STATISTICAL ANALYSIS OF THE FLOW STRUCTURE OF MINIATURE END-PLATE POTENTIALS UNDER THE INFLUENCE OF FACTORS AFFECTING TRANSMITTER RELEASE

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Analysis of the spike flow structure of miniature end-plate potentials (MEPP), reflecting spontaneous secretion of transmitter in the neuromuscular junction [7, 9], could contribute to our understanding of the mechanisms of transmitter release (exocytosis) through the presynaptic membrane. On the basis of the quantum-vesicular hypothesis and assuming a sufficiently chaotic character of movement of the vesicles in the cytoplasm and the random character of their interaction with the presynaptic membrane, it can be postulated that deviations of the MEPP flow from the Poisson character may be evidence of some degree of organization of the interaction sites (transmitter release).

The object of this investigation was to analyze the time flow of MEPP and assess the distribution of MEPP amplitudes in order to obtain evidence of the character of release, the course of which was deliberately modified by the action of ouabain, an inhibitor of Na, K-ATPase, which produces an increase in the frequency of MEPP through the abolition of electrogenic cation transport [3], of tetanus toxin which blocks transmitter exocytosis through the presynaptic membrane and thus reduces the frequency of MEPP [1], and of 4-aminopyridine, which inhibits the transmembrane flow of potassium ions and changes the character of release of the synaptic transmitter [2].

EXPERIMENTAL METHOD

Tracings of MEPP were obtained by intracellular recording of synaptic activity in an isolated preparation of the phrenic nerve and a strip of diaphragm muscle (temperature 35°C). Ouabain and 4-aminopyridine were added to the external solution in concentrations of $1 \cdot 10^{-4}$M and tetanus toxin ($2 \cdot 10^4$ MLD for mice) was injected into the diaphragm 3 h before isolation of the preparation.

A statistical study was made of deviations of the time flow of MEPP from the Poisson distribution. For this purpose first-order histograms of interspike intervals (ISI) were plotted. The hypothesis of an exponential distribution of ISI was tested by the chi-square criterion of goodness of fit. The expected number of events falling in the i-th bin of the histogram, with width Δt, is:

$$n_i = N\left(\exp\left(-\frac{(i-1)\,\Delta t}{M[x]}\right) - \exp\left(-\frac{i\Delta t}{M[x]}\right)\right),\ i = 1, \ldots, k,$$

where k is the number of histogram bins, N the total number of intervals, M[x] the mean interval. The number of degrees of freedom of the chi-square distribution is $k - 2$.

To determine the precise form of deviation of the ISI distribution from exponential, the value of Sherman's statistic [5] was calculated:

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$$\omega_N = \sum_{i=1}^{N} \frac{|X_i - M[x]|}{2(N+1)M[x]},$$

where X_i (i = 1, ..., N) is the ISI sequence.

According to the null hypothesis of exponentiality of the ISI distribution [11] $M[\omega_N] = 1/e \approx 0.368$,

$$D[\omega_N] = \frac{0,05908}{N} - \frac{0,07145}{N^2} + 0(1/N^3).$$

When $\omega_N < M[\omega_N]$ the empirical series contain an excess of intervals of close to the mean lengths but a deficiency of those considerably greater or much shorter than the mean. When $\omega_N > M[\omega_N]$ the empirical series contains an excess of both long and short intervals. In the first case, the distribution is called "concentrated," in the second case "diffuse" [12]. To determine the precise form of the distribution the logarithm of the reliability function is constructed. The reliability function:

$$R(x) = 1 - F(x),$$

where F(x) is the distribution function, for an exponential distribution has the form $\exp\left(-\frac{t}{M[x]}\right)$, and its logarithm $\ln R(x) = -\frac{t}{M[x]}$. The shape of the curve of the logarithm of the reliability function is known for various types of distributions generated by recovery processes, etc. [4, 5].

To analyze the amplitude distribution of MEPP, histograms of amplitudes were plotted and means and standard deviations were calculated. Conformity to the normal distribution was assessed. Two characteristics were chosen as criteria; the coefficient of asymmetry and the coefficient of excess.

EXPERIMENTAL RESULTS

Steadiness of the MEPP flows was assessed. The null hypothesis concerning steadiness of ISI distribution was tested, the competing hypothesis being the presence of a trend of the type $\lambda(t) = e^{\alpha + \beta t}$, where $\lambda(t)$ is the intensity of the Poisson flow. In that case, the value:

$$U = \frac{\sum t_i/(N-1) - t_0/2}{t_0\sqrt{1/12(N-1)}},$$

where t_i denotes moments of appearance of events, t_0 the total time of observation, N the total number of events, is distributed normally with zero mean and unitary dispersion [5]. For the samples tested, assessment of the value U is distributed approximately normally if the null hypothesis indicates absence of a trend. Assessments of U agree sufficiently well with the null hypothesis at a 5% level of significance.

In the absence of pharmacologic intervention, testing conformity of the ISI distribution (Fig. 1a) to exponential revealed a significant difference between these distributions, with a level of significance $\alpha < 0.05$. The size of the sample was N = 708, the mean interval $\bar{x} = 0.39$ sec, the standard deviation $\sigma = 0.39$ sec, and the mean frequency $\lambda = 2.56$ sec^{-1}. The curve of the logarithm of the reliability function was concave in its initial parts and its appearance changed sharply at approximately $x_i = 1.8$ sec, evidently indicating a mixed type of ISI distribution. The appearance of the curve of the logarithm of the reliability function, incidentally, corresponded neither to the appearance of the ln R(x) curve of a branched Poisson process, which has been suggested as the process of transmitter release [12], nor to that of the ln R(x) curve for the model of periodic transmitter release [8, 12].

Three series of ISI were investigated during the action of ouabain: 1) N = 361, $\bar{x}$ = 30.42 msec, σ = 30.79 msec λ = 22.87 sec^{-1}; 2) N = 382, $\bar{X}$ = 32.40 msec, σ = 32.31 msec, λ = 30.86 sec^{-1}; 3) N = 499, $\bar{X}$ = 24.20 msec σ = 22.73 msec, λ = 41.32 sec^{-1}. For samples 2 and 3, deviation of the ISI distribution (Fig. 1B) from exponential was significant ($\alpha < 0.05$). A low value of Sherman's statistic ω_N was obtained, compared with $M[\omega_N]$, with a level of significance of $\alpha < 0.01$ for three samples, evidence of the "concentration" of the ISI distribution. The curve of the logarithm of the reliability function is concave, similar to the shape of the initial part of the ln R curve in the absence of pharmacologic intervention.

4-Aminopyridine. N = 280, $\bar{X}$ = 0.61 sec, σ = 0.64 sec, λ = 1.64 sec^{-1}. The hypothesis of the exponential character of the ISI distribution (Fig. 1C) also is rejected with a level of significance of $\alpha < 0.05$. The shape of the curve of the logarithm of the reliability function indicates a change in ISI distribution. The initial linear region of the ln R curve suggests an exponential distribution of short and average intervals, and the terminal part of the ln R curve is convex.

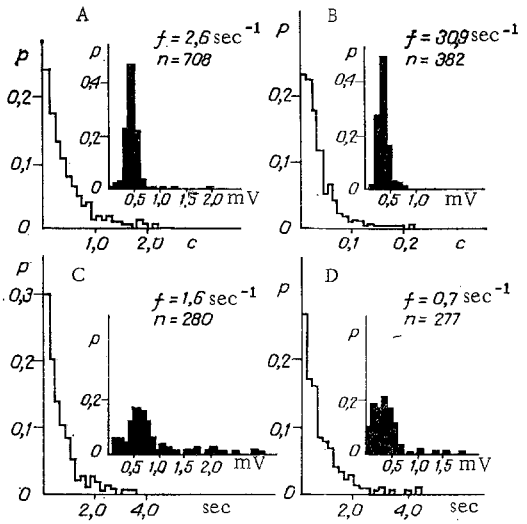

Fig. 1. Changes in character of distribution of intervals and amplitudes of MEPP in single muscle fibers under various influences. Frequency histograms of amplitudes (black columns) and intervals (unshaded columns) for normal state (A), ouabain (B), 4-aminopyridine (C) and tetanus toxin (D). Abscissa, amplitude or duration of interval; ordinate, probability of appearance of amplitudes or intervals of corresponding magnitude, rat phrenic nerve–diaphragm preparation, 35°C. f) Frequency of MEPP; n) number of MEPP.

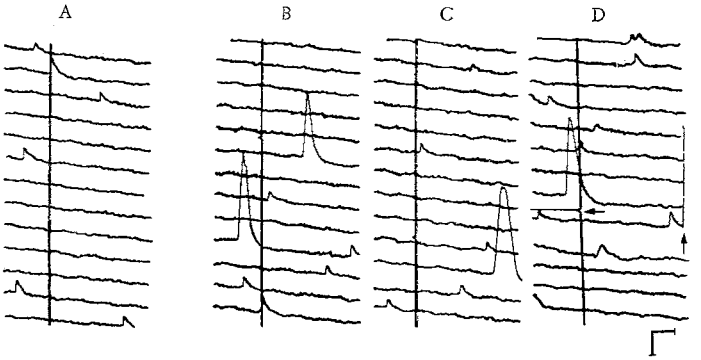

Fig. 2. Change in character of spontaneous synaptic activity under the influence of 4-aminopyridine. A) Before, B, C, and D) 100 sec after addition of 4-aminopyridine (1×10^{-4} M) to Tyrode solution. Longitudinal tracing represents transmembrane potential, transverse represents alternating component (MEPP) with high amplification. Calibration below, for longitudinal tracing: amplitude 40 mV, time 100 msec; for transverse tracing: amplitude 1 mV, time 10 msec. Arrow indicates appearance of spontaneous action potential. Rat phrenic nerve–diaphragm preparation, 35°C.

Tetanus Toxin. $N = 277$, $\bar{X} = 1.41$ sec, $\sigma = 1.49$ sec, $\lambda = 0.71$ sec^{-1}. The hypothesis of the exponential character of the ISI distribution (Fig. 1D) is rejected with a level of significance $\alpha < 0.05$. The curve of the logarithm of the reliability function has an initial linear and final convex region.

Investigation of statistical independence of the lengths of ISI by constructing serial correlograms showed that the intervals are not statistically independent, but the character of this dependence is not clear.

The distribution of amplitudes of MEPP under normal conditions and during all forms of pharmacologic intervention has a positive coefficient of asymmetry, significantly different from zero ($\alpha < 0.01$). Normally, (Fig. 1A), and under the influence of ouabain (Fig. 1B), the coefficient of excess is positive ($\alpha < 0.01$), i.e., the distribution has a more pointed peak than the Gaussian, evidence of a larger number of near-average amplitudes. Normally "giant" MEPP are very rarely observed. Under the influence of tetanus toxin (Fig. 1D), the standard deviation was approximately twice the normal value and the coefficient of excess was negative and significantly different from zero ($\alpha < 0.01$), i.e., the distribution has a flatter peak than the normal distribution. "Giant" MEPP are observed. Under the influence of 4-aminopyridine (Fig. 1C), the standard deviation was about five times greater than normally. The distribution of amplitudes has a long "tail" at its right end.

Early attempts at statistical analysis of the MEPP flow revealed its random, close to Poisson, character [5, 6], but more recent studies have shown deviations of the MEPP flow from the simplest type, evidence of the greater orderliness of the process of transmitter release [8]. These facts have led to the concept of periodic liberation of transmitter through a limited number of sites [7, 8], and of priority or "dead" time after each release. It is considered that deviations from the Poisson distribution are due to the grouping of events ("giant" MEPP, "bursts" of spikes) and, ultimately, to the absence of independence between events [10, 12]. These data agree well with the model of a branched Poisson process, where each random primary release of transmitter is followed with a certain probability by additional release from the same site [12].

In the present investigations, as has already been stated, significant deviations of the release process from the Poisson distribution were found in practically all experimental situations. Meanwhile, depending on the character of deviations of the ISI distribution from exponential, the flows can be divided into two groups: 1) normal and ouabain; 2) tetanus toxin and 4-aminopyridine. Normally, in the region of short and average lengths of ISI the number of intervals of approximately equal length is greater than is assumed for the exponential distribution. With an approximately tenfold increase in the intensity of the MEPP flow under the influence of ouabain, this orderliness becomes even more marked in character. It can be tentatively suggested that it reflects a definite organization of the presynaptic membrane. In both cases, deviations in the amplitude of distribution from Gaussian are in the same direction (Fig. 1A, B). However, these deviations do not contradict the hypothesis of the quantum character of release.

The character of distribution of ISI of the second group (tetanus toxin and 4-aminopyridine) is evidence of the more complex structure of the process determining spike activity: Starting out from the assumption of branched processes, this may be a question of modification of the type of branching, and even the presence of two sufficiently independent processes, differing in their organization, cannot be ruled out. These deviations found at the critical point of the process may perhaps help to explain its true structure. In this respect it is interesting to note that it is in this group that more substantial deviations of the amplitude characteristics of MEPP, not in harmony with views on the standard size of the quantum, are found (Fig. 1C, D). They are particularly noticeable in the case of 4-aminopyridine [2], when the polymodal type of distribution curve is well marked, sometimes shifted into the region of lower values, and "giant" MEPP, capable of evoking an action potential, are observed on the tracings (Fig. 2). More recently, the idea has been developed that under normal conditions the standard size of the quantum response (the MEPP) is determined by the synchronous release of a relatively constant number of subquanta of transmitter by the "active zone" of the presynaptic membrane [10, 13]. The effects of tetanus toxin and 4-aminopyridine found in the present experiments agree with this view if it is assumed that the standard character of spontaneous synchronization is disturbed in one respect or other by the action of the agents used.

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