cdot10^{-5}$ g/ml, did not change the dynamics of the initial decline in transmitter release during repetitive stimulation. After 3-4 min of stimulation, transmitter secretion ceased completely (Fig. 2C) and did not return to its initial level after cessation of stimulation.

The rapid decrease in transmitter secretion lasting several tens of seconds is a process of utilization of transmitter available for release [7]. Replenishment of the reserves of accessible mediator takes place from the mobilization reserves, which also become exhausted during repetitive stimulation. Hence the slow decrease in mediator release following the rapid decline reflects exhaustion of the mobilization reserves. Stabilization of transmitter secretion after stimulation for 2-3 min ("plateau") is evidence that the rates of replenishment and loss of mediator during this time were equal. Disappearance of the "plateau" state under the influence of hemicholinium-3 suggests that the "plateau" reflects the rate of acetylcholine synthesis. Transmitter reserves were estimated by the method in [7]. The number of quanta released during 50 stimulations of the motor nerve was plotted as a function of the total number of quanta secreted (Fig. 3). A straight line was drawn through the first two or three points, and its intersection with the horizontal axis gave the value of the reserves of accessible mediator. A second straight line was drawn through points 4-10, and intersection of this line with the abscissa gave the total of accessible and mobilization reserves. By subtracting the accessible transmitter reserves from the total, the mobilization reserves could be calculated. The rate of synthesis was determined by counting the number of responses during stimulation for 1 sec in the "plateau" state. According to the results of seven experiments, reserves of accessible transmitter varied from 32 to 47 quanta, with a mean value of 40.0 ± 2.4 quanta, and the mobilization reserves amounted to 228.6 ± 32.3 quanta (120-320

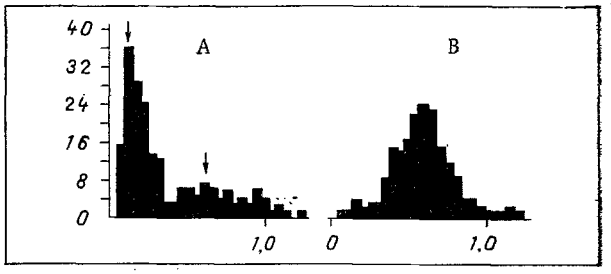


Fig. 1. Histograms of distribution of amplitudes of single-quantum EPPs. Abscissa, amplitude of EPP (in mV); ordinate, number of observations. A, B) Recordings by electrode with impedance of 1 and 15 MΩ, respectively, from one neuromuscular synapse. Frequency of stimulation 10 Hz. Arrows indicate two populations of EPPs.

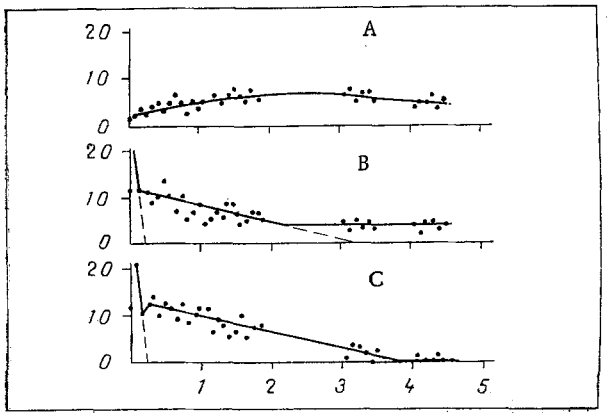


Fig. 2

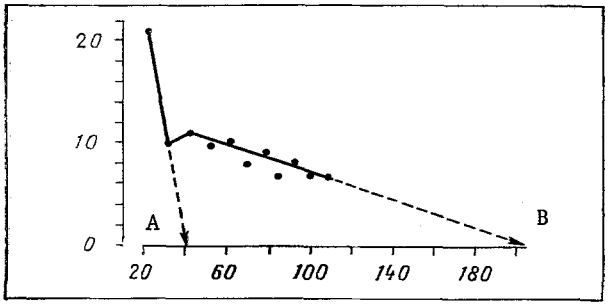


Fig. 3

Fig. 2. Time course of transmitter secretion during repetitive stimulation. Abscissa, duration of stimulation (in min); ordinate, number of quanta of transmitter secreted in response to 50 stimulations. A) Low initial level of secretion (m = 0.06), B) higher initial level of secretion (m = 0.24), C) action of hemicholinium-3 in concentration of $2 \cdot 10^{-5}$ g/ml for 1 h. Frequency of stimulation 10 Hz. Broken lines indicate fast and slow decay of mediator release. Circles on vertical axis show initial level of secretion at frequency of stimulation of 0.5 Hz.

Fig. 3. Determination of parameters of transmitter turnover. Abscissa, total sum of secreted quanta; ordinate, number of quanta released during 50 stimulations. Broken lines — extrapolation of fast and slow decay. Arrow A indicates reserves of accessible mediator, arrow B denotes total of accessible and mobilization reserves. Frequency of stimulation 10 Hz. Results of a single experiment..

quanta). The rate of synthesis of transmitter varied from 0.3 to 1.0 quanta/sec (mean 0.60 ± 0.03 quanta/sec).

Recent electron-microscopic investigations have shown that the morphological correlate of the release point is the "active zone" of the nerve ending [5]. The active zone lies across the nerve ending and is a thickening of the presynaptic membrane. Synaptic vesicles, each of which contains a quantum of transmitter, are arranged in two rows along the edge of the active zone. The number of vesicles in the active zone (30-50 [4]) coincides with the reserves of accessible mediator determined in the present experiments (32-47). Hence, it canbe postulated that the reserves of accessible mediator are contained in vesicles in the immediate vicinity of the active zone.

Binomial statistics shows that only one quantum of transmitter is released from one release point. Evidently, all synaptic vesicles in the active zone are about equally capable of exocytosis, but the critical (necessary for release) $Ca^{++}$ concentration is created only in one vesicle. The possibility likewise cannot be ruled out that release of a single quantum blocks the release of the other quanta from this point [3]. Within the limits of this model the probability of release of a quantum of transmitter must depend on the number of vesicles and the $Ca^{++}$ concentration at the release point.

Replenishment of the reserves of accessible transmitter at the release point takes place from the mobilization reserves. Calculations showed that the mobilization reserves consist of 120-320 quanta. It can be tentatively suggested that these quanta are located in vesicles at a distance from the release point, and are transported into the strategic area of the active zone when the reserves of accessible mediator are used up. Replenishment of the mobilization reserves takes place on account of acetylcholine synthesis.

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