ACTION OF TETANUS TOXIN ON NEUROMUSCULAR TRANSMISSION

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The authors of a number of works [2, 11, 22, 23, 26, et al.] have advanced the hypothesis that tetanus toxin may increase the excitability of muscles and end-plates and may even throw the latter into a state of automatic exciatation. It has been assumed that these mechanism underlie the muscular rigidity observed in tetanus. Harvey's hypothesis [23], which holds that tetanus toxin, being a cholinesterase inhibitor, causes continual excitation of end-plates as a result of accumulation of acetylcholine, has attracted special attention. This hypothesis led to a whole series of confirmatory works, but their results were very inconsistent.

We may now consider the central origin of the muscular rigidity to be solidly established [4-6, 28, 29]. However, the problem of the action of tetanus toxin on the peripheral neuromuscular apparatus has lost none of its importance. We still do not know whether this toxin affects neuromuscular transmission or the characteristics of the changes which develop in this case. Elucidation of these problems is also of general interest in connection with the study of the pharmacological properties of tetanus toxin and the solution of certain problems of neuromuscular physiology.

EXPERIMENTAL METHOD

Our experiments were conducted on spinal (C_7-Th_1) cats without anesthesia (ether was administered only during preparation of the animal). Toxin in a dose of 1/100 MLD was injected fractionally into the left gastrocnemius muscle 6-8 h and 1.5-6 days before the experiment. We studied the electrical responses of the muscle (action potentials) on isolated and rhythmic indirect stimulation at various frequencies under normal conditions, during poisoning with tetanus toxin, and under the supplemental action of proserine (0.25 ml/kg administered intravenously) and d-paracurarine (0.3 mg/kg administered intravenously). The muscle was stimulated indirectly through the peripheral end of the sciatic nerve (the branches leading to other muscles were transected) with supramaximal square pulses lasting 0.1 msec. A volley of 10 pulses at different frequencies was employed in rhythmic stimulation. In the experiments involving administration of d-paracurarine we determined the rate at which neuromuscular transmission was blocked under normal conditions and in tetanus-toxin poisoning, testing conductivity every 2 sec. The action potentials of the muscle were led off by the method of Eccles and O'Connor [19].

A total of 45 animals were used in the experiments: 10 in the experiments with proserine (5 in the control and 5 in the experiment—1.5 h after administration of the toxin) and 35 in the experiments with d-paracurarine (10 in the control and 20 in the experiment—6-8 h and 1.5, 3, and 6 days after administration of the toxin, 5 animals in each group).

The results of our investigations were uniform and we may consequently limit ourselves to a general description of the data.

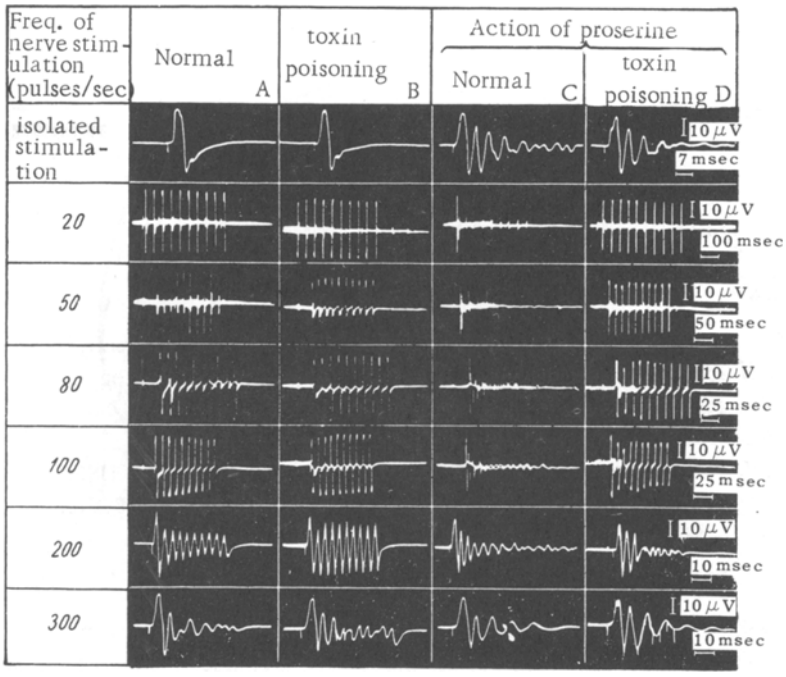

Fig. 1. Electrical responses of normal and tetanus-toxin poisoned gastrocnemius muscle to indirect rhythmic stimulation at various frequencies under the action of proserine (1.5 days after administration of toxin).

EXPERIMENTAL RESULTS

An isolated indirect stimulation produced an isolated action potential in both the normal and "tetanized" muscles (Fig. 1a and b); the "tetanized" muscle did not exhibit the multiplicity of responses to a single stimulation characteristic of the action of cholinesterase inhibitors [1, 7, 14, 18, 23].

A multiplicity of responses appeared after administration of the cholinesterase inhibitor proserine (Fig. 1c and d). However, the effect of proserine on the "tetanized" muscle was substantially less marked, occasionally being almost entirely lacking. This was especially obvious on rhythmic indirect stimulation.

Thus, comparison of the effects of tetanus toxin and the cholinesterase inhibitor which we tested, proserine [14], revealed both a similarity and a functional antagonism in the actions of these substances.

Experiments involving supplemental administration of d-paracurarine enabled us to establish that there is a functional synergism in the effects of tetanus toxin and this drug. It is manifested in the fact that a curare block develops more rapidly in the presence of tetanus intoxication (Fig. 2). While under our experimental conditions the rate at which neuromuscular transmission was blocked under the influence of d-paracurarine was 195±15 sec, 6-8 h after administration of the toxin it was increased by a factor of almost 1.5 (144±15 sec). The rate of curare-block development increased with the duration of intoxication (the difference was statistically reliable). We observed a parallel progressive decrease in the initial amplitude of the action potential of the "tetanized" muscle in response to indirect stimulation.

It may be seen from the material presented that tetanus toxin actually affects neuromuscular transmission. However, this action is not analogous to that of cholinesterase inhibitors. The multiplicity of responses which Harvey noted [23] in a "tetanized" muscle on indirect stimulation and which enabled him to hypothesize that tetanus toxin has the properties of cholinesterase inhibitors apparently resulted from the peculiarities of his experimental conditions and, perhaps, from increased specimen excitability, a phenomenon which may also be observed in normal muscle [19, 22].

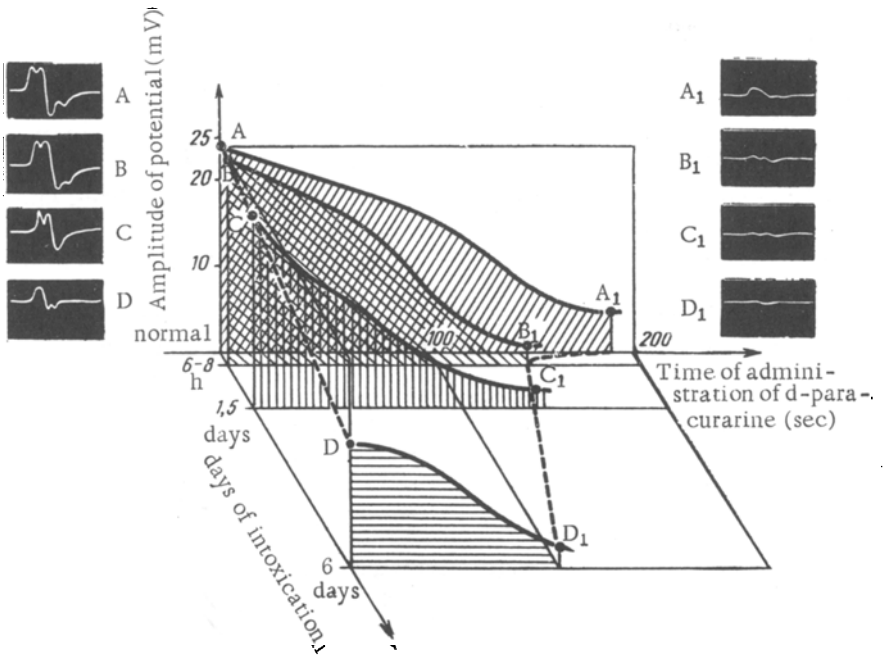

Fig. 2. Dynamics of the changes in the action potentials of the gastrocnemius muscle under the influence of d-paracurarine under normal conditions and at various intervals after administration of toxin. A-D) Responses of muscle to indirect stimulation before administration of d-paracurarine; A_1-D_1) at instant of development of block.

The data obtained enable us to conclude that tetanus toxin causes a disruption of neuromuscular transmission.

It is at present difficult to say in which portion of the process this disruption occurs or what its mechanisms are (the problem is now being studied). It may theoretically be assumed that each of the stages of synaptic transmission (the presynaptic apparatus, the end-plate, and the transition between the end-plate and the muscle fiber), as well as the muscle fiber itself, may be acted upon by tetanus toxin. Possible activation of cholinesterase has also been suggested. It is quite probable that the mechanism by which neuromuscular transmission is disrupted in tetanus intoxication is an impairment of mediator discharge by the presynaptic apparatus. This hypothesis is in accord with the experimental results of Ambache et al. [12], who showed that tetanus toxin disrupts cholinergic transmission in the rabbit iris, attenuating acetylcholine discharge, as well as with data indicating that a "tetanized" muscle does not lose its capacity to react to acetylcholine and carbocholine [12, 23].

The results of the experiments involving administration of proserine and d-paracurarine are in good agreement if we assume that neuromuscular transmission is disrupted under the influence of tetanus toxin and, in turn, substantiate this assumption: if tetanus toxin causes a disruption of neuromuscular transmission, it is clear that the depolarization block which develops as a result of the excess of acetylcholine under the action of proserine must be attenuated when the muscle is poisoned with toxin; for the same reason, a curare block, produced as a result of insufficient end-plate depolarization, develops more rapidly in a "tetanized" than a normal muscle.

The theory that tetanus toxin disrupts neuromuscular transmission may also explain a peculiarity which we detected in the responses of the "tetanized" muscle to indirect rhythmic stimulation at a comparatively high frequency (see Fig. 1b). This peculiarity lay in the fact that, in contrast to the normal muscle, the poisoned muscle was capable of responding with increasing action potentials to the first few stimuli in the rhythmic series and maintained its responses at the frequency pessimal for the normal muscle (see Fig. 1a and b).

This phenomenon may be explained in the following manner. It is well known that a neural impulse leaves behind an increased probability that synchronous discharge of acetylcholine quanta will occur under that action of the following pulse [24]. As a result, when the nerve is subjected to brief rhythmic stimulation at a rather high (but not extreme) frequency, before the presynaptic block develops [3, 8, 10, 21] the mediator mobilization and discharge

mechanisms are activated [21]. Under normal conditions, when all the muscle fibers and their synaptic apparatuses are in a comparatively uniform functional state, this mechanism promotes a pessimal muscle reaction because of the almost simultaneous development of a depolarization end-plate block in all or a majority of the motor units. In poisoning, when there are substantial differences in the functional state of the synaptic apparatuses of various muscle fibers, rhythmic stimulation promotes involvement in the reaction of those fibers which did not react to the first few stimuli because of disrupted synaptic transmission. The drawing of new muscle fibers into the reaction is manifested in the total action potential of the poisoned muscle and maintenance of the same relative response level on further stimulation as a result of the intermittent activity of the individual muscle fibers and groups of fibers.

This type of muscle reaction to indirect rhythmic stimulation is not observed solely in tetanus intoxication. It is also noted in other cases of relatively reversible functional disruption of neuromuscular transmission not produced by end-plate depolarization [9, 15, 25].

That tetanus toxin causes a disruption of neuromuscular transmission is of interest in several respects. First, it explains the long-known fact that, despite the rigidity, muscular atrophy develops in local tetanus, resulting from denervation of the muscle. It must be noted that Harvey [23] pointed out the presence of denervation phenomena in the affected muscle.

The disruption of neuromuscular transmission which occurs under the influence of tetanus toxin enables us to compare its action with that of botulin toxin, which is known to disrupt transmission by hampering mediator discharge [13, 15, 16, 27]. This comparison is of interest because of the similar amino acid composition of the 2 toxins and the fact that they are products of the vital activity of anaerobic Clostridia. Such comparisons have already been made in the literature [13, 17, 21].

In connection with the data presented above special interest inheres in the fact that tetanus toxin, like strychnine, is believed to be an agent which specifically disrupts postsynaptic inhibition [17, 20, 21]. It is assumed [20, 21] that this is effected by liberation of a special inhibitory mediator in the inhibited synapses. This makes a pressing problem of the specific mechanisms by which tetanus toxin acts on synaptic apparatuses of varying functional importance, and, on the other hand, forces us to turn to the question of the mechanisms underlying their activity.

If the hypothesis that an inhibitory mediator is liberated in inhibited synapses is confirmed and it is shown that tetanus toxin acts on the presynaptic apparatus, it must be acknowledged that this toxin can block the discharge of mediator, regardless of the nature of the latter. The results of our investigations enable us to assume that unusual denervation phenomena may develop in the central nervous system in tetanus intoxication.

SUMMARY

Comparison of the effects produced by tetanus toxin and proserine (neostigmine) demonstrated in experiments on cats that tetanus toxin was not a cholinesterase inhibitor. There exists a functional antagonism between the action of the tetanus toxin and that of proserine. At the same time, functional synergism was found to exist between the action of the toxin and that of d-paracurarine as evidenced by the fact that curare block developed more rapidly in the toxin-poisoned muscle than in the normal one. With increase of intoxication periods there was a drop in the action potential amplitude in the muscle, and curare block developed more rapidly. A characteristic response of the intoxicated muscle to indirect rhythmic stimulation with a fairly high frequency (pessimal for the normal muscle) was an increase in the action potential during the initial stimuli, and a relative maintenance of its value during the later stimuli.

The results of this work permit a conclusion that tetanus toxin disturbs the neuromuscular transmission. The possible mechanisms of this effect are discussed. It is very likely that tetanus toxin influences the excretion of the mediator by the presynaptic apparatus.

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