MECHANISM OF CHANGES IN THE POLARIZATION OF CROSS-STRIATED MUSCLE FIBERS IN EXPERIMENTAL BOTULISM AND DENERVATION

V. V. Mikhailov and V. V. Morrison

UDC 616.981.553-092.9-07:616.74-073

In rats with generalized botulism the decrease in membrane potential (MP) of the skeletal muscles is nonspecific in character: it is associated with the development of hypoxia, and when artificial respiration is applied the MP level of the skeletal muscles returns to normal. In local botulism of one hindlimb in rats and frogs the level of polarization of the muscle fibers is reduced just as after denervation. However, the concentrations of catecholamines and electrolytes differ considerably in poisoned and denervated muscles. In poisoned and denervated skeletal muscles different changes in the value of MP also are found after administration of ATP, cysteine, and phenylephrine.

In botulism the tetanic neuromuscular structures are selectively damaged while the tonic structures remain intact. At the same time the receptive zone is widened and sensitivity to mediators is increased [2, 8, 12]. Degeneration is absent in thick tetanic nerve fibers, while growth of nerve fibrils is often observed in the region of the end-plates [6, 12]. Nevertheless, some workers [5, 12] regard the changes in the functional properties of the skeletal muscles in botulism as the result of their complete denervation.

It was accordingly interesting to determine how the changes in the level of polarization and in the concentration of electrolytes and catecholamines differ in skeletal muscles poisoned with botulinus toxin and denervated by division of the regional nerve trunks.

EXPERIMENTAL METHOD

Experiments were carried out on frogs (Rana ridibunda) weighing 50-80 g and on albino rats weighing 170-280 g. Type C botulinus toxin (1 MLD for mice = 0.0006 mg of the dry substance) was injected into the leg muscles of the frogs in a dose of 0.2 mg/100 g. With this dose the frogs developed a general paralytic syndrome after 4-5 days. The rats received an injection of 0.0001 mg/100 g of the toxin into the leg muscles and a dose of 0.01 mg/100 g intraperitoneally. In the first case, local botulism of the muscles of the limb into which the toxin was injected developed 3-4 days after poisoning, while in the second case a general paralytic syndrome developed 18-20 h after injection of the toxin. The leg muscles were denervated by dividing the sciatic nerve in the upper third of the thigh.

The membrane potential (MP) of the muscle fibers in the frogs was determined in the isolated tetanic sartorius muscle and in a tonic bundle of the ileofibularis muscle, while in rats, which were anesthetized with pentobarbital in a dose of 3-4 mg/100 g body weight, it was determined in the mixed gastrocnemius muscle. The MP was recorded by glass microelectrodes with a tip less than 1 μ in diameter, filled with 2.5 M KCl solution. The microelectrodes were inserted into the test muscles at a distance of 3-4 mm from the point of entry of the nerve. The biopotentials were led to the input of a type S1-4 oscilloscope and recorded photographically from the screen. The total potassium and sodium concentrations in the muscles were determined by flame photometry and the catecholamine concentration fluorometrically.

A. A. Bogomolets Department of Pathological Physiology, Saratov Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 1, pp. 25-30, January, 1973. Original article submitted June 16, 1971.

TABLE 1. Changes in Membrane Potential of Cross-Striated Muscle Fibers in the Course of Experimental Botulism and after Denervation (in mV)

Species of animal	Series of experiments	Tetanic m. sartorius						Tonic bundle m. ileofibularis			
		Number of fibers	$M \pm m$	P_1	P_2	P_3	P_4	Number of fibers	$M \pm m$	P_1	P_2
Frog	Control	182	77,3±2,0					129	67,6±1,3		
	Control + adrenalin	103	75,6±0,86					122	70,0±0,84		
	Control + acetylcholine	106	84,1±0,91					115	58,5±0,84		
	Botulism: paresis	101	68,6±0,97	>0,1				117	67,9±0,7	>0,1	
	local paralysis	107	70,7±0,63	<0,002				117	63,2±0,9	<0,01	
	paralytic syndrome	108	63,5±1,05	<0,001				125	61,7±0,76	<0,001	
	paralytic syndrome + adrenalin	100	72,0±0,92		<0,001			106	71,8±0,91		<0,001
	paralytic syndrome + acetylcholine	100	61,7±0,78		>0,1			101	56,6±0,79		<0,001
		Mixed m. gastrocnemius									
Rat	Control	110	70,0±0,1								
	Control + ATP	89	70,9±1,13	>0,5							
	Control + cysteine	100	72,0±0,83	>0,1							
	Control + phenylephrine	84	79,2±1,17	<0,001							
	Inactivated botulinus toxin	102	69,9±1,0	>0,5							
	Generalized botulism without artificial respiration	113	58,7±0,84	<0,001							
	Generalized botulism with artificial respiration	112	71,8±0,93	>0,5							
	Local botulism:										
	botulism 48 h	104	70,0±0,85	>0,5							
	botulism 4 days	110	61,7±1,07	<0,001		>0,05					
	botulism 14 days	107	58,8±0,85	<0,001		>0,5					
	botulism 14 days + ATP	85	67,0±0,95				<0,05				
	botulism 14 days + cysteine	88	68,4±0,97				<0,001				
	botulism 14 days + phenylephrine	81	74,1±1,16				<0,05				
	Denervation 4 days	102	58,9±1,03	<0,001							
	Denervation 14 days	106	59,0±1,05	<0,001							
	Denervation 14 days + ATP	80	64,0±1,2								
	Denervation 14 days + cysteine	100	61,9±1,02								
	Denervation 14 days + phenylephrine	91	70,6±1,09								

Note. P_1) Compared with control; P_2) compared with corresponding stage of poisoning; P_3) compared with denervation at the same times; P_4) compared with denervation and administration of the corresponding drug.

TABLE 2. Changes in Concentrations of Electrolytes (in meq/g dry tissue) and Catecholamines (in μg/g fresh tissue) in Skeletal Muscles in Botulism and after Denervation

Species of animals	Type of muscle	Series of experiments	Potassium				Sodium			
			n	$M \pm m$	P_1	P_2	n	$M \pm m$	P_1	P_2
Frog	Tetanic m. sartorius	Control	12	0,559±0,023			12	0,09±0,003		
		Botulism:								
		a) paresis	11	0,424±0,015	<0,001		11	0,078±0,004	<0,02	
		b) local paralysis	11	0,322±0,018	<0,001		11	0,079±0,003	<0,05	
		c) paralytic syndrome	12	0,289±0,012	<0,001		12	0,062±0,003	<0,01	
	Tonic m. rectus-abdominis	Control	12	0,402±0,02			12	0,095±0,007		
		a) paralytic syndrome	13	0,23±0,013	<0,001		13	0,072±0,004	<0,01	
Rat	Mixed m. gastrocnemius	Control	13	0,556±0,047			13	0,107±0,007		
		Generalized botulism	11	0,324±0,013	0,001		11	0,072±0,002	0,001	
		Local botulism:								
		a) early 4 days	10	0,27±0,021	<0,001	<0,001	10	0,145±0,015	<0,05	<0,05
		b) late 14 days	10	0,311±0,013	<0,001	<0,001	10	0,115±0,014	>0,2	>0,5
		Denervation:								
		a) 4 days	11	0,429±0,005	<0,01		11	0,118±0,001	>0,1	
		b) 14 days	11	0,398±0,007	<0,001		11	0,126±0,004	<0,05	

Table 2. (cont'd)

Species of animal	Type of muscle	Series of experiments	Adrenalin *				Noradrenalin*			
			n	M±m	P_1	P_2	n	M±m	P_1	P_2
Frog	Tetanic m. sartorius	Control	16	0,39±0,009			16	0,62±0,04		
		Botulism:								
		a) paresis	10	0,39±0,036	>0,5		10	0,28±0,02	<0,001	
		b) local paralysis	10	0,45±0,054	>0,05		10	0,32±0,049	<0,001	
		c) paralytic syndrome	13	0,52±0,044	<0,01		13	1,42±0,124	<0,001	
	Tonic m. rectus-abdominis	Control	15	0,35±0,039			15	0,73±0,108		
		a) paralytic syndrome	12	0,22±0,023	<0,01		12	0,89±0,089	>02	
Rat	Mixed m. gastrocnemius	Control	14	0,96±0,092			14	0,63±0,086		
		Generalized botulism	11	0,56±0,033	<0,001		11	1,17±0,047	<0,001	
		Local botulism:								
		a) early 4 days	13	0,65±0,139	<0,05	<0,001	13	0,66±0,11	>0,5	>0,2
		b) late 14 days	10	0,49±0,066	<0,01	<0,001	10	0,22±0,077	<0,01	<0,01
		Denervation:								
		a) 4 days	10	0,19±0,025	<0,001		10	0,56±0,065	>0,5	
		b) 14 days	10	0,17±0,018	<0,001		10	0,54±0,045	>0,2	

*Mixed with other catecholamines fluorescing with 520 nm interference filters

Note. P_1) Compared with control; P_2) compared with denervation at the same times.

EXPERIMENTAL RESULTS

As the results in Tables 1 and 2 show, during development of the generalized paralytic syndrome in the rats the value of MP and the concentrations of potassium, sodium, and adrenalin in the muscles fell progressively, while at the same time the noradrenalin concentration rose. Similar changes were found also in frogs, but in these animals with generalized botulism both catecholamines accumulated in the tetanic sartorius muscle, while in the tonic rectus abdominis muscle the noradrenalin concentration was unchanged while the adrenalin concentration fell a little. At this stage of poisoning irrigation of the paralyzed muscles with solutions of adrenalin and acetylcholine in a concentration of 1×10^{-6} g/ml, which is usually used to study the effect of mediators on the polarization level [1, 3], led to a corresponding increase or decrease in the MP value.

Generalized botulism is characterized by hypoxia, i.e., a state in which the level of polarization of the cell membranes is lowered [7]. It was therefore decided to investigate to what extent this fact determines the partial depolarization of the muscle fibers in total paralysis of the skeletal muscles in botulism. As Table 1 shows, compensation of the respiratory failure by artificial ventilation of the lungs restores the normal level of polarization of the gastrocnemius muscle fibers of the poisoned rats at the stage of depression of spontaneous respiration, thus showing that the changes in lesions of this form are reversible.

In local botulism the lowering of the polarization level and the deficiencies of potassium and adrenalin accompanied by an excess of sodium and a normal concentration of noradrenalin in the muscle were observed only 2-3 days after the development of paralysis. These changes remained constant later (until 2 weeks) with the exception that in the late stages the concentration not only of adrenalin, but also of noradrenalin in the paralyzed muscles was lowered (Table 2). Similar changes were found in frogs.

In both generalized and local botulism the decrease in MP was thus regularly linked with a selective decrease in the potassium concentration in the muscle tissue; no correlation was found between the level of MP and the catecholamine concentration in the poisoned muscles despite the ability of the biogenic amines to modify the polarization level of muscle fibers [10]. This last property is usually attributed to disturbance of the activity of the sodium pump [11], a system functioning with the participation of ATP [9]. Table 1 shows that 15-20 min after intraperitoneal injection of ATP (10 mg/kg, a dose delaying the development of muscular degeneration after division of the nerve [4]) the level of polarization of the fibers of the poisoned muscles rose considerably. A similar effect was observed after the corresponding injection of the therapeutic dose of phenylephrine (0.1 mg/kg) and a specially chosen dose of cysteine (10 mg/kg). These results show that a reversable deficiency of ATP and sulfhydryl groups probably arises in the affected muscles, when the muscle fibers are still capable of increasing the polarization of their membranes in response to the action of the stable sympathomimetic agent phenylephrine.

However, the mixed character of the muscle did not permit the action of the various drugs on phasic and tonic muscle fibers separately in rats to be differentiated.

In technically similar experiments on rats after division of the nerve trunks it was found that, despite the decrease in MP in the denervated mixed muscle, the decrease in the potassium level in the early and late stages after division of the nerve was less marked than in the same muscle when poisoned with botulinus toxin. The sodium concentration, on the other hand, was unchanged in the early period after denervation, and later it increased. A more marked deficiency of adrenalin developed in the denervated muscles than in the poisoned muscles, but the noradrenalin concentration in these muscles was normal. Injection of ATP and cysteine into the rats increased the MP value very slightly in the denervated muscles, and only phenylephrine restored the MP level to the control values.

It can be concluded from these results that changes in the polarization of muscle fibers and in the content of catecholamines and electrolytes in the skeletal muscles in botulism and after denervation are dissimilar in character. The reason for this is evidently because in botulism mainly the activity of the tetanic fibers is depressed and the activity of the tonic neuromuscular structures remains intact, whereas after denervation the trophic influence of both phasic and tonic nerve fibers on the effector is completely abolished.

LITERATURE CITED

1. M. Yu. Klevets and M.F. Shuba, in: Synaptic Processes [in Russian], Kiev (1968), p. 92.
2. V. V. Mikhailov, in: The Infectious Process. Pathogenesis. Experimental Therapy [in Russian], Moscow–Saratov (1966), p. 66.

3. A. I. Shapovalov, Cellular Mechanisms of Synaptic Transmission [in Russian], Moscow (1966).
4. D. L. Ferdman, in: Problems in the Biochemistry of Muscles [in Russian], Kiev (1954), p. 113.
5. D. B. Drachman and F. C. A. Romanul, Arch. Neurol. (Chicago), 23, 85 (1970).
6. L. W. Duchen and S. Strich, Quart. J. Exp. Physiol., 53, 84 (1968).
7. J. Hubbard and Y. Loyning, J. Physiol. (London), 185, 205 (1966).
8. A. C. Guyton and M. A. MacDonald, Arch. Neurol. Psychiat. (Chicago), 57, 578 (1947).
9. R. D. Keynes, in: Regulation of the Inorganic Ion Content of Cells. Ciba Foundation Study Group 5, Boston (1960), p.77.
10. K. Kuba, J. Physiol. (London), 211, 551 (1970).
11. S. Locke and H. C. Solomon, J. Exp. Zool., 166, 377 (1967).
12. S. Thesleff, J. Physiol. (London), 151, 598 (1960).