SINGLE NERVE TERMINAL OF THE FROG SATORIUS MUSCLE: ULTRASTRUCTURAL FEATURES AND TRANSMITTER SECRETION

E. N. Bezgina, L. A. Kashanova,
D. A. Moshkov, and A. L. Zefirov

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The neuromuscular synapse of fast frog muscle fibers is known to consist of several thin, unmyelinated terminals, each of which extends on the surface of the muscle fiber for a distance of 100-300 μ. Until recently it was considered that the terminal of intact neuromuscular synapses is a relatively unchanging and homogeneous structure along the whole length of which the characteristics of transmitter secretion are approximately identical. However, data recently were obtained which question the validity of these views. It was found that processes of growth and regression terminals are constantly taking place in synapses, depending of the season and function lobes [13]. It has also been found that the nerve terminal is functionally heterogeneous along its length and had considerable differences in the characteristics of transmitter release and the distribution of ionic channels [1, 3, 5, 11].

The available ultrastructural data suggest that correlation exists between the nonhomogeneity of transmitter secretion and the morphological heterogeneity of the terminal [4, 7-10]. To clear up this matter completely a combined study was made both of the ultrastructure and of the parameters of transmitter release in different parts of a single frog nerve terminal.

EXPERIMENTAL METHOD

Experiments were carried out on the sartorius muscle of pond frogs (*Rana ridibunda*) in the winter period. For morphological study the stretched muscle was fixed in a 2% solution of glutaraldehyde in cacodylate buffer, pH 7.2-7.4, for 2 h, and then postfixed for 1 h in a 1% solution of OsO_4 in the same buffer. From 5 to 10 min after immersion of the tissue in OsO_4, nerve terminals became distinguishable on the superficial muscle fibers under a binocular loupe. At this time wedges of muscle tissue were excised so that an unbranched terminal was located on one of the superficial fibers, and the proximal and distal parts of the terminal faced the wide and narrow ends of the tissue fragment. Serial sections with an identified terminal were glued to an Epon block, from each of which a series (30-50) of ultrathin sections was cut on the LKB-3 ultramicrotome. Three terminals of different lengths were studied: terminal 1 (T1) — 150 μ, terminal 2 (T2) — 180 μ, and terminal 3 (T3) — 200 μ. Negatives for each terminal were analyzed separately under a final magnification of 91,000. The perimeter of the nerve terminal, the extent of the synaptic contact, and the number of synaptic vesicles were determined for each section.

In the electrophysiological experiments spontaneous and evoked postsynaptic potentials were recorded extracellularly by glass microelectrodes filled with NaCl solution (3 M), with a resistance of 5-20 MΩ. The signals were recorded at 5-10 points of the nerve terminal, located 10-20 μ apart, by gradually moving the electrode from the proximal toward the distal portions of the synapse. The criteria of displacement of the electrode were visual observation of the lat myelin segment of the motor axon and muscle fiber on which the nerve ending was situated, and the change in shape of the presynaptic spike arising in response to stimulation of the motor nerve [1, 3]. In the proximal portions of the terminal the presynaptic spike was electrically negative, whereas in the distal portion this was positive. Evoked responses were averaged by means of an automated system based on the D3-28 microcomputer. The quantum composition of the end-plate potentials (EPP) was determined by counting the

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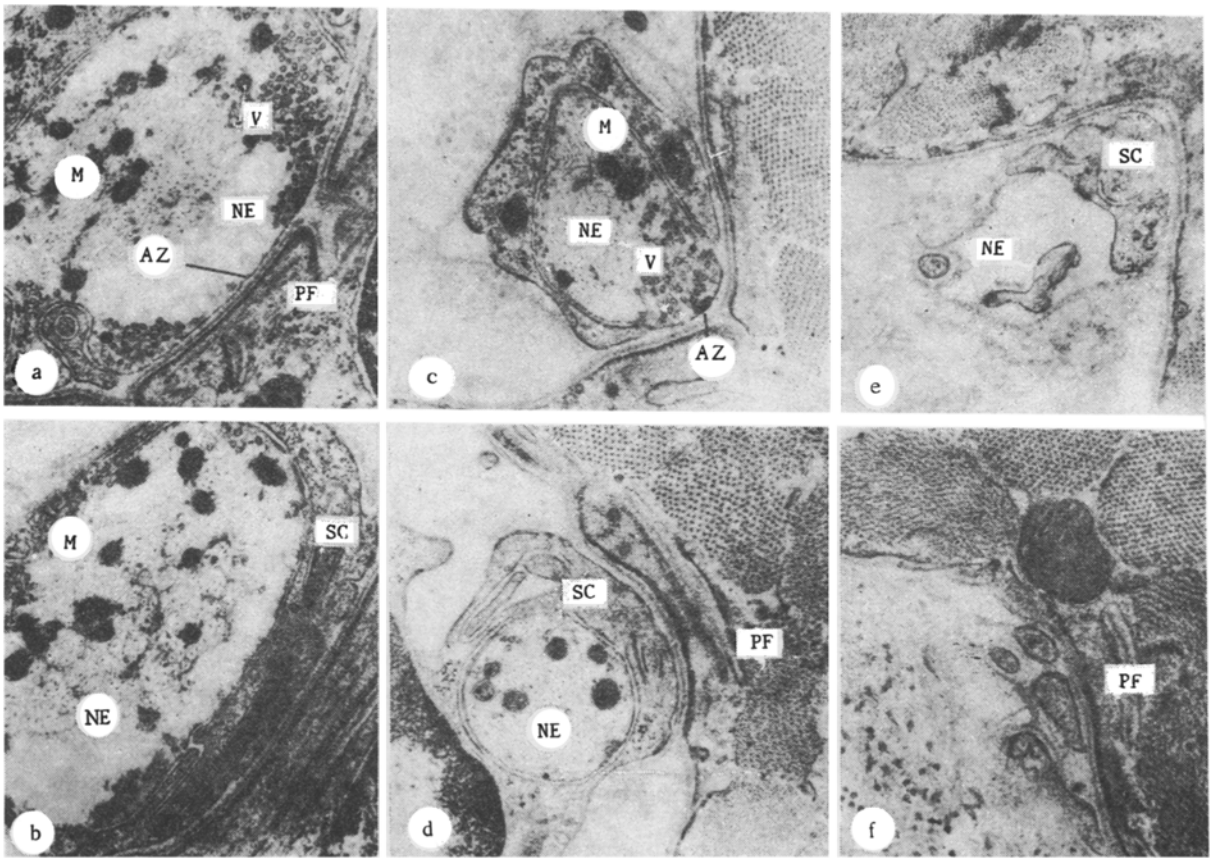

Fig. 1. Ultrastructure of a single nerve terminal of the frog satororius muscle (25,000×). a, b) Region of nerve ending (NE) at a distance of 60 μ from the last myelin segment; c, d) the same, at a distance of 120 μ; e, f) 180 μ. a, b) Regions containing active zones (AZ), synaptic vesicles (V), and mitochondria (M); c, d, e) terminal separated from muscle fiber by circumference of Schwann cell (SC); f) synaptic pit on muscle fiber with remains of postsynaptic folds (PF) and processes of Schwann cell.

number of quanta of transmitter secreted in response to 300-900 stimulations. The significance of differences was estimated by Student's t test.

EXPERIMENTAL RESULTS

The fine structure of the single nerve terminal was found to agree in general features with data in the literature [4, 6, 7, 10]. However, the study of serial sections through the terminal revealed some new details of its structure.

The nerve terminal decreased considerably in thickness from the proximal toward the distan portions (Fig. 1a-e). The maximal perimeter of the proximal portions of the terminal, for instance, was 9.5 ± 3.5 μ for T1, 8.0 ± 0.8 μ for T2 and 8.8 ± 1.1 μ for T3. In the distal portions the perimeter was considerably smaller, namely 3.9 ± 0.6, 4.5 ± 1.61, and 4.3 ± 0.7 μ for T1, T2, and T3, respectively ($p < 0.01$). After the nerve terminal a distance of 15-30 μ on the muscle fiber was occupied by a Schwann cell and a characteristic depression with postsynaptic folding (Fig. 1f). This depression is evidence of reduction of the length of the terminal in winter frogs. Besides the decrease in volume of the terminal from the proximal toward the distal portions, the extent of the synaptic contact (which we defined as the region of the presynaptic membrane not separated from the muscle fiber by a Schwann cell) also was reduced. For the proximal portions of the terminal the extent of synaptic contact was: 3.8 ± 1.3 μ for T1, 2.1 ± 0.3 μ for T2, and 3.5 ± 0.5 μ for T3, whereas in the distal portion it was 1.5 ± 0.3, 1.2 ± 0.6, and 1.4 ± 0.3 μ, respectively ($p < 0.01$).

Considerable differences also were found in the number of synaptic vesicles. The total number of synaptic vesicles in the plane of transverse sections of the proximal portions of the terminal in the region of the active zones (AZ) was 155 ± 59 for T1, 125 ± 16 for T3, and

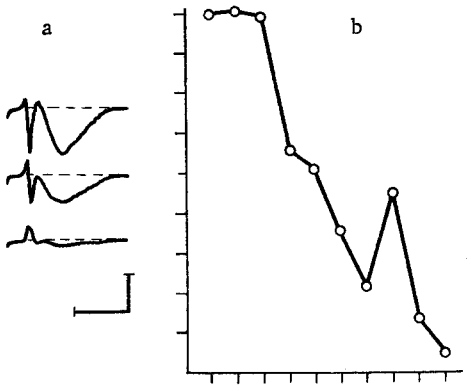

Fig. 2. Time course of changes in evoked transmitter release along the course of a nerve terminal. Extracellular microelectrode moved gradually from a visible segment of myelin to one side, and single-quantum EPP arising in response to 600 stimulations of the motor nerve were recorded. Recordings made at 100 points of the nerve ending at a distance of 10-20 μ apart. a) Averaged responses to 100 stimulations at 1st (derivations from a myelinated region of the motor axon), 5th, and 10th recording points. Along the course of the terminal the presynaptic spike becomes inverted and the averaged EPP is reduced; d) time course of changes in quantum composition of EPP along course of terminal. Abscissa, No. of recording point; ordinate, quantum composition of EPP. Extracellular Ca^{++} concentration, 0.3 mM; Mg^{++}, 4 mM.

75 ± 37 and 41 ± 29, respectively, for the terminal portions ($p < 0.05$). The number of vesicles in the plane of transverse section of the terminal in the regions between AN was considerably smaller.

Reconstruction of the synapse based on analysis of serial sections showed that the terminal consists of several varicose expansions. These expansions were separated by regions of the terminal that were completely isolated from the muscle fiber by processes of a Schwann cell (Fig. 1b, d). The expansions differed in size, and their diameter and extent were considerably reduced at the ends of the terminal. AZ, numerous synaptic vesicles, and mitochondria were concentrated in these expansions, whereas regions of the terminal surrounded by a Schwann cell contained only a few vesicles and no AZ whatsoever. Each expansion, in turn, was found to divide into segments, formed by thin digitiform processes of the Schwann cell, and each such segment contained 1-3 AZ.

It can be concluded from these results that a nerve terminal forms an interrupted synaptic contact. In other words, the terminal consists of several elementary synaptic contacts, separated from each other by projections of the Schwann cell. Each elementary synaptic contact consists of a group of active zones and has the appearance of an expansion of the nerve terminal. These elementary synaptic contacts evidently form the "bulb-like appearance of the terminal, visible under the light microscope [12, 14]. The discovery of varicose expansions of the terminal and the grouping of AZ indicates that transmitter secretion takes place only in certain parts of the terminal, located some distance apart. The presence of these spatially isolated regions of quantal transmitter release may explain the polymodality in the distributions of amplitude of single-quantum postsynaptic potentials observed on extracellular recording [2].

Extracellular recording of postsynaptic potentials in different parts of the nerve ending showed a sharp fall in the level of transmitter release from the proximal toward the distal portions of the terminal. Within the same terminal a 3-28-fold decrease in evoked transmitter release was observed (Fig. 2). In 10 experiments, with a low Ca^{++} ion concentration (0.3 mM) the quantum composition of EPP was 0.195 ± 0.029 in the proximal portions of the terminal and 0.038 ± 0.008 in the distal portions ($p < 0.05$). A similar situation also was observed with respect to spontaneous transmitter release: the frequency of miniature EPP was 5-20 times less in the distal portions than in the proximal.

Comparison of the ultrastructural and electrophysiological data leads to the conclusion that the decrease in evoked and spontaneous transmitter release in the distal portions of the nerve ending is connected with the morphological features of the terminal and, in particular, with a sharp decrease in its diameter and in the extent of the synaptic contact. Since AZ run across the terminal and since synaptic vesicles, capable of exocytosis and of releasing a quantum of transmitter into the synaptic cleft, are arranged in two rows along the edge of AZ [6, 9, 10], the decrease in diameter of the terminal and in the area of synaptic contact in the

distal portions leads to a decrease in size of AZ and a decrease in the number of vesicles in them. All these changes are expressed as a decrease in the probability of release of a quantum of transmitter. From this standpoint we can explain the decrease in the intensity of both evoked and spontaneous transmitter release in the end portions of the terminal. The sharp decrease in the number of synaptic vesicles away from the presynaptic membrane, in the distal portions of the terminal, suggests that in these parts the terminal is less able to make good the utilized transmitter, i.e., that utilized in transmitter mobilization processes. Electrophysiological observations indicating more marked depression of the quantum composition of EPP in the distal portions of the nerve ending confirm this hypothesis [1].

It has been suggested that voltage-dependent Ca channels also are arranged along the border of AZ [10], and consequently, that their number is determined by the area of AZ. A second cause of the decrease in evoked transmitter release in the distal portions of the terminal may therefore be a decrease in the intracellular Ca^{++} concentration, connected with lower values of the inward Ca current. The possibility likewise cannot be ruled out that the density of voltage-dependent Na and K channels is reduced in the distal portions [3, 11], and this leads to reduction of the depolarization level of the presynaptic membrane during a spreading action potential.

It can thus be concluded from these results that the single frog nerve terminal is a morphologically and functionally heterogeneous formation, and that differences in transmitter secretion observed in different parts of the terminal are due to the real structural differences between these parts.

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