MECHANISM OF DISTURBANCES OF ELECTRICAL ACTIVITY OF SPINAL CORD NEURONS IN EXPERIMENTAL BOTULISM

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Ventral root potentials of segments L_7–S_1 and electrical activity of single neurons in these same segments of the spinal cord were investigated in cats with local botulism. Development of mild pareses of the affected limb was accompanied by a decrease in amplitude of the monosynaptic ventral-root potentials and by loss of ability of some motoneurons to generate monosynaptic action potentials. Later, as complete paralysis developed, loss of mono- and polysynaptic reflex discharges associated with a sharp decrease in excitability of the α-motoneurons was observed. The latter lose their ability to generate action potentials in response to synaptic and antidromic stimulation. During investigation of the background and evoked electrical activity of interneurons no visible changes were found in their functional state in either the early or late stages of experimental botulism.

The results indicate selective damage to the spinal cord α-motoneurons by botulinus toxin.

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We have previously shown [5] that simple and complex spinal reflexes are blocked in botulism. Activity of the spinal motor centers is lost first, and this is followed as a result of degenerative processes in the nerve trunk by disappearance of phasic and preservation of tonic nervous influences on the skeletal muscles [5, 6].

Because of these results it was interesting to determine changes in the functional state of spinal cord neurons of different types in the course of experimental botulism.

EXPERIMENTAL METHOD

Experiments were carried out on cats weighing 2.5-3.5 kg, some of which were healthy and the rest poisoned with type A botulinus toxin (1 MLD for mice is equivalent to 0.00001 mg of the dried toxin), injected intramuscularly in a dose of 0.3-0.4 mg/kg in Ringer's solution into one hind limb. Paralysis of the affected limb developed 72-96 h after injection of the toxin. Investigation of evoked electrical activity of the ventral roots and of single neurons of the spinal cord was carried out on the side of injection and on the intact side 24, 48, 72, and 96 h after injection of the toxin. Spontaneous activity of the interneurons was investigated against the background of the developing paralytic syndrome of the affected limb at the stage of early (4-5 days after poisoning) and late paralysis (14-16 days after poisoning).

Recording of the ventral root potentials and intracellular recording of the action potentials (AP) of single spinal cord neurons were carried out by the usual methods [2, 4, 8]. Motoneurons were identified by their antidromic and monosynaptic AP generated in response to stimulation of limb nerves: nerves to the gastrocnemius muscle, posterior tibial and peroneal nerves.

EXPERIMENTAL RESULTS

The investigations showed that changes in electrical activity of the ventral roots of the spinal cord appear in the preparalytic stage of botulinus poisoning. They consist of a decrease in amplitude of the monosynaptic potentials to 1.03 ± 0.2 mV ($P < 0.001$) compared with the control (1.74 ± 0.07 mV). As a rule the amplitude of the polysynaptic discharges was indistinguishable from the control results. Development of paralysis of the affected limb was accompanied by total loss of the ventral root potentials on the side of injection of the toxin.

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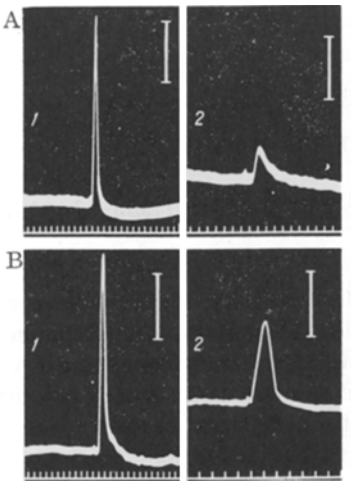

Fig. 1

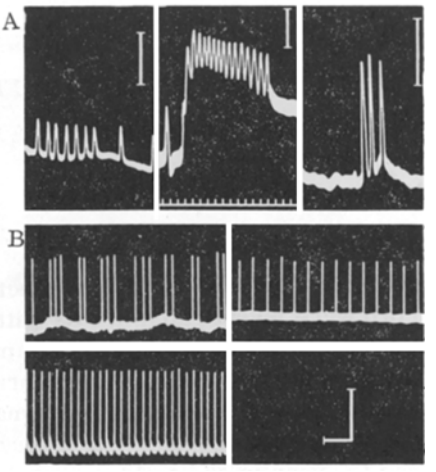

Fig. 2

Fig. 1. Responses of motoneurons to monosynaptic and antidromic stimulation in local botulinus poisoning. A: 1) Monosynaptic AP of motoneuron in nucleus of nerve to gastrocnemius 24 h after injection of toxin; 2) monosynaptic EPSP of motoneuron in nucleus of nerve to gastrocnemius 48 h after poisoning; B: 1) antidromic AP of motoneuron in nucleus of posterior tibial nerve 24 h after poisoning; 2) IS-component of antidromic AP of motoneuron of posterior tibial nerve 72 h after intramuscular injection of botulinus toxin. Calibration: amplitude 25 mV, time marker 500/sec.
Fig. 2. Electrical activity of spinal cord interneurons against a background of botulinus paralysis of one hind limb (96 h after injection of toxin, affected side). A) Evoked discharges of interneurons; B) several types of spontaneous activity of interneurons. Calibration: amplitude 25 mV, time marker 50 msec.

Because of these results, in the next series of experiments, changes in the functional state of single spinal cord motoneurons were studied by means of microelectrodes at times when the generation of ventral root potentials was disturbed. The functional state of the motoneurons on the side of injection of the toxin showed no change from the control 24 h after injection, before the appearance of clinical manifestation of poisoning. Later 48 h after intramuscular injection of the toxin as pareses of the muscles of the affected limb developed, significant disturbances of the AP of individual motoneurons were found. About one-third of the monosynaptically stimulated motoneurons generated only an excitatory postsynaptic potential (EPSP) of amplitude just below threshold (Fig. 1). The remainder of the motoneurons under the influence of this type of stimulation generated AP similar in amplitude to the control.

In the preparalytic stage of experimental botulism, besides a decrease in amplitude of the ventral root monosynaptic spikes, one-third of the motoneurons showed defects in the synaptic mechanisms of EPSP generation. Most motoneurons at this stage of poisoning remained capable of AP generation irrespective of the type of stimulation.

Against the background of paralysis of the affected limb 72 h after poisoning we found that the ability of the motoneurons of the affected motor nuclei to become excited synaptically was almost completely lost. The number of motoneurons capable of antidromic excitation also was considerably reduced and most of them generated only the IS-component of the spike (Fig. 1). With the dose of toxin chosen, a full range of movements was preserved on the intact side and the electrical activity of the motoneurons was unchanged.

More profound injury to the motoneurons was discovered 96 h after injection of the toxin: only a few motoneurons responded to antidromic stimulation by an IS-component. No AP were generated either when the strength of stimulation was increased or when the type of stimulation was changed. At this stage of poisoning changes were also found in the activity of motoneurons on the opposite side: about 10% of the monosynaptically stimulated motoneurons generated only EPSP instead of AP. However, these changes were not significantly reflected in the range of limb movements on the intact side.

TABLE 1. Distribution of Spontaneous Activity of Spinal Cord Interneurons by Character of Rhythm and Frequency of Discharges in Healthy Animals and Animals Poisoned with Botulinus Toxin (in percent of total number of recorded neurons)

Series of experiments	Side of investigation	Number of animals	Number of cells	Character of rhythm			Frequency of discharges						
				single discharges		grouped discharges	less than 9	10-19	20-29	30-39	40-49	50-59	more than 60
				regular rhythm	irregular rhythm								
Control		12	174	23	58.5	18.5	12.6	24.6	20.1	14.4	10.3	8.1	9.8
Botulinus poisoning	Paralyzed		163	21.5	62.5	16	10.4	26.4	20.8	18.4	11.6	7.4	5.0
Early paralysis	Intact	10	95	22	61.7	16.3	13	27	19.5	12.5	9.7	8.8	10.0
Late paralysis	Paralyzed		175	20	63.7	16.3	17.7	22.8	22.3	15.4	7.4	5.7	8.6
	Intact	9	80	23	59.8	17.2	15.1	23.4	22.5	12.3	10.0	7.0	9.7

The duration of the latent period during antidromic and orthodromic stimulation was not significantly changed at all stages of botulinus poisoning.

It can be concluded from these results that the appearance of pareses and paralyses in botulism is associated with progressive injury to the α-motoneurons of the spinal cord. The fact that in preparalytic stages of botulinus poisoning there is dissociation between toxic damage to the processes of monosynaptic and polysynaptic excitation of the motoneurons can be explained by differences in the location of synaptic structures responsible for these processes on the soma and dendrites [7].

Because of the important role of spinal cord interneurons in maintenance of the optimal level of excitability of the motor centers of the spinal cord [3], the next stage of this investigation was to discover whether the decrease in excitability of the spinal cord motoneurons in botulism is connected with changes in spontaneous and evoked activity of the interneurons in the affected segments of the spinal cord.

As a first step we investigated the spontaneous spike activity of interneurons in segments L_7–S_1 of the spinal cord of healthy animals and of animals receiving injection of botulinus toxin. As Table 1 shows, the spontaneous activity of most recorded interneurons in both the early and late stages of the paralytic syndrome consisted of irregular spindle discharges. The frequency of the discharges usually remained stable during recording and it varied in individual neurons from 2 to 150/sec, most commonly being 10-30/sec. The spontaneous activity of some interneurons was characterized by grouped discharges (Fig. 2, B). The largest number of neurons possessing spontaneous activity was recorded in the region of the posterior horns and intermediate zone of the gray matter of the spinal cord. If the distribution of spontaneous spike activity is compared by the character of the rhythm and frequency of the generated discharges in the experimental and healthy control cats and consideration is paid to data in the literature [4], it will easily be seen that the spontaneous activity of the interneurons is not visibly changed in experimental botulism. Consequently, neither the synaptic mechanisms responsible for excitation of spontaneously discharging interneurons nor the ability of these interneurons to generate action potentials is significantly disturbed as a result of the action of botulinus toxin.

The study of evoked activity of the interneurons showed that both in healthy animals and in those poisoned with botulinus toxin in the preparalytic and paralytic stages of poisoning, AP were generated after different latent periods (2.2-27 msec) and they varied considerably in amplitude (20-67.5 mV). The discharges were mainly grouped, the frequency of the groups and the number of spikes in the discharge varying within wide limits, reaching highest values in reflex discharges of the Renshaw cells (frequency

up to 1440/sec, number of impulses in discharge up to 24; Fig. 2, A). Similar variability of responses of spinal cord interneurons has been observed by other workers [1, 2, 4, 9-11].

The results thus demonstrate the absence of a pathogenic action of botulinus toxin on the interneuron system. This confirms the conclusion that in botulism the paralytic syndrome arises as a result of selective damage to the α-motoneurons of the anterior horns of the spinal cord.

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