ACTIVATION OF SYNAPTIC PROCESSES IN THE MYONEURAL JUNCTION POISONED BY TETANUS TOXIN IN RESPONSE TO REPETITIVE NERVE STIMULATION

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UDC 616.981-551-07:616.8-091.81-07

Synaptic processes in neuromuscular synapses of the rat diaphragm, poisoned with tetanus toxin, were activated by stimulation of the motor nerve. An increase in spontaneous synaptic activity (an increase in the frequency of miniature end-plate potentials from 0.01 to 100-200/sec) and partial recovery of synaptic conduction were oberved during and after stimulation. The character of the pathogenic action of the toxin on the presynaptic structures in various types of synapses is discussed.

The primary component of the lesion to the synaptic system of a muscle poisoned by paralytic doses of tetanus toxin, just as in a disturbance of central inhibition in tetanus, is located in the presynaptic structures [1, 9, 13]. Inhibition of the spontaneous secretion of the mediator and the accumulation of synaptic vesicles in the axon terminals suggest that the action of tetanus toxin is to disturb the liberation of mediator through the presynaptic membrane [6]. With this type of disturbance, repetitive stimulation of the motor nerve, which is known to activate the secretion of mediator in neuromuscular synapses of the innervated muscle [11], might perhaps prove effective. Under these conditions, investigation of the mediator function of poisoned synapses, an important means of assessing their functional state, could also help to explain the nature of the phenomenon discovered previously, that during indirect repetitive stimulation of a poisoned muscle, the amplitude of its electrical responses is maintained or even increased (compared with its initial value), and not reduced as is found under normal conditions [3, 4].

The object of the present investigation was to study the state of the synaptic system of a muscle poisoned with tetanus toxin during and after stimulation of its nerve.

EXPERIMENTAL METHOD

Male August rats weighing 150-180 g were used. Electrical activity of the muscle was recorded intracellularly and extracellularly on an isolated nerve-muscle preparation consisting of the phrenic nerve and a strip of the diaphragm muscle, immersed in a chamber at constant temperature through which there was a constant flow of carbogen-saturated Tyrode's solution (NaCl 8 g, KCl 0.2 g, $CaCl_2$ 0.2 g, $MgCl_2$ 0.1 g, $NaHCO_3$ 1 g, NaH_2PO_4 0.05 g, glucose 2 g to 1 liter of solution).

The transmembrane potentials in the synaptic zone were recorded by glass microelectrodes filled with 2.5 M KCl solution, with a tip less than 0.5 μ in diameter and an impedance of 10-100 MΩ. These microelectrodes have long, flexible tips so that they could be held in the stimulated muscle. The potentials were amplified and recorded on a Disa-Indicator oscilloscope and a special matching system [10]. The electromyogram was recorded by concentric Disa unipolar and bipolar electrodes. The phrenic nerve was kept in a moist chamber and stimulated by square pulses 0.2 msec in duration.

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Laboratory of Pathophysiology of Toxico-Infections, Institute of Normal and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. Transalted from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 5, pp. 22-26, May, 1972. Original article submitted October 28, 1971.

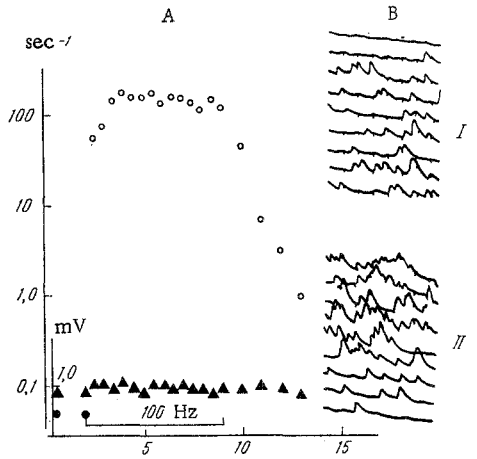

Fig. 1

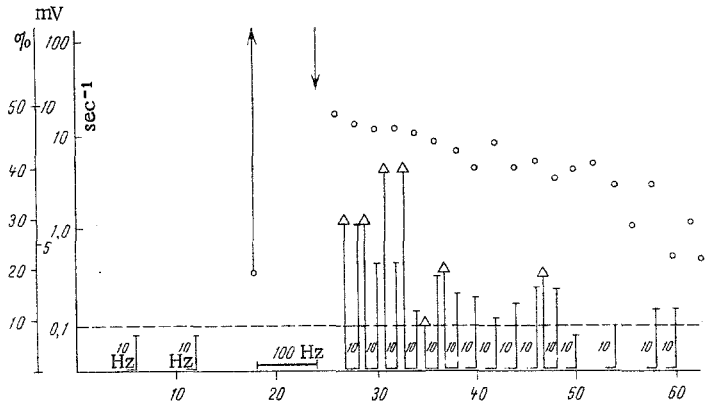

Fig. 2

Fig. 1. Increase in spontaneous synaptic activity in myoneural junction poisoned with tetanus toxin during repetitive stimulation of motor nerve. A) change in frequency and amplitude of MEPPs during nerve stimulation. Abscissa, time (in sec); ordinate, circles show mean values of MEPP frequency, triangles show mean amplitudes of MEPPs; line denotes duration of nerve stimulation (frequency 100 Hz); B) MEPPs recorded at beginning (I) and end (II) of stimulation. Evoked EPPs absent (see artefacts of stimulation).

Fig. 2. Activation of synaptic processes in period after stimulation Abscissa: lines denote duration of testing (10 Hz) and conditioning (100 Hz) stimulation (in sec), arrows show unassessable increase in MEPP frequency during stimulation (Fig. 1); ordinate: circles show mean values of MEPP frequency; broken line shows level of mean amplitude of MEPP (quantum value); vertical line with triangle represents frequency of AP generation; vertical line with horizontal stroke shows mean amplitude of EPP during testing stimulation. Markers on the abscissa: hertz.

The toxin (Batch No. 9 obtained from native toxin of batch No. 255, strain No. 471, Moscow Institute of Vaccines and Sera) was injected in a dose of 3×10^5 MLD in a volume of 0.3 ml into the left half of the cupola of the rat diaphragm in situ. The nerve-muscle preparation was isolated 3-3.5 h after injection of the toxin. Some details of the method were described previously [6, 8].

EXPERIMENTAL RESULTS AND DISCUSSION

As was shown previously [6, 9], the dynamics of development of the lesion, especially with the method of poisoning chosen, leads to disturbances of the neurosecretory process of unequal degree in the different synapses of the poisoned muscle, and for this reason only a shift of the main peak of distribution of the fibers by mean frequencies of miniature end-potentials (MEPPs) toward lower values enable the character of the damage to the synaptic system of the muscle to be estimated. In the present investigation the effects of repetitive stimulation of the motor nerve (frequency 50-100 Hz) for the most representative group of fibers, with inhibited spontaneous synaptic activity (mean frequencies of MEPPs 0.1-0.01/sec), were determined and are described in this paper.

An increase in spontaneous synaptic activity was observed during nerve stimulation in most fibers tested. The frequency of the MEPPs reached a maximum in the course of several seconds, after which the increased spontaneous activity was maintained during stimulation and for a long period after its end (Figs. 1 and 2). Activation of synaptic activity was not necessarily accompanied by the appearance of evoked postsynaptic potentials (Fig. 1B). No change in the mean amplitude of the MEPPs during their potentiation was found, which shows that the quantum response was unchanged and that this value can be used to assess the quantum composition of the evoked end-plate potentials (EPPs).

Facilitation of evoked synaptic activity in some cases could be detected both after (Fig. 2) and during stimulation (Fig. 3).

In the period after stimulation the state of the synaptic mechanism facilitating the conduction of excitation was tested by stimulating the nerve at fixed time intervals by short (1-2 sec) series of stimuli of rel-

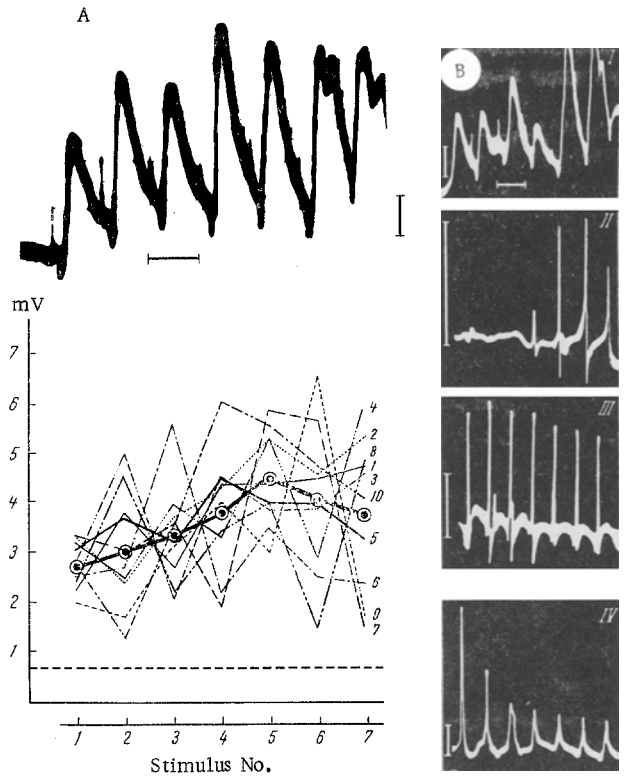

Fig. 3. Potentiation of synaptic conduction during high-frequency stimulation. A) change in amplitude of EPPs in a consecutive series of stimuli. Ordinate, amplitudes of consecutive EPPs during 10 short series of high-frequency (50 Hz) stimulation of nerve (individual series are denoted by numbers from the right and by the corresponding curves; averaged values are shown by double circles and thick lines; broken line shows "quantum" value). One of the records from which measurements were made is shown above the graph; B) facilitation of synaptic conduction in neuromuscular synapse during repetitive stimulation of nerve: i) EPP in single fiber (intracellular recording); ii) extracellular recording of integral electrical responses from a few muscle fibers (subthreshold stimulation of nerve by bipolar electrode); iii) responses of large group of fibers from strip of diaphragm muscle; iv) inhibition of responses in normal muscle. (Recording conditions similar to iii). Calibration of amplitude 1 mV for i and 0.5 mV for ii-iv.

atively low frequency (5-10 Hz). The increase in mean amplitude of the EPPs in the testing series of pulses and, consequently, in the mean quantum composition was evidence of activation of the synchronous liberation of mediator. The random character of each EPP appearance in the testing series, which could be estimated in small and large samples, leads to a certain probability of appearance of action potentials (APs). The frequency of AP appearance is thus determined by at least three factors: the magnitude of the single quantum response, the value of the quantum composition (M) reflecting the state of the mechanisms for synchronous liberation of the mediator, and the threshold of excitation of the muscle fiber. A few quanta are evidently sufficient to produce excitation of thin fibers (Fig. 2). With regard to this experiment the following factors must be borne in mind:

1) approximately equal AP amplitudes, for reasons which will be clear, were disregarded during the evaluation of the mean EPP amplitudes, so that this estimate was a little too low, for it did not include some of the large EPPs;

2) testing volleys of stimuli, despite their low frequency, evidently activate synaptic processes and, at the same time, have a conditioning effect, so that the quantitative evaluation of the connection between the degree of facilitation of synaptic conduction and activation of the spontaneous secretion of mediator, becomes indeterminate. The parallel between the development of these functional changes will be sufficiently evident, although it must be pointed out that the maximum of facilitation did not coincide with the beginning of the post-stimulation period but was linked with a certain "optimal" level, close to the normal level of spontaneous secretion.

During high-frequency stimulation of the nerve to the poisoned muscle, indeterminacy of the situation also took place. In this case (Fig. 3), in the course of repetitive volleys, the magnitude of each response was random in character (in absolute value and also, evidently, in quantum composition); but because of the activating effect of each volley, the mean amplitudes of a successive series of pulses showed a tendency to increase. This mechanism may evidently lead to restoration of transmission in a series of fibers, and when the integral electrical response of a small group of muscle fibers was recorded, to an increase in amplitude (Fig. 3). At a certain stage of poisoning, this stochastic character of the restoration of conduction (the attainment of the threshold of excitation of the muscle fibers) occurred in many synapses as intermittent activity of the fibers, and it led to preservation of the integral electrical response during the repetitive volley (Fig. 3). Under normal conditions the decrease in amplitude of the integral response during stimulation could be connected not only with presynaptic mechanisms, but also with postsynaptic trace processes due to excessive liberation of mediator [14, 17]. In the poisoned muscle, inhibition of the synchronous liberation of the mediator led to a higher frequency of appearance of small interspike intervals in individual fibers for responses to repetitive stimulation and, consequently, to a better performance of the whole muscle in responding to the high frequency of nervous volleys. As a whole these effects can simulate, as was mentioned previously [3-5], increased lability of neuromuscular transmission in the poisoned muscle.

Under these conditions of poisoning of the muscle, repetitive nerve stimulation can thus considerably activate neurosecretion in the synapses and can partially restore the mediator function of the synaptic system connected with it. The possibility of considerable and prolonged potentiation of spontaneous synaptic activity is in agreement with the views developed previously regarding the character of disturbance of neurosecretory processes in tetanus poisoning [1, 6].

Tetanus toxin probably has a uniform pathogenic action of this type on different forms of synaptic endings in the centers and at the periphery [2, 7, 12, 13, 16]. Differences between the effect of the toxin in synapses of different types may be due, in particular, to differences in their function. For instance, excitatory effects mediated by discrete conduction of excitation in the electrogenic component (the value of the threshold level), which has a large reserve of reliability in the peripheral synapses, are more resistant to the toxin (high reliability of conduction in the myoneural junction in the diaphragm is due to the large excess of the EPP over the threshold of excitation of the muscle fiber; see Fig. 2). The modulatory effects of the inhibitory synapses, which are directly connected with the quantity of mediator secreted, must be more sensitive to inhibition of neurosecretion by tetanus toxin. In this case the structural localization of the synaptic formations in the relation to path of spread of the toxin, the character of the barriers, and so on, may play an important role.

It can be assumed that the inhibition of mediator liberation through the presynaptic membrane is a general, but possibly not the only, mechanism of action of tetanus neurotoxin, and that the specific character of its effects in synapses of different nature is determined by the differences between their structural and functional organization rather than by the chemical properties of the secreted mediator.

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