3. P. Andersen, T. V. P. Bliss, and K. K. Skrede, Exp. Brain Res., 13, 208 (1971).
4. R. Dingledine, L. L. Iversen, and E. Breuker, Eur. J. Pharmacol., 47, 19 (1978).
5. T. Duka, M. Wuster, P. Shubert, et al., Brain Res., 205, 181 (1981).
6. T. Dunwiddie, A. Mueller, M. Palmer, et al., Brain Res., 184, 311 (1980).
7. C. Gall, N. Brecha, H. J. Karten, et al., J. Comp. Neurol., 198, 335 (1981).
8. H. L. Haas and R. W. Ryall, J. Physiol. (London), 308, 315 (1980).
9. R. G. Hill, Neurosci. Lett., 21, 217 (1981).
10. J. Sh. Hong and R. Schmid, Brain Res., 205, 415 (1981).
11. R. C. Meibach and S. Maayani, Eur. J. Pharmacol., 68, 175 (1980).
12. A. M. Tielen, F. H. Lopes da Silva, W. J. Mollevanger, et al., Exp. Brain Res., 44, 343 (1981).
13. W. Zeiglgänsberger, E. D. French, G. R. Siggins, et al., Science, 205, 415 (1979).

POLYMODALITY OF DISTRIBUTION OF MINIATURE END-PLATE POTENTIAL AMPLITUDES DUE TO THE ACTION OF SPATIALLY SEPARATE AREAS OF TRANSMITTER RELEASE

A. L. Zefirov

UDC 612.816.3:612.822.1.018

KEY WORDS: miniature end-plate potentials; scatter diagram; histogram of amplitude distribution; polymodality of distribution.

Investigations have shown that during recording of miniature end-plate potentials (MEPP) two populations of signals are found — of low and high amplitude [2, 5]. Low-amplitude signals, accounting for 2-5% of all MEPP, form an independent peak at the beginning of the histogram of distribution, whereas high-amplitude signals constitute the main population of spontaneous potentials and constitute classical MEPP. Peaks, multiples of the mean amplitude of the small MEPP, were found on histograms of high-amplitude signals [6, 8]. These experimental data lay at the basis of the subquantum hypothesis of transmitter secretion in the neuromuscular synapse. According to this hypothesis, small MEPP are the response to liberation of one subquantum of acetylcholine, whereas high-amplitude signals, corresponding to classical MEPP, are formed through synchronous release of a certain number of subquanta [6].

It is also known that a quantum of mediator is released in particular areas of the nerve ending, or release points, which are arranged some distance apart [3, 7]. The membrane of the muscle fiber has a high input resistance, and accordingly the signal arising in response to secretion of a quantum of mediator, as it spreads over the membrane, dies away [4]. In this paper the hypothesis is submitted and proved that peaks on histograms of amplitudes of spontaneous synaptic potentials may be due to the activity of spatially separate areas of transmitter secretion in the nerve ending.

EXPERIMENTAL METHOD

Experiments were carried out on the sartorius muscles of small frogs at room temperature. During the experiment the preparation was fixed in a bath containing continuously flowing Ringer's solution of the following composition (in mM): NaCl 115.0, KCl 2.0, $CaCl_2$ 0.3, $MgCl_2$ 2.0, $NaHCO_3$ 2.4.

To record MEPP two glass microelectrodes filled with 3 M KCl solution, with a resistance of 7-10 MΩ, were used. Under a binocular microscope a myelinated nerve twig running along the surface of the muscle was dissected. Microelectrodes were inserted into a muscle fiber near this nerve twig, 50-100 μ apart. The distance between the electrodes was measured by means of an ocular micrometer. The criterion that the microelectrodes were in the region of a synapse was recording of MEPP with a leading edge about 1 msec in duration.

Spontaneous potentials recorded by two microelectrodes were amplified by means of two amplifiers and recorded from the screen of a two-channel oscilloscope on photographic film by means of an FOR-2 camera. For convenience of recording and measuring, signals from one elec-

Department of Normal Physiology, Kazan' Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 5, pp. 6-8, May, 1983. Original article submitted December 29, 1982.

trode were inverted. After determination of the amplitude of the signals, scatter diagrams were drawn. The amplitude of the signal from the first electrode was plotted along the abscissa, that from the second electrode along the ordinate. The scatter diagrams and histograms of amplitude distribution were plotted on the basis of analysis of 200-400 MEPP.

EXPERIMENTAL RESULTS

Scatter Diagrams of Amplitudes of MEPP Recorded Intracellularly. MEPP recorded simultaneously by two intracellular electrodes in the most characteristic experiments are illustrated in Fig. 1a. The distance between the electrodes was 100 μ. On the scatter diagram of the signals recorded (Fig. 1b) several independent populations of points could be distinguished. One such group of points will be described below as a field. In 15 experiments the number of fields varied from 3 to 8. All fields on the scatter diagram can be divided into three groups (Fig. 1b): 1) fields whose points are grouped around the 45° line, i.e., fields consisting of signals of approximately equal amplitude (field 3); 2) fields lying at the side and, most frequently, at a certain angle to the 45° line (fields 2 and 4), the number of which varied from one to three. They could be symmetrically or asymmetrically arranged relative to the line bisecting the right angle between the axes of coordinates; 3) fields shifted to the side of the low values, toward the origin of the axes of coordinates (fields 1 and 5).

In some experiments, in which the mean amplitude of MEPP was low, as was observed when the values of membrane potential were low and in large muscle fibers, the fields were superposed on each other and it was difficult to distinguish them. Experiments of this kind were not taken into account.

The results can be explained on the basis of the following arguments: 1) Signals recorded by the two electrodes arise in response to transmitter secretion in a certain area of the nerve ending; 2) the amplitude of signals arising in the area of release is not identical but varies around a certain mean value; 3) having arisen at a certain point of the synapse, the signal decays according to an exponential rule with a certain spatial constant. The same signal may therefore differ in amplitude when recorded by the two electrodes.

Within these constraints the fields on the scatter diagram of MEPP amplitudes represent the response of spatially separate areas of release to transmitter secretion. For example, in Fig. 1b field 3 is a release area lying between the recording electrodes, fields 4 and 2 are release areas near the first and second electrodes, respectively, and fields 1 and 5 are distant release areas, far away from the recording electrode. These fields are evidently not separate release points. Transmitter release points in the neuromuscular synapse are known to lie 1-2 μ apart [3, 7]. It may accordingly be supposed that the fields found on MEPP amplitude scatter diagrams reflect the working of a group (accumulation) of release points. On the basis of these data the distance between the transmitter release areas can be estimated approximately. Since we found 2-4 fields in the top part of the diagram, when the distance between the electrodes was 100 μ, the release areas must be arranged with an interval of several tens of microns.

As Fig. 1b shows, fields on the scatter diagram were represented by different numbers of points. Usually fields formed by small numbers of points were located at the origin of the axes of coordinates (fields 1 and 5), whereas fields with many points were located in the top right hand corner of the diagram (fields 2, 3, and 4). It can be concluded from these data that the probability of release of a quantum of mediator is the same in different parts of the nerve ending [1]. Since fields 1 and 5 are the end areas of the neuromuscular synapse, it can be tentatively suggested that the distal areas have less probability of release of a quantum of transmitter.

Histograms of Distribution of MEPP Amplitudes. Histograms of amplitudes of MEPP recorded in the experiment illustrated in Fig. 1 are shown in Fig. 2. Histograms of distribution of MEPP amplitudes were drawn for each field on this scatter diagram. Signals recorded by the first and second electrodes were analyzed separately. The distribution of amplitudes of signals forming a particular field on the scatter diagram was found to be usually monomodal; modal values of signal amplitudes differed in different fields. In some cases distributions of amplitudes of signals from different fields were similar in type and had equal modal values. For example, distributions of MEPP amplitudes in areas 3 and 4 during analysis of signals from the first electrode (Fig. 2a) were for practical purposes indistinguishable.

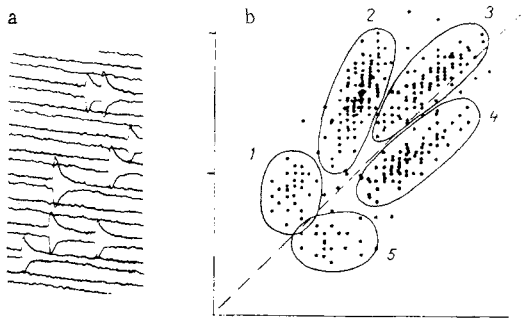

Fig. 1. Recording of MEPP from synaptic region of frog sartorius muscle fibers by means of two intracellular electrodes. a) Traces of MEPP. Signals from one microelectrode were inverted. Distance between electrodes 100 μ. b) Scatter diagram of amplitudes of MEPP (in mV): abscissa, amplitudes of MEPP from first electrode; ordinate, from second electrode. Fields are numbered and outlined. Results of a single experiment.

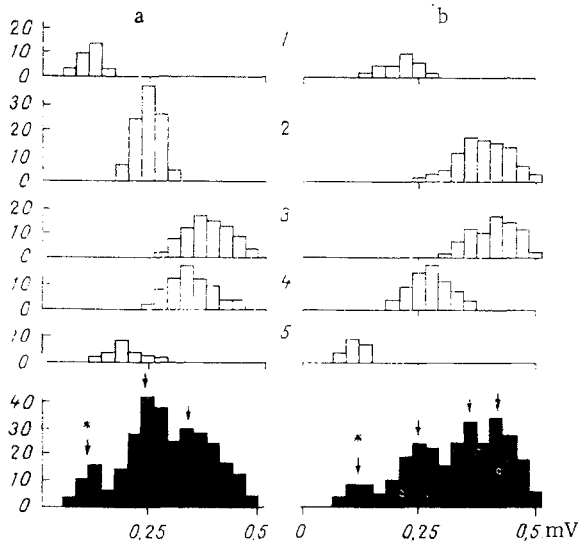

Fig. 2. Histograms of distribution of MEPP amplitude during simultaneous recording by two intracellular electrodes. a) MEPP recorded from first electrode, b) from second electrode. 1-5) **Histograms** of distribution of MEPP amplitudes forming corresponding fields on scatter diagram. Bottom histograms shaded black represent distribution of amplitudes of all MEPP recorded. Peaks on histograms of distribution indicated by arrows. Asterisks denote populations of low-amplitude signals. Abscissa, amplitudes of MEPP (in mV); ordinate, number of observations. The same experiment as in Fig. 1.

The combined histogram of amplitudes of all MEPP recorded by one electrode contains several peaks which coincide in their distribution with modal values of amplitudes of signals from different fields. When modal values of different fields coincided, the number of peaks on the combined histogram was less than the number of fields on the scatter diagram.

The number, size, and arrangement of peaks on the combined histogram of amplitudes of MEPP recorded separately by the first and second microelectrodes differed, and for that reason the shape and polymodality of distribution of MEPP amplitudes when recorded intracellularly depend to a large extent on the position of the electrode in the region of the synapse and the topography of the release areas.

The population of low-amplitude signals forming a separate peak at the beginning of the histogram (Fig. 2) is particularly interesting. This population of signals was formed by fields 1 and 5 on the scatter diagram and was due to activity of distal areas of the nerve ending.

Secretion of quanta of transmitter from spatially separate areas of the nerve ending may thus lead to the appearance of a population of low-amplitude MEPP and of polymodality in the distribution of MEPP amplitudes. The results contradict the subquantum hypothesis of transmitter release in the neuromuscular synapse.

LITERATURE CITED

1. A. L. Zefirov and E. L. Stolov, Neirofiziologiya, 14, 233 (1982).
2. J. D. Cooke and D. M. J. Quastel, J. Physiol. (London), 228, 377 (1973).
3. J. Del Castillo and B. Katz, J. Physiol. (London), 132, 630 (1956).
4. P. Fatt and B. Katz, J. Physiol. (London), 115, 320 (1951).
5. M. E. Kriebel and C. E. Gross, J. Gen. Physiol., 64, 85 (1974).
6. M. E. Kriebel, F. Llados, and D. R. Matteson, J. Physiol. (London), 262, 553 (1976).
7. A. Wernig, Brain Res., 118, 63 (1976).
8. A. Wernig and H. Stirner, Nature, 269, 820 (1977).

EFFECT OF ANIMALS' PRE-EXPERIMENTAL EMOTIONAL STATE ON THE SYSTEMIC HEMODYNAMICS

S. A. Martyuk and V. A. Tsyrlin

UDC 612.13-06:613.863

KEY WORDS: hemodynamics; reflex regulation; emotional stress.

Emotional stress is accompanied by marked hemodynamic changes: elevation of the arterial pressure (BP), an increase in heart rate (HR) and in cardiac output [2, 5, 7]. Prolonged elevation of BP is observed even after brief emotional stress [3]. However, the role of central regulation of the circulation in the mechanism of onset of this hypertension and in neurogenic control of the hemodynamics after termination of emotional stress has not been explained.

The object of this investigation was to compare the initial hemodynamics and reflex changes in the circulation in cats in acute experiments on animals whose emotional state before the experiment differed.

EXPERIMENTAL METHOD

Altogether 70 cats were used. Seventeen cats which, before the experiment, had been in a tranquil state were kept in a cage for 10-15 min, 30 min before the beginning of operative preparation for the experiment, with a dog, precautions being taken to ensure that no direct contact between the animals was possible. Of 53 cats not exposed before the experiment deliberately to emotional stress, 28 animals were described as quiet, domesticated, and the rest as aggressive, malicious, and wild.

Under halothane anesthesia the femoral arteries and veins of all the animals were catheterized, and the left greater splanchnic and renal nerves were isolated extraperitoneally. After the end of dissection, muscle relaxants were injected and the artificial respiration system connected. BP, HR, and electrical activity of one branch of the renal nerve were recorded 60 min after the end of anesthesia, in the initial state and during stimulation of the

Department of Pharmacology, Central Research Laboratory, I. P. Pavlov First Leningrad Medical Institute. Department of Experimental Cardiology, Research Institute of Cardiology, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR P. N. Veselkin.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 5, pp. 8-11, May, 1983. Original article submitted September 23, 1982.

0007-4888/83/9505-0570$07.50