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APPEARANCE AND SPREAD OF EXCITATION IN THE FROG MOTOR NERVE ENDING

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During the conduction of excitation in nerve fibers changes of potential along the membrane are heterogeneous, and local currents arise between neighboring areas, which on the one hand lead to conduction of the impulse into resting parts of the nerve fiber and, on the other hand, exert a significant effect on the development of the action potential (AP) in the part already excited [5, 9].

Processes of generation and spread of excitation in motor nerve endings, which are terminal ramifications of the motor axon and are responsible for the transmission of excitation from nerve to skeletal muscle, have not yet been elucidated. Until quite recently it was considered [6-10] that the AP spreads along the nerve ending of both warm- and cold-blooded animals actively and without decrement, but that depolarization of the presynaptic membrane induces a flow of Ca^{2+} ions into the axoplasm of the ending and secretion of the mediator [4, 5, 11].

However, recently it was found, when ionic currents of a mouse nerve ending were recorded [7, 10], that the endings do not contain sodium channels, and they are depolarized passively due to local currents from the Ranvier node and the preterminal segment. It was later shown [1, 2, 12] that frog motor nerve endings can generate excitation and cause its active spread, although the value of the sodium current falls along the course of the nerve ending.

In the investigation described below local application of tetrodotoxin (TTX), a specific sodium channel blocker, to different parts of the nerve ending and Ranvier nodes, the generation and spread of excitation in the frog motor nerve ending and the role of these processes in mediator secretion were studied.

EXPERIMENTAL METHOD

Experiments were carried out on nerve-muscle preparations of the cutaneosternal muscle of *Rana ridibunda* in the winter period, during continuous perfusion of the preparation with solution of the following composition (in mM): NaCl – 115.0, KCl – 2.0, $CaCl_2$ – 0.4-0.6, $MgCl_2$ – 2.0, $NaHCO_3$ – 2.4, pH 7.2-7.4, temperature 18-20°C.

Evoked electrical responses of the nerve ending and subsequent end-plate currents (EPC) were recorded extracellularly by means of glass microelectrodes with a tip having an internal diameter of 1-2 μ, and filled with the perfusion solution. Microelectrodes with a tip under 1 μ in diameter, filled with TTX in a concentration of 100 μM (resistance 100-200 MΩ), were used for iontophoretic application [3].

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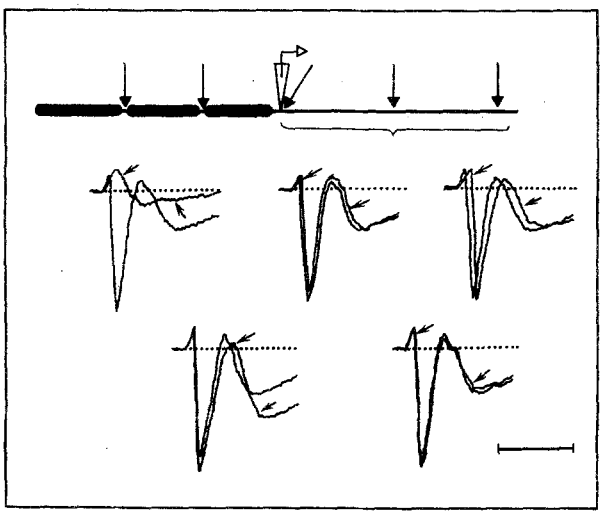

Fig. 1. Effect of local application of TTX on electrical responses of proximal segments of synapse. Scheme of experiment shown above. Recording electrode located on nerve ending at distance of 5-10 μ from last segment of myelin. Sites of local application of TTX indicated by arrows. Below — averaged responses to 50 stimulations of motor nerve before and 30 sec after (arrows) beginning of TTX application. Dotted lines indicate zero levels.

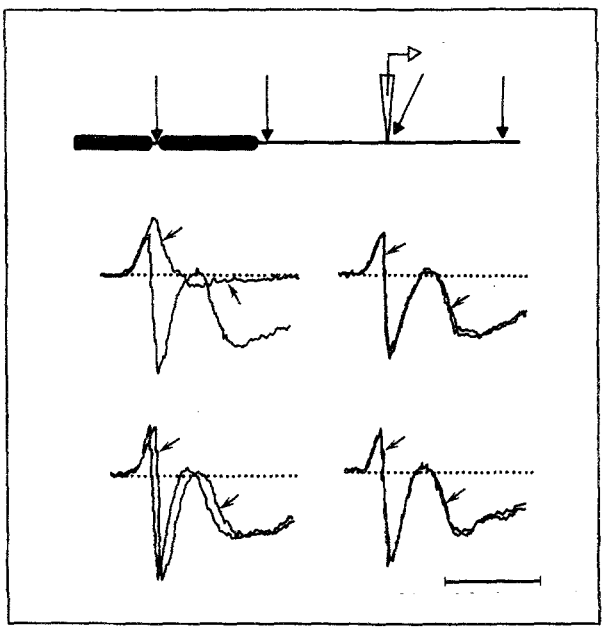

Fig. 2. Effect of local application of TTX on electrical responses of central segments of synapse. Electrode located 80 μ from last segment of myelin. Legend as to Fig. 1.

Under a polarization-interference microscope (magnification 300) superficial nerve endings, with few branches, except one or two terminal twigs, and with the last two segments of myelin and Ranvier nodes clearly visible, were sought. Under visual control the recording electrode was applied to the nerve ending and pressed against it. There were three series of experiments, in which the recording electrode was located: 1) on the proximal segment of the ending (5-10 μ from the last segment of myelin); 2) on the central segment (the middle of the ending, 50-80 μ from the last segment of myelin); 3) on the distal (termi-

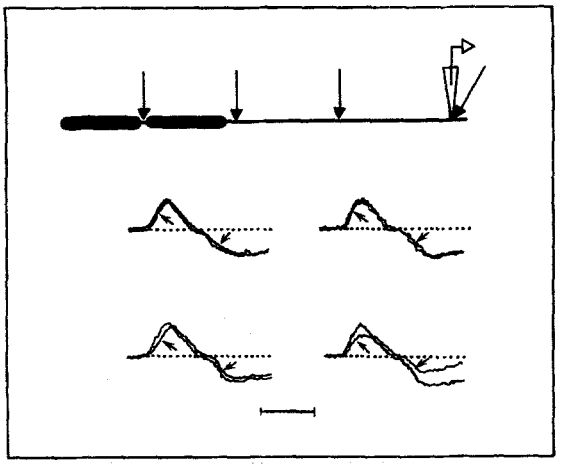

Fig. 3. Effect of local application of TTX on electrical responses of distal segments of synapse. Legend as to Figs. 1 and 2.

nal) portion of the ending. Endings 120-160 μ long were studied. The applying electrode was located at different distances from the recording electrode toward the nerve ending or nodes of Ranvier. Iontophoresis of TTX from the electrode was carried out by applying a positive potential to it.

The motor nerve was stimulated by square pulses of above threshold amplitude, and with a duration of 0.15-0.30 msec, from an electronic stimulator. Evoked electrical responses were amplified and averaged by means of an automated system based on the DZ-28 microcomputer. Averaged responses (20-100 realizations) were recorded on paper by means of an automatic x–y writer.

EXPERIMENTAL RESULTS

In the proximal segment of the nerve ending stimulation of the motor nerve led to the appearance of a triphasic response consisting of the first and third positive phases and the second high-amplitude negative phase (Fig. 1). On application of TTX to the recording electrode (Fig. 1a) some increase in amplitude of phase 1 and a sharp reduction of amplitude or disappearance of the negative phase 2 were observed. These experiments confirm previous findings [1-3, 9, 12] and lead to the conclusion that the negative phase of the response reflects the inward sodium current of the nerve ending. The effect of TTX was accompanied by a sharp reduction of mediator secretion, expressed as disappearance of the EPC. Application of TTX to both the penultimate and ultimate Ranvier nodes (Fig. 1b, c) led to prolongation of the first positive phase of the response and to the later appearance of the second negative phase; the amplitude of EPC remained unchanged. It will be clear from Fig. 1b, c that the effect of application of TTX to the last Ranvier node was more marked than that of its application to the penultimate node, and it was accompanied by a greater shift of the negative phase of the response to the right. It can be concluded from these results that the first positive phase of the response is a passive depolarizing current, due to electrical activity of the Ranvier nodes. Application of TTX to the central segment of the ending (Fig. 1d) caused an increase in amplitude and widening of the second negative phase of the response. These changes were accompanied by a marked increase of mediator secretion in the proximal segments (an increase in amplitude of EPC). The results are evidence that during the spread of excitation under natural conditions, preceding segments of the nerve ending have a significant influence on the processes of electrogenesis in the proximal segments excited previously. It can be tentatively suggested that the inward sodium current of the central segments of the nerve ending leads to the appearance of outgoing loops of current, which cause shortening and diminution of the inward current of the proximal segments. This last state of affairs leads to reduction of the amplitude and duration of AP of the proximal segments of the ending, to weakening of the inward calcium current, and to a decrease in mediator secretion. Application of TTX to the distal segments of the ending (Fig. 1e) caused no changes whatever in the amplitude and shape of the responses, evidence that the terminal segments of the nerve ending have no influence on the proximal segments.

In the central segment of the nerve ending the extracellularly recorded responses were biphasic in shape (Fig. 2). In response to application of TTX to the recording site (Fig. 2a) the first positive phase was enlarged and the second phase and also the EPC disappeared virtually completely. Blocking of sodium conductance of the last Ranvier node (Fig. 2b) had no significant effect on electrical activity of the central segments. Application of TTX to the proximal segments caused slowing of development of the first phase of the response and the later appearance of the second negative phase (Fig. 2c). It can be concluded from these results that electrotonic depolarization of the central segments of the ending takes place mainly on account of passive outward currents from the proximal segments of the ending. Application of TTX to the distal segments of the nerve ending did not change the shape of responses of the central segments or secretion of mediator (Fig. 2d). Since the distance between the tested proximal and central, and also central and distal segments in the present experiments was about equal, it can be postulated that distal segments had no effect on the central segments because of the sharp reduction or absence of the inward sodium current in the distal segments.

In the distal segment of the nerve ending a virtually single electropositive first response was recorded (Fig. 3). Local application of TTX to the recording site affected neither the shape of the response nor secretion of mediator (Fig. 3a). Application of TTX to the more proximal segments of the ending led to some reduction of the monophasic response and of the amplitude of EPC; a stronger effect was observed, moreover, to application to the central segments of the ending (Fig. 3c, d). Blocking sodium conductance of the Ranvier node did not change the shape of the response (Fig. 3b). These results suggest that the inward sodium current is absent in the terminal segments of the ending, and they are depolarized by inward currents mainly in the central and also in the proximal segments of the ending.

Comparing these findings with data in the literature, we can conclude that the processes of generation and spread of excitation in the motor nerve ending of warm- and cold-blooded animals differ significantly. In warm-blooded animals, which have compact and short nerve endings, local currents from the last Ranvier node and the penultimate segment can induce complete depolarization of the whole ending and the secretion of mediator from it [10]. We can thus understand the absence of sodium channels in the mouse nerve ending [7]. Local passive currents from the Ranvier node of the penultimate segment in frog synapses, which have longer nerve endings, cannot depolarize the whole ending, and for that reason the spread of excitation takes place actively over the greater part of the ending. Only the terminal segments of the ending do not contain sodium channels, and they are depolarized passively.

It can be concluded from these experimental results that during the spread of excitation, segments of the frog motor nerve ending exert a marked influence on one another. The more proximal segments cause depolarization of the preceding segments and conduction of AP into an unexcited part of the ending. The effect of preceding segments is to reduce and shorten depolarization of previously excited segments, and this leads to a decrease of mediator secretion. As the AP advances toward the distal segments the effect of the preceding segments gradually diminishes, due to weakening and disappearance of the inward sodium current at the final part of the ending.

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