ELECTRICAL ACTIVITY IN DIFFERENT PARTS OF FROG NERVE ENDINGS

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The immediate cause of transmitter secretion in the neuromuscular synapse is depolarization of the presynaptic membrane by a spreading action potential (AP). On the basis of the results of experiments with extracellular recording of AP in the frog nerve ending it has been suggested that AP spreads actively and without decrement along the whole length of synaptic terminals [7, 9].

Meanwhile it has been shown that distal portions of the terminal have a lower level of evoked transmitter release than proximal parts [1, 2]. This may be due to differences in the character of spread of AP in the terminal parts of the synapse. This suggestion is confirmed by the recently discovered differences in the distribution of ion-selective and K-channels along the course of the nerve ending [3, 8, 11].

This paper describes the study of electrical responses in different parts of the frog motor nerve ending and analysis of the effect of tetrodotoxin on presynaptic currents in the nerve terminal.

EXPERIMENTAL METHODS

Experiments were carried out on isolated nerve-muscle preparations of the sartorius muscle and sciatic nerve of *Rana ridibunda* at room temperature. The preparation was perfused continuously with Ringer's solution of the following composition (in mM): NaCl) 115.0; KCl) 2.0; $CaCl_2$) 0.3-0.6; $MgCl_2$) 2-4; $NaHCO_3$) 2.4 evoked pre- and postsynaptic currents were derived by extracellular glass microelectrodes with a resistance of 2-10 MΩ, filled with 2 M NaCl solution of Ringer's solution.

The motor nerve was stimulated by square electrical pulses 0.1-0.3 msec in duration and of above-threshold amplitude. The criteria that the electrode was actually on the nerve terminal were described in detail by the writers previously [1, 2]. Responses were averaged for 50-200 realizations by means of an automated system based on DZ-28 microcomputer. The signal measurement interval was 20 μsec. The results of averaging were recorded on an N-306 graph plotter.

Microelectrodes filled with tetrodotoxin (TTX) in a concentration of $3 \cdot 10^{-4}$ M, with a resistance of over 100 MΩ, were used for iontophoretic application.

EXPERIMENTAL RESULTS

Terminal nerve twigs of frog phasic muscle fibers are of considerable length (100-300 μ) and are terminal ramifications of the myelinated motor axon [6]. Under the ordinary light microscope only the last segment of myelin can be seen and the terminal branches are invisible. In the present experiments, therefore, the terminal myelinated segment of the axon served as guide for the nerve ending [1, 2, 9].

On approximation of the extracellular microelectrode to the nerve ending stimulation of the motor nerve led to the appearance of an electrical response of the nerve ending (an AP), followed by an end-plate potential (EPP). Typical averaged responses of a nerve ending in different parts of the synapse are shown in Fig. 1. In the proximal part of the nerve ending (Fig. 1A) at a distance of a few tens of microns from the last myelin segment, a triphasic response (+−+) could be recorded, in which the second, electronegative phase, pre-

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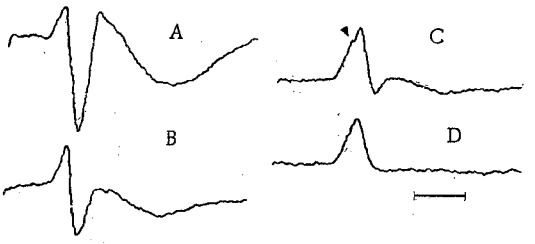

Fig. 1. Electrical responses in different parts of nerve ending. A-D) Electrical responses recorded extracellularly at 4 points of a terminal at distances from 50 μ (A) to 100 μ (D) from last myelin segment. Averaged responses from 50 realizations. Frequency of stimulation 1 Hz. Results of single experiment.

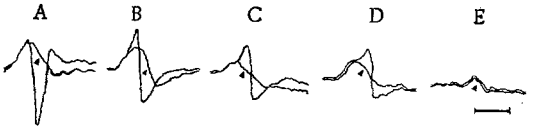

Fig. 2. Effect of local application of TTX in different parts of the synapse. Effect of TTX indicated by arrows (explanation in text). A-E) Results of different experiments.

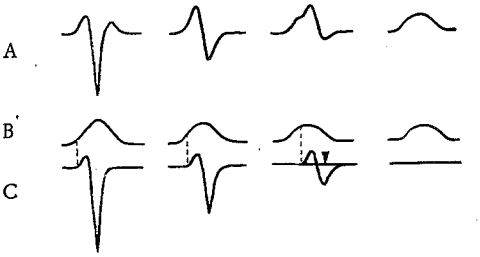

Fig. 3. Diagram of TTX-sensitive and TTX-insensitive components in different parts of synapse. A) Typical responses along course of nerve ending (from left to right — from proximal to distal parts of synapse). B) TTX-insensitive component (effect of TTX), C) TTX-sensitive component (obtained by subtracting B from A). Broken lines — time of superposition of TTX-sensitive indicates alternate disappearance of TTX-sensitive component.

dominated. On movement of the extracellular electrode along the terminal away from the last myelin segment, some enlargement of the first phase and a decrease in amplitude of the second phase, and disappearance of the third phase of the triphasic response were observed (Fig. 1B). The electrical response became biphasic (+−). This was followed by a gradual reduction of the negative phase of the signal. Nearer to the terminal parts of the synapse variations in shape of the responses were observed from one stimulation to the next; in some cases a characteristic inflection (arrow in Fig. 1C) was observed on the ascending part of the response. Two components could be distinguished in these responses: the first — positive, the second — biphasic. The first component was constantly present to every stimulation, but the second occurred rarely, and was superposed on the peak of the first. Superposition of the second component on the first caused the appearance of the inflection de-

scribed above on the ascending part of the combined signal. On recording from the end regions of the terminal, only monophasic (+) responses were observed. These responses were of low amplitude and were absolutely identical with the first component of the biphasic response (Fig. 1D). From the proximal to the distal parts of the synapse there was a considerable decrease in transmitter release, reflected in a reduction in amplitude of the averaged EPP (Fig. 1).

As an additional control of the position of the electrode on the nerve ending, after the electrophysiological experiments the terminal ramifications were stained with methylene blue. These observations confirmed that triphasic responses are observed when the electrode is located in the initial parts of the terminal, and biphasic responses with predominance of the electropositive phase, and monophasic (+) responses are observed in distal parts.

A change in shape of the electrical responses along the course of the nerve ending (from predominantly negative to predominantly positive) can be explained by the "open end" effect of the the terminal in the presence of active penetration of AP without decrement into the nerve ending [9]. However, another explanation is possible. The electropositive response in the distal parts of the synapse may indicate passive depolarization of the presynaptic membrane on account of a conduction block in the end of the terminal [5]. To verify these explanations, the effect of the specific blocker of Na channels, tetrodotoxin [10], on the shape of the responses of the nerve ending was investigated. In these experiments, the extracellular electrode was applied initially to a particular part of the nerve ending. Next the electrode, filled with TTX, was applied as close to possible to the recording electrode. To expel TTX from the microelectrode a positive potential was applied to it. During local application of TTX to proximal parts of the nerve ending a dramatic decrease in the electronegative phase of the triphasic response and its later appearance were observed. The amplitude of the positive phase was unchanged or was increased a little. After application for 2-3 min the response was converted into a purely monophasic response (+), resembling that of the terminal parts of the nerve ending (Fig. 2A). The effect of TTX was reversible: Cessation of its application led after 3-5 min to complete recovery of the amplitude and shape of the responses.

If the recording electrode recorded a biphasic response, TTX led to reduction of the electropositive phase and disappearance of the electronegative (Fig. 2B, C). Under these circumstances the response became extended monophasic in character (Fig. 2C, D). In experiments in which two components could be distinguished in the response of the nerve ending, TTX led to disappearance of the second, biphasic component. TTX had no effect on monophasic (+) responses of the terminal parts of the synapse (Fig. 2E).

Analysis of the effect of local application of TTX to the recording point showed that in all parts of the nerve terminal two components could be distinguished: sensitive and insensitive to TTX. The TTX-insensitive component could be detected during the action of TTX, whereas the TTX-sensitive component could be obtained by subtracting TTX-insensitive component from the response before the action of TTX. A scheme of the electrical responses in different parts of the nerve ending, with distinction of TTX-sensitive and TTX-insensitive components, is illustrated in Fig. 3. The TTX-insensitive component was roughly the same in different parts of the nerve ending, although toward the distal parts its amplitude decreased (Fig. 3B). The TTX-sensitive component was biphasic in shape with a well-defined negative phase. Along the course of the nerve ending there was a gradual decrease in the amplitude of this component, and closer to the end of the terminal it was intermittently absent. From the proximal toward the distal parts of the synapse, moreover, it appeared later (Fig. 3C). Whereas in proximal parts the TTX-sensitive component appeared on the ascending part of the TTX-insensitive component, in distal parts it appeared on its peak (Fig. 3B, C). This led to the appearance of an inflection on the ascending part of the responses. In distal parts of the synapse the TTX-sensitive component was absent altogether.

The results suggest that TTX-insensitive component is a passive outward current generated by the advancing AP, whereas the TTX-sensitive component mainly reflects the inward Na current at the recording point. These two currents are depolarizing: The first depolarizes the membrane of the nerve ending to the threshold level, whereas the second is the generator peak of AP. If this explanation is accepted, along the course of the nerve ending the Na current decreases, and appears later (after the beginning of the outward current). This later appearance is due in all probability to the increase in the threshold level of appearance of AP. We know that a decrease in the inward Na-current and an increase in the threshold

level are observed on a reduction in Na-permeability of the membrane [4]. It can accordingly be postulated that along the course of the nerve ending there exists a gradient of Na-channels, and in the distal parts of the terminal Na-channels are absent.

It can accordingly be concluded from these results that AP spreads along the nerve ending with a decrement, but in the end regions of the terminal depolarization of the presynaptic membrane takes place passively. These characteristics of electrogenesis of the nerve ending can explain the lower level of transmitter secretion in the distal parts of the synapse.

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