EFFECT OF TETANUS TOXIN ON MORPHOLOGICAL AND FUNCTIONAL STATE OF SYNAPSES ON SPINAL MOTONEURONS

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One of the features distinguishing the action of tetanus toxin is a disturbance of postsynaptic inhibition of spinal cord motoneurons [7, 10, 15, 17, 23]. However, the mechanisms of this disturbance remain unknown. The state of the synapses on motoneurons in the affected segments likewise has not been explained. Its study is of great importance not only to the understanding of the mechanism of action of tetanus toxin, but also in the wider neurophysiological plane in connection with investigations of the morphological and physiological properties and the localization of synapses of different functional significance.

The object of the present investigation was to examine these problems.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 180-200 g. Tetanus toxin was injected into the gastrocnemius muscle in a dose of 1/20 -1/25 MLD per rat. When given in this way, as the results of previous investigations [5, 7] showed, the toxin enters the ventral horns of the lumbar and sacral segments along bundles of motor fibers. The animals were used in the experiment 72 h after injection, when they presented a picture of severe local tetanus of the hind limb.

Electrophysiological investigations were carried out by the usual method [3] to study the state of mono- and polysynaptic reflexes and also certain types of post-synaptic inhibition of extensor motoneurons: direct inhibition and inhibition during stimulation of high-threshold muscle afferents. The magnitude of monosynaptic reflexes from segments L5 and L6 on both sides were compared in animals with local tetanus and in healthy rats (3 groups of experiments, 30 animals). The investigation was carried out on unanesthetized animals in which the spinal cord was divided 24 h before the experiment at the level T1-T2.

TABLE 1. Number of Impregnated Synapses on Bodies and Dendrites of Motoneurons

Series of experiments and variants of count	Number of motoneurons investigated: healthy animals	rats with local tetanus: same side as tetanus	rats with local tetanus: opposite side	Location of synapses	Mean number of synapses: healthy animals (1)	rats with local tetanus: same side as tetanus (2)	rats with local tetanus: opposite side (3)	P
I 1st	—	100	100	Body	—	17,63±0,78	25,45±1,18	$P_{2-3}<0,001$
				Dendrites	—	18,27±0,71	18,82±0,23	$P_{2-3}>0,9$
2nd	—	125	125	Body	—	17,28±0,71	20,42±0,55	$P_{2-3}<0,02$
				Dendrites	—	17,04±0,62	15,65±0,61	$P_{2-3}>0,1$
II	418	212	216	Body	20,68±0,32	17,45±0,44	21,24±0,64	$P_{1-2}<0,001$ $P_{1-3}>0,4$ $P_{2-3}<0,001$
				Dendrites	17,99±0,36	20,50±0,60	19,15±0,55	$P_{1-2}<0,001$ $P_{1-3}>0,1$ $P_{2-3}>0,1$

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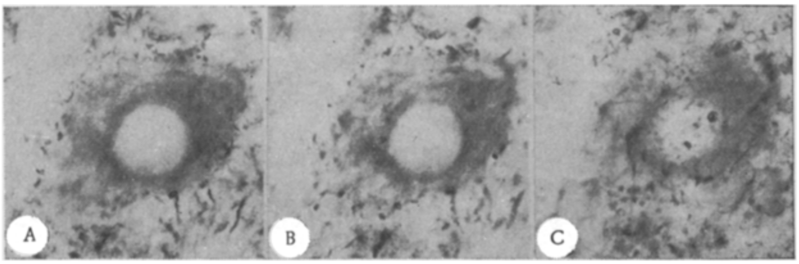

Fig. 1. Impregnated ring-shaped synapses on body and dendrites of a motoneuron of the 6th lumbar segment of the spinal cord of a control rat. A-C) Different planes of cross section of motoneuron. Golgi–Deineka, objective 90 (oil immersion), ocular 10.

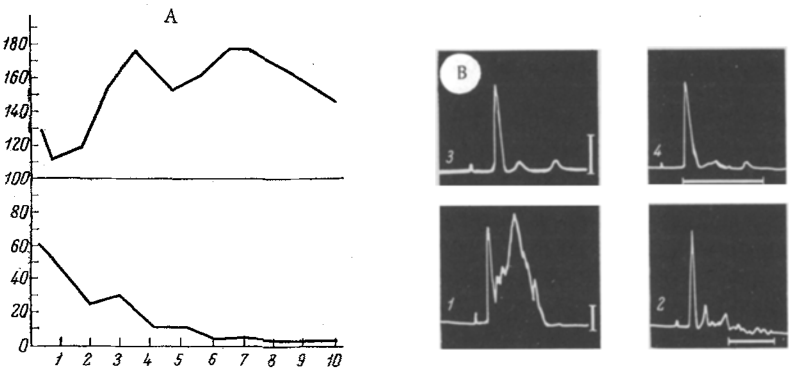

Fig. 2. State of inhibition of monosynaptic and polysynaptic reflexes in local tetanus. A) curves of inhibition of monosynaptic reflexes caused by stimulation of the tibial nerve, during stimulation of the peroneal nerve of a healthy animal (below) and in local tetanus (above). Abscissa–interval (in msec) between conditioning and test stimuli, ordinate–change in amplitude of monosynaptic reflex (in percent of original value); B) reflex responses to stimulation of the tibial nerve in a rat with local tetanus on the side of injection of the toxin (1), on the opposite side (2), and in a healthy rat on the left (3) and right (4) side. Time marker 10 msec, calibration 1 mV.

For the morphological investigation the animals were decapitated and the lumbar enlargement of their spinal cord was impregnated with silver by the Golgi–Deineka method. The motoneurons of the lateral nucleus of the ventral horn of segments L5 and L6 were investigated in serial sections 15μ in thickness. The number of ring-shaped synapses was counted separately on the bodies and dendrites of the motoneurons in 3 planes (Fig. 1); in the plane of the nucleolus (B) and in planes situated directly above (A) and below (C) it. Synapses on the dendrites of the motoneurons were counted throughout the length of these processes, not exceeding the longer diameter of the body of the particular motoneurons. In the experiments of series I, two variants of counting were used: in the first the number of synapses was counted on the large motoneurons, the greatest diameters of whose bodies were approximately the same (every 10th section was used; altogether 20 sections from each of 5 animals); in the second the number of synapses was counted on 5 motoneurons lying at random in a field of vision (every 40th section was used; altogether 5 sections from each animal). In the experiments of series II all motoneurons of the lateral nucleus of the ventral horn encountered in the sections were investigated (every 20th section was used; altogether ten sections from each of 5 control and 5 experimental animals).

EXPERIMENTAL METHOD

In agreement with results obtained by other authors experimenting on cats [10, 15, 23], the present experiments showed that in rats with local tetanus the types of postsynaptic inhibition investigated were disturbed. Instead of inhibition, facilitation of the monosynaptic reflexes was recorded, sometimes amounting to a very considerable degree (Fig. 2, A). It may be assumed that in both cats and rats, not only these but also other types of postsynaptic inhibition are disturbed. Another characteristic sign was a sharp increase in the polysynaptic reflexes evoked by stimulation both of the cutaneous nerves and of high-threshold muscle afferents (Fig. 2, B). These results are in agreement with those of experiments on other animals [10, 15, 16, 23]. At the height of development of tetanus, polysynaptic activation became practically constant, resulting in an almost continuous efferent flow.

The results of the study of monosynaptic responses using statistical analysis showed that at this stage of tetanus poisoning the monosynaptic reflexes were not significantly affected. Their magnitude varied to one side or the other, but in the animals of different groups these variations lay within the same limits. In relation to the present investigation the important feature is that no regular decrease in the monosynaptic reflexes was found at this stage of poisoning. As mentioned above, instead of inhibition, facilitation of the monosynaptic reflexes was observed, but in response to combined monosynaptic and polysynaptic stimulation, which reflects the character of activation of the motoneurons in tetanus more adequately [7], the monosynaptic reflexes could increase in the process of stimulation.

The results of the morphological investigation are given in Table 1, which shows that in local tetanus the number of impregnated synapses on the bodies of the motoneurons was reduced on the side of the tetanus. This decrease was found in the experiments of both series I and II, and was statistically significant. The number of impregnated synapses on the dendrites was practically the same when the side of the tetanus and the opposite side were compared (experiments of series I) and showed a statistically significant increase when the "tetanus" side was compared with both sides of the spinal cord of the healthy animals (experiments of series II). The reason for this difference is that in animals with local tetanus the mean number of impregnated ring-like synapses on the dendrites of the motoneurons on the opposite side was greater than in healthy animals (experiments of series II).

The morphological data thus indicate that in local tetanus the number of impregnated ring-like synapses in the affected segments of the spinal cord is reduced on the body of the motoneurons and increased on their dendrites.

Data in the literature [14, 19] indicate that impregnation of the ring-like synapses reveals a specific structural unit of the cytoplasm in the presynaptic enlargement of the axon – the neurofilaments – on account of their affinity for silver. It may be assumed that the changes discovered in the number of impregnated synapses reflect a change in the affinity of these neurofilaments for silver. Since a functional load causes an increase in argentophilia of synaptic structures [1, 2, 9], the increase observed in the number of impregnated ring-like synapses on the dendrites of the motoneurons in local tetanus may indicate that they are in an active state. Conversely, the decrease in number of impregnated synapses on the body of the motoneurons may be evidence that some axosomatic synapses are in an inactive state.

Comparison of the morphological data with the results of electrophysiological analysis suggests that these inactive synapses may be inhibitory synapses, for only the processes of postsynaptic inhibition of the motoneurons are disturbed. However, many excited synapses in local tetanus are in a state of increased activity, so that polysynaptic reflexes are greatly increased. Consequently, after impregnation with silver, the number of synapses belonging to these reflex arcs must be increased.

The results obtained and the conclusions drawn from them are in agreement with Gesell's hypothesis [18] and recent observations [11-13] demonstrating the selective localization of inhibitory synapses on the soma of the neuron and of excitatory synapses on the dendrites.

When this investigation had been carried out and the preliminary results published [7, 8], a report appeared of an electron-microscopic investigation of synapses on rat motoneurons during tetanus poisoning [22]. Considerable changes in ultrastructure were found in the axosomatic synapses, corresponding to the observations made with the optical microscope described above. At the same time, the fine structure of the axodendritic synapses was not significantly changed. The authors cited consider that their results support the view that inhibitory synapses are located on the body of neurons.

The results of the present investigation may be interesting also in connection with the study of mechanisms of action of tetanus toxin, and especially mechanisms of disturbance of postsynaptic inhibition in tetanus. They may be regarded as results which provide some evidence in support of the earlier hypothesis that the disturbance of synaptic transmission in tetanus poisoning may be due to changes in the presynaptic apparatus, as a result of which synthesis or release of the mediator is disturbed [6, 7, 15]. On the other hand, the results may also be important for the further analysis of the problem of localization of functionally different synapses, especially inhibitory synapses, the morphological and physiological properties of which remain largely unexplained.

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