ELECTROPHYSIOLOGICAL ANALYSIS OF DISTURBANCES IN REFLEX ACTIVITY OF THE SPINAL CORD IN EXPERIMENTAL BOTULISM TYPE A IN WARM - AND COLD-BLOODED ANIMALS

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It was determined by us [2, 3, 4] in the investigation of the action of botulism toxin on the nervous system that exclusion of nervous influences on tissue results from the damaging effect of the toxin on afferent divisions of nerve centers. However, the mechanographic method used by us in published reports enabled us in the case of botulism to obtain only an incomplete idea of the actual functional disturbances in the nervous elements.

In this connection the problem of the current project was the study of electrical activity of the anterior roots of the spinal cord when stimulated reflexively in the presence of a unilateral involvement of cord segments produced by small doses of botulism toxin with resulting "local" form of botulism – paralysis of one of the hind legs. The second, unparalyzed, side served as a control. Aside from this, it was of interest to clarify the functional condition of the peripheral motor nerve conductors in the zone of botulism paralysis. In the role of indices characteristic of the functional condition of motor nerve trunks we used lability and duration of the phases of absolute and relative refractivity.

The literature indicates that the magnitude of the given indices reacts delicately to metabolic changes in nerve trunks [6].

In relation to the electrical activity of striated muscles and nerves, it was made clear that botulism toxin does not produce any changes in oscillograms of motor nerves and does not slow down transmission of excitation waves along them [7, 8, 9]. This was confirmed on the A, B, C fibers of the sciatic, phrenic and vagus nerves [7]. However, when neuromuscular block due to botulism develops, recording of the muscular electropotentials showed that when a motor nerve is stimulated by two maximal stimuli with an interval between of 10 to 300 milliseconds, the second stimulus causes a greater muscle activity current than the first. The authors explain such of fact by summation of excitations in an area located somewhere near myoneural junctions.

EXPERIMENTAL METHODS AND RESULTS

Experiments were carried out on primed and unprimed cats and frogs. Priming was produced by the administration of botulism toxin type A (1 Mld = 0.00005 mg), dissolved in saline solution, into the gastrocnemius muscle in the following doses: cats – 0.25 mg/kg body weight (5,000 mouse Mld , frogs – 0.15 – 0.2 mg per 35-40 gm body weight (3,000 – 4,000 mouse Mld). In cats receiving the indicated doses, "local" botulism developed 2-3 days later – a complete paralysis of the muscles of the involved hind leg. In frogs, however, "local" botulism also developed in 2-3 days, but by the 4th – 5th day it went on, as a rule, to a "generalized" form, i.e. general paralytic botulism syndrome. Recording of activity currents from the anterior roots of the spinal cord and investigation of lability and of refractivity phases were carried out in cases of "local" botulism on both sides – the paralyzed and, as a control, the unparalyzed, but in cases of "general" – only on one side.

Under deep chloroform-ether anesthesia the spinal canal was opened at the lumbar level and the anterior roots of the spinal cord at the level of the 6th and 7th lumbar segments were severed; the skull of the animal was then trephined for decerebration. Following decerebration the anesthesia was discontinued and nerve twigs to the gastrocnemius were severed. One to 2 hours after decerebration the central end of the divided anterior root of the 7th lumbar segment was placed on platinum electrodes connected with the entry to the amplifier and the central end of the divided muscle nerve twig placed on the electrodes connected with the exit of the generator of the universal apparatus constructed by us for electrophysiological investigations [5].

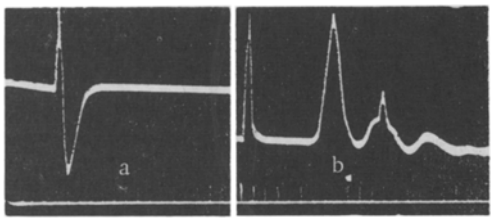


Fig. 1. Electrical activity of the anterior roots of the 7th lumbar segment of the spinal cord of a cat on the paralyzed (a) and contralateral side (b). Time mark — 1,000 cycles per second.

Mono- and polysynaptic and electrotonic potentials recorded in the anterior roots of the spinal cord were produced by stimulation of the central ends of the divided nerve twigs with rectangular stimuli of varying amplitude. Similar methods were often employed by domestic investigators [1]. Phases of relative and absolute refractivity were investigated by stimulating the trunk of the greater tibial and lesser tibial nerves with paired impulses with regulated intervals between them. The beginning of the phase of relative refractivity was recognized by the decrease of the second activity current on the oscillogram, the beginning of the phase of absolute refractivity — by the complete disappearance of the current. The beginning of transformation of the stimulation rhythm was recognized as the index of lability. Activity currents of the anterior roots of the spinal cord and of the motor nerves were photographed with a small camera ("Exacta," "Zenith") from the screen of the oscilloscope of the universal apparatus with momentaneous or repeated unwinding. In some of the experiments currents of nerve activity were recorded with the aid of a trained oscillograph of the type MPO-2.

Almost an analagous method was used in experiments on frogs. Under deep chloroform-ether anesthesia the spinal canal of the animals was opened and the anterior roots at the level of the 7th and 8th segments of the cord were severed. The latter were divided at the entrance to the intervertebral orifices. Anesthesia was discontinued and 2-3 hours after dissection the part of the experiment dealing with potentials of activity of the central ends of the divided anterior roots was carried out. Electrical activity of the root was provoked, as in the experiments with cats, by stimulation of the central ends of the divided nerve twigs to the gastrocnemius muscle of the ipsilateral side with single rectangular impulses. Investigation of the refractivity phases did not differ from analogous experiments on cats.

A total of 59 experiments was performed upon cats and 94 upon frogs.

In the first series of observations we investigated the character of electrical activity of the anterior roots of the primed segments of the spinal cord by stimulating the ipsilateral sensory nerve twigs of the completely paralyzed extremity. As controls we used analogous data obtained from the anterior roots of unprimed areas of spinal cord or from normal animals. Inasmuch as paralyses of the involved extremity develop only on the 2nd or 3rd day after intramuscular injection of botulism toxin (in sublethal doses), recording of electropotentials of the anterior roots was not done until after this period. In investigating the electropotentials of the anterior roots a total of 18 experiments were performed on cats (from the 4th to the 15th day after priming) and 14 similar experiments on frogs (from the 2nd to the 7th day after administration of the toxin). In all animals a form of "local" botulism developed — paralysis of the involved hind leg. Experiments showed that in warmblooded as well as in coldblooded animals once paralysis of an extremity develops it is impossible to record, during any of the indicated periods, any electrical activity in the anterior roots of the primed segments of the spinal cord; at the same time on the symmetrical unparalyzed side similar activity undergoes no noticeable changes.

Figure 1a shows that stimulation of the central end of a divided nerve twig to the gastrocnemius muscle of the paralyzed extremity with a single shock of a rectangular current on the 5th day after the administration of botulism toxin is not accompanied by the potential of activity in the anterior root of the primed segment of the spinal cord; all that is observed is the artefact of the loop of the stimulating current. In Fig. 1b is shown the control oscillogram of the current of activity in the anterior root of the spinal cord of the same animal, but on the unparalyzed side from which it is evident that development of mono- and polysynaptic potential in the anterior root is produced by stimulation of the sensory nerve twigs of the gastrocnemius muscle.

Similar fall of electrotonic currents of activity in the anterior roots was noted on the paralyzed side in frogs with "local" botulism; on the unparalyzed side the electrotonic reactions of the anterior roots of the spinal cord were preserved. It must be pointed out that the magnitude of the latent periods and the character of the electrical activity of the anterior roots on the unparalyzed side of animals with "local" botulism were found to be entirely similar to corresponding controls obtained in 2 experiments on normal cats and in 5 experiments on frogs, which in turn did not numerically differ from data to be found in the literature [1].

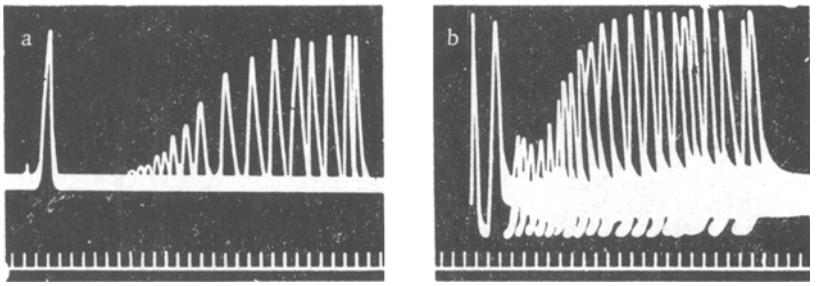


Fig. 2. Phases of refractivity of the lesser tibial nerves of the cat's paralyzed (a) and unparalyzed (b) extremity on the 22nd day after priming. Time mark – 1,000 cycles per second.

Thus, experimental results with "local" botulism clearly indicate that sausage poison causes the paralytic syndrome as a result of exclusion of motor neurons of the anterior roots of the spinal cord which blocks transmission of excitation to motor nerve conductors.

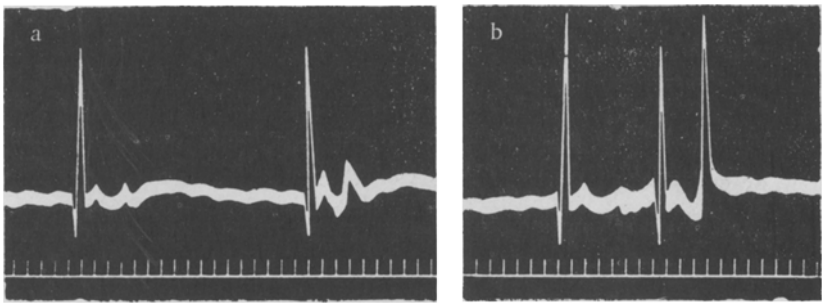
Further it became interesting to determine whether any change takes place in the functional properties of motor nerve trunks following the affection of their " trophic" centers – nerve cells – with botulism toxin. Data from the literature testifies to the fact that the oscillogram of a nerve primed with botulism toxin does not differ from that of a normal nerve; however, we were unable to find any information in the literature concerning changes in functional properties of nerve conductors in botulism. In this connection we investigated the duration of phases of absolute and relative refractivity at various intervals following the development of botulism paralysis, and in some experiments – immediately the lability of the greater or lesser tibial nerve in the zone of "local" botulism. As controls we used analogous indices obtained on nerves of the contralateral unparalyzed side or in experiments on normal animals.

A total of 34 experiments were carried out on cats (from the 3rd to the 25th day after priming) and 32 experiments on frogs (from the 2nd to the 7th day after administration of toxin).

Experiments on both species of animals revealed similar regularity: early after the development of the paralytic botulism syndrome (up to the 4th – 6th day in cats and up to the 2nd day in frogs) the duration of the refractivity phases and the lability of motor nerves remain close to normal. Thus, the duration of the phase of relative refractivity of the greater and lesser tibial nerves on the unparalyzed side in cats was 4 – 8 milliseconds and of absolute – 0.8 – 1.8 milliseconds. In the unparalyzed sciatic nerve of frogs the duration of the phase of relative refractivity was 12 – 16 milliseconds, and absolute – 2.2 – 3.2 milliseconds. Identical values were obtained in 5 experiments on normal cats and in 8 experiments on frogs. In the zone of botulism paralysis during the early periods following priming referred to above, the duration of the relative phase of refractivity of the motor nerves in cats was 9 – 13 milliseconds, and absolute 1.8 – 3.2 milliseconds; in frogs the first increased to 17, the second – to 3.7 – 5 milliseconds. The lability of the paralyzed nerves underwent a change too: in normal nerve, transformations of the excitation rhythm were noted at a frequency of approximately 500 cycles per second, but in the paralyzed nerve – at a frequency of 450 cycles per second and less.

Beginning with the 7th – 8th day after development of paralysis in cats and with the 3rd – 4th day in frogs, refractivity phases progressively continue to increase and reach the maximum: in cats – on the 10th – 14th day and in frogs on the 5th – 6th day. The phase magnitude remains relatively stable during the later stages of the investigation. During the current stage of intoxication the duration of the phase of relative refractivity of motor nerves in the cat is 18 – 22 milliseconds and of absolute refractivity – 6 – 12 milliseconds; in frogs the former reaches 28 – 32 milliseconds and the latter – 8 - 10 milliseconds.

In Fig. 2 a, one can see that there is a considerable increase in the phases of relative and absolute refractivity in the zone of "local" paralysis in cats following intramuscular injection of botulism toxin type A in the dose of 0.3 ml $1 \cdot 1^{-3}$ per 1 kg body weight into left hind leg. In Fig. 2 b is presented an oscillogram of the lesser tibial nerve on the unparalyzed side of the same animal. The duration of the refractivity phases is within normal limits. Thus, despite the ability of a motor nerve in the presence of botulism to conduct excitation, its functional properties are significantly altered. An important role in the dynamics of changes in functional properties of motor nerves in the form of substantial increase in refractivity phases and decrease in lability is played by the disturbance of trophic effects of spinal nerve centers whose activity is the first to be destroyed by botulism toxin. In this connection it can be assumed that alteration in the functional properties of motor nerve conductors is due to severe disturbances in their metabolism.



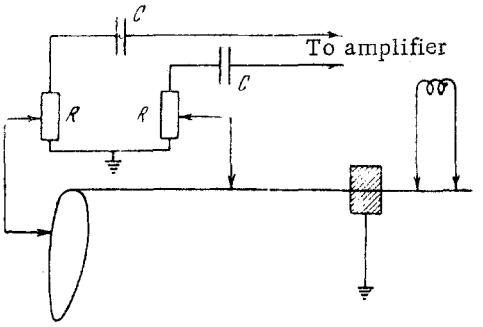


Fig. 3. Summation of excitation in the transmission of two nerve electropotentials from the nerve to the muscle in a zone of botulism paralysis; a) absence of transmission of two nerve activity currents from nerve to muscle within an interval of 36 milliseconds; b) appearance of transmission of excitation from nerve to muscle as a result of summation within an interval of 13 milliseconds between impulses. Time mark – 500 cycles per second.

In the last series of experiments on frogs poisoned with botulism toxin the purpose was to determine whether the phenomenon of summation in the transmission of only the second impulse through the myoneural junction when the nerve is presented with two stimuli is associated with alteration in the functional condition of the paralyzed motor nerve. In 19 experiments, as presented in Fig. 3, we recorded simultaneously the activity currents from the greater tibial nerve and the gastrocnemius muscle.

Experimental results indicated that in the development of botulism paralyses, the disturbance of transmission of excitation from nerve to muscle is observed only 4 – 6 days after priming, i.e. at a time when recording of significant alterations in the functional properties of motor nerve trunks can be made. According to our data, summation in the transmission of two nerve activity currents occurred with an interval between them of 12 to 54 milliseconds (Fig. 3, a, b). In experiments performed on the 2nd – 3rd day after priming, as in the 16 controls on normal animals, the phenomenon of summation was not observed inasmuch as the appearance of nervous activity current was always accompanied by an electrical response of the muscle.

Partial disturbance in conductivity of excitation through the myoneural lamina in paralytic botulism syndrome is apparently associated with pronounced alterations in functional properties of motor nerve conductors only.

On the strength of the electrophysiological data obtained, we can come to the conclusion that botulism toxin paralyzes the motor centers of the spinal cord first. Such a paralysis of the nerve center is accompanied apparently by a severe disturbance in its trophic effect on peripheral motor nerve conductors which results in gradual development of significant alterations in functional properties of nerve trunks and myoneural junctions.

SUMMARY

The effect of botulism toxin on the electric acitivity of the spinal cord was studied by the electrophysiological method. The author has demonstrated on frogs and cats that in "local" botulism the mono-polysynaptic and electrotonic reactions are absent in the anterior roots of the spinal cord. The functional condition of motor nerves was examined. It was revealed that with development of botulism poisoning, especially in late stages, a significant prolongation of the phases of relative and absolute refractivity and decrease of lability take place.

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