MECHANISM OF FUNCTIONAL REORGANIZATION OF PARALYZED INTERCOSTAL MUSCLES IN RATS WITH EXPERIMENTAL BOTULISM

V. V. Mikhailov and V. F. Yakovlev

UDC 616.98:579.852.13]-092.9-06:616.745.6-009.11-07:616.74-018.61-073.97

KEY WORDS: botulism; intercostal muscles; resting membrane potential; action potential.

Patients with botulism usually die because they develop paralysis of the respiratory muscles and an asphyxia syndrome [7]. Artificial ventilation of the lungs can prevent them from dying, but spontaneous respiration is not always restored under these conditions [6]. The reason is evidently that in botulism most of the nerve cells in the motor nuclei innervating the respiratory muscles are damaged and dystrophic changes develop in the respiratory muscles themselves.

Since botulinus toxin (BT) selectively injures phasic motoneurons, especially in a state of functional activity [4], it was decided to study how the functional state of the phasic fibers of the external and internal intercostal muscles, which normally differ in their degree of motor activity, is changed in paralysis due to botulism.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-200 g were used. Under sterile conditions and pentobarbital anesthesia (40 mg/kg body weight) the intercostal muscles of the 2nd-4th intercostal spaces were exposed and BT was injected into them in a sublethal dose of 200 mouse MLD (1 MLD for mice is 0.0005 mg of dry BT), after which the wound was sutured in layers. The animals were used in the acute experiments at various times (3-4 and 11-12 days) after poisoning, when local unilateral paralysis of the intercostal muscles had developed, in the absence of any signs of external respiratory failure. The rats were anesthetized with pentobarbital, the corresponding intercostal spaces were exposed, the intercostal muscles were stretched, and the ribs fixed. The skin surrounding the wound was formed into a bath which was filled with warm (37°C) mineral oil. A glass microelectrode with a tip about 1 μ in diameter, filled with 2.5 M KCL solution (resistance 5-15 MΩ), was inserted by means of a micromanipulator into the fibers of the intercostal muscles and connected to the input of cathode follower, incorporated in a bridge circuit [1]. Intracellular stimulation was applied through the same electrode with square pulses of constant current (pulse duration 50 msec, current 1-30 nA). The resting membrane potential (RMP) and action potential (AP) were photographed from the screen of the CRO by means of an FOR-2 camera.

EXPERIMENTAL RESULTS

As the results in Table 1 show the external and internal intercostal muscles contain fibers with different electrogenic properties. The relative percentages of the different fibers was not the same in the intercostal muscles: The fraction of fibers with high RMP was clearly much higher in the internal intercostal muscles.

In botulism the electrogenic properties of the fibers and the relative proportions of different types of fibers do not change at once. By the 4th day after poisoning, when paralysis was due to a disturbance of transmission in the central segment of the reflex arc [2], changes were minimal and consisted of merely a small increase in membrane excitability of all types of fibers. A different picture was observed on the 12th day after poisoning, i.e., at times of development of neurodegenerative changes in motoneurons [3]. Table 1 also shows that normalization of electrogenic properties was virtually complete in groups of fibers with low and average RMP, whereas in the group of fibers with high RMP the disturbances progressed, especially in the external intercostal muscles: The mean level of RMP fell and depolarizing currents of 30 nA did not induce AP generation.

Department of Pathological Physiology, N. A. Semashko Moscow Medical Stomatologic Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Adо.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 6, pp. 666-668, June, 1981. Original article submitted August 22, 1980.

TABLE 1. Electrophysiological Characteristics of Fibers of External and Internal Intercostal Muscles in Rats with the Local Form of Botulism (M ± m)

Experimental conditions	Fibers	Intercostal muscle	RMP, mV	n	%	AP, mV	Rheobase current, nA	Critical level of depolarization, mV	n
Control	With low RMP	External	54,0±2,0	25	19	54,1±1,6	19,1±1,4	12,4±0,5	19
		Internal	56,7±2,5	7	6	60,1±1,7	20,1±1,4	16,4±1,7	5
	With average RMP	External	73,2±0,9	65	49	82,1±2,0	26,3±0,8	15,1±0,9	14
		Internal	71,7±0,9	35	33	91,6±2,7	21,4±1,6	18,3±1,9	12
	With high RMP	External	97,4±1,1	45	32	99,4±4,9	16,8±4,2	19,2±0,9	12
		Internal	113,4±2,2	64	61	114,5±1,2	26,1±1,1	19,2±1,4	19
4th day of paralysis	With low RMP	External	52,1±4,3	13	27	59,2±1,9	6,2±1,2*	7,0±0,6*	13
		Internal	52,7±2,6	6	9	60,2±1,9	10,7±1,2*	18,4±0,6	6
	With average RMP	External	70,4±1,3	20	42	79,3±3,5	8,1±4,3*	7,1±1,0*	19
		Internal	69,2±1,4	25	36	86,4±5,2	9,1±1,4*	18,7±0,9*	13
	With high RMP	External	91,3±4,7	24	31	91,6±1,8	7,9±1,6*	8,4±1,2*	16
		Internal	97,3±4,1	28	55	114,2±2,6	10,4±1,2*	18,7±0,5	19
12th day of paralysis	With low RMP	External	46,1±1,0	51	40	53,1±0,9	22,0±1,3	16,1±3,0	21
		Internal	53,1±1,4	13	15	57,1±1,6	29,1±0,8*	22,1±1,9	14
	With average RMP	External	66,8±1,0	26	45	68,4±2,6*	28,7±0,7	18,4±4,1	26
		Internal	70,9±1,6	44	52	90,0±1,3	28,1±1,4	26,1±1,4*	14
	With high RMP	External	85,1±1,3*	19	15	—	30,0	—	14
		Internal	92,4±1,6*	28	33	107,77±1,4	29,3±0,5	24,3±2,1	18

Legend. RMP levels of fibers with low RMP under 59 mV, average RMP 60-79 mV, and high RMP over 80 mV; *P < 0.05.

Changes in the internal intercostal muscles were less marked and the muscle fibers generated APs in response to depolarizing currents.

The change in the functional properties of different types of fibers of the intercostal muscles in botulism thus resembles to some extent the changes described in other parts of the skeletal musculature [5]. These changes are evidently the result of development of injury and degeneration in the respiratory motoneurons whose characteristics resemble those of the phasic motoneurons of the anterior horns of the spinal cord. The fact that the changes were more severe in the external intercostal muscles than in the internal can probably be explained on the grounds that motoneurons of the former are more active, and this predisposes them to injury by BT.

LITERATURE CITED

1. P. G. Kostyuk, Microelectrode Techniques [in Russian], Kiev (1960).
2. V. V. Mikhailov and V. V. Korolev, Byull. Éksp. Biol. Med., No. 11, 24 (1968).
3. V. V. Mikhailov and V. Vas. Mikhailov, Byull. Éksp. Biol. Med., No. 11, 21 (1975).
4. V. V. Mikhailov and S. D. Mikhailova, Byull. Éksp. Biol. Med., No. 1, 36 (1964).
5. V. V. Morrison, Byull. Éksp. Biol. Med., No. 9, 262 (1977).
6. A. M. Popova, Klin. Med., No. 9, 89 (1969).
7. H. Sonntag and C. Meyer-Beurgdorf, Can. Anaesth. Soc. J., 19, 559 (1971).