ROLE OF DISTURBANCE OF AXOPLASM SYNTHESIS IN THE NERVE CELL IN THE MECHANISM OF INJURY OF PERIPHERAL NEUROMUSCULAR JUNCTIONS BY BOTULINUS TOXIN

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In botulism the pathogenic action of the toxin extends mainly to the motor "tetanic" neuromuscular junctions, while the "tonic" junctions retain their activity. It has been found that the "tetanic" neuromuscular junctions are injured invariably through involvement of the neural centers [1, 5]. It is not yet clear, however, what is the role of the nerve cells of the centers in the mechanism of injury of the peripheral neuromuscular junctions by botulinus toxin.

In the present study the changes in the speed of movement of the axoplasm were investigated and compared with the disturbance of the functional state of single medullated nerve fibers in the course of botulinus poisoning.

EXPERIMENTAL METHOD

Experiments were carried out on frogs of the species Rana ridibunda, poisoned with botulinus toxin (1 MLD for mice, 0.00001 mg of dry toxin) and procuran. The poisons were injected intramuscularly: the toxin in a dose of 0.1 mg/70 g, procuran in a dose of 1 ml (0.002 g)/70 g body weight. Acute experiments were carried out on spinal preparations of poisoned and nonpoisoned animals. Movement of the axoplasm was recorded by means of a type MKU-1 motion picture camera. Using Tasaki's method [4], from 2 to 5 single thick medullated fibers were dissected from the sciatic nerve. Next, with a magnification of 630×, automatic filming took place for 2-3 h at a speed of 1 frame every 30 sec. Later, using a type K-301 projector, the exposed film was examined at a speed of 24 frames/sec on a screen measuring 1.0 × 1.3 m. Altogether 356 medullated nerve fibers were used in the experiments, 41 of them as controls.

The indices of the functional state of the single nerve fiber were the duration of the phases of relative and absolute refractoriness and the velocity of conduction of the nervous impulse. The method of dissection of the single fibers was the same as that used when taking motion pictures. The single nerve fiber was placed on the active platinum electrode, the indifferent electrode (a silver plate) was inserted into the animal's mouth, and the stimulating platinum electrodes were placed on the sciatic nerve as close as possible to the spinal cord. The nerves were stimulated by rectangular pulses of current from a generator of paired impulses with a radiofrequency output [2].

Altogether 70 single fibers were investigated in different animals, 23 of them as controls.

EXPERIMENTAL RESULTS

Synthesis of the cytoplasm in the nerve cell and its transport along the axon constitute the most important mechanism maintaining the functional capacity of the nerve fiber [1]. This fact was used for the analysis of the causes of loss of the phasic component of the nervous influence on the skeletal muscle in late forms of botulinus paralysis.

In the experiments of series I the changes in the velocity of transport of the axoplasm in single medullated nerve fibers of the sciatic nerve were studied in the course of development of botulinus poisoning. As Table 1 shows, the speed of movement of the axoplasm began to change long before the development of a generalized botulinus paralytic syndrome. In six experiments performed 18-22 h after injection of the toxin the velocity of movement of the axoplasm in the frogs which had developed hardly perceptible pareses of the skeletal muscles had

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TABLE 1. Changes in the Velocity of Movement of the Axoplasm in the Course of Botulinus Poisoning in Frogs (in mg/day)

Character of procedure	Clinical signs of injury	n	M±m	P_1	P_2
Control		41	312.2±5.35		
Procuran (0.002g/70 g body weight, into gastrocnemius muscle)	Paralysis of the whole skeletal musculature	33	300.6±6.19	0.5	
Botulinus toxin (0.1 mg/70 g body weight, into gastrocnemius muscle)	Stage I (mild paresis)				
	a) 18-22 h after injection	6	2026.1±14.1	0.001	0.001
	b) 22-26 h after injection	10	1040.1±21.2	0.001	0.001
	c) 25-28 h after injection	25	550.2±10.4	0.001	0.001
	Stage II (marked paresis)				
	a) 36-48 h after injection	33	311.0±8.9	0.5	0.5
	b) 48-72 h after injection	27	175.0±7.1	0.001	0.001
	Stage III (generalized paralytic syndrome) 96 h or more	181	0	—	—

Note. Here and in Table 2: P_1—comparison with control; P_2—comparison with procuran.

increased to more than 6 times the control figure. In the later stages (after 36-72 h) the velocity began to fall gradually, and in the stage of the generalized paralytic syndrome (after 96 h or more) movement of the axoplasm ceased altogether.

It may be postulated that the disturbance of the synthesis of the axoplasm and of its movement along the axon was connected with the development of asphyxia, arising as a result of the paralysis of external respiration in the frogs poisoned with botulinus toxin. Because of this a control series of experiments was carried out, in which the animals were injected with paralytic doses of procuran. These experiments showed, however, that in the frogs paralyzed with procuran the velocity of axoplasm transport in the single medullated nerve fibers was essentially indistinguishable from its velocity in the control animals. Hence, the results of the experiments on the different models of the generalized paralytic syndrome showed that in botulism the disturbance of synthesis of the axoplasm and the arrest of its movement along the nerve fibers were independent of the asphyxia forming one component of the poisoning. The fact that the velocity of movement of the axoplasm was directly dependent on the periods of injury by the botulinus toxin was evidence of the very conspicuous staged structure of the disturbances of the ability of the nerve cell to synthesize cytoplasm: in the preparalytic period of poisoning, synthesis was regularly increased, but in the stage of paralysis it stopped. This is in agreement with the authors' previously published data [2], showing that botulinus toxin exerts its action primarily on the motor cells of the anterior horns of the spinal cord.

In the experiments of series II an attempt was made to determine the functional changes arising in the single nerve cells at various stages of the disturbances of axoplasm transport. It is clear from Table 2 that in the preparalytic stage of botulinus poisoning, the indices of the phases of refractoriness and of the velocity of conduction of the nervous impulse were essentially indistinguishable from those in the control animals, even in the case when the velocity of transport of the axoplasm in the nerve fiber showed a marked increase. It may be concluded from this that the functional state of the single nerve fiber within certain limits is independent of an increase in the velocity of movement of the axoplasm within it.

The results of the experiments on frogs in the late stage of botulinus poisoning showed that the appearance of tonic contractions of the skeletal muscles in response to indirect stimulation was invariable associated with marked changes in the functional properties of the single medullated nerve fibers: the refractoriness was considerably increased, while the velocity of spread of the nervous impulse was lowered. Comparison of the indices of refractoriness and of the velocity of conduction of the nervous impulse in experiments such as these with the existing data [3] suggests that in botulism the thick medullated nerve fibers come to resemble in their functional properties the thin fibers, only slightly medullated, conducting at a slow velocity. Evidently, the main cause of these changes is the blocking of the movement of the axoplasm from the body of the nerve cell, and the low level of nutrient materials necessary for maintaining rapid transmission of the nervous impulses in the nerve fiber. A supplementary series of experiments on frogs poisoned with procuran confirmed the fact that the asphyxia arising during the development of the generalized paralytic syndrome is not responsible for the appearance of the above-mentioned changes in the functional properties of the single medullated thick nerve fibers.

TABLE 2. Changes in the Functional State of the Single Medullated Nerve Fiber in Botulism

Character of procedure	Clinical signs	n	Velocity of conduction of excitation (in m/sec)			Absolute refractory phase (in m/sec)			Relative refractory phase (in m/sec)		
			$M \pm m$	P_1	P_2	$M \pm m$	P_1	P_2	$M \pm m$	P_1	P_2
Control	—	23	58,0±0,77			1,23±0,071			4,72±0,118		
Procuran (0.002 g/70 g body wt., into gastrocnemius muscle.)	Paralysis of skeletal muscles	13	61,0±1,35	<0,5		1,4±0,102	<0,5		4,73±0,202	<0,5	
Botulinus toxin (0.1 mg/70 g body wt., into gastrocnemius muscle)	Early stage (paresis)	13	59,1±1,37	<0,5	<0,5	1,3±0,07	<0,5	<0,5	4,67±0,183	<0,5	<0,5
	Late stage (paralysis)	21	32,5±0,79	<0,001	<0,001	4,22±0,118	<0,001	<0,001	12,0±0,404	<0,001	<0,001

As this analysis of the disturbances of transport of the axoplasm and of the functional properties of the single medullated thick nerve fibers shows, in botulism the large neurons of the anterior horns of the spinal cord are the main target for the pathogenic action of the toxin. In the course of development of botulinus poisoning the primary changes affect synthesis of the axoplasm in the large neurons, and these subsequently cause disturbances of the mechanism of rapid transmission of nervous impulses along the thick medullated fibers. These disturbances, in turn, evidently cause the development of tonic contractions of the skeletal muscles in response to indirect stimulation.

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