MECHANISM OF DISTURBANCE OF REGENERATION OF THICK MEDULLATED NERVE FIBERS IN BOTULISM

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Division of a nerve leads initially to acceleration of the flow of axoplasm in the regenerating axons, followed by a decrease in its velocity. Botulinus toxin damages regenerating fibers much more rapidly than intact. Division of a nerve during botulism does not stimulate axoplasm transport in the regenerating nerve fibers.

Poisoning with botulinus toxin is characterized by a disturbance of axoplasm synthesis in the tetanic motor neurons. This is one cause of the paralytic syndrome, the partial blocking of transmission from nerve to muscle, and the appearance of tonic contractions in response to indirect stimulation of the affected muscles [3, 9]. On the other hand, both in human subjects and animals with botulism, there is a very long period of recovery of the contractile function of the muscles, much longer than the time required for restitution of the nerve fibers after division of nerve trunks [7, 11, 12].

All these observations suggest that the slow recovery of motor activity of the skeletal muscles after botulism is due to a disturbance of the regenerative capacity of the affected nerve cells because of a deficiency of axoplasm.

To test this hypothesis it was decided to study changes in the flow of axoplasm and in the functional state of the thick medullated nerve fibers during regeneration in intact animals and animals poisoned with botulinus toxin.

EXPERIMENTAL METHOD

Experiments were carried out on frogs (Rana ridibunda). Under sterile conditions the peroneal nerve was divided at the level of the upper third of the leg 1-28 days before the acute experiment. In the early stages after the operation botulinus toxin type A (1 M.L.D. for mice = 0.00001 mg of the dry substance) was injected into the thigh muscles on the side of the operation in a dose of 0.3 mg/50 mg body weight. In some experiments the trunk of the peroneal nerve was divided in frogs receiving a preliminary injection of botulinus toxin in the same dose.

The flow of axoplasm in the thick medullated nerve fibers, the indices of their refractoriness, and the velocity of conduction of the nervous impulses were recorded in acute experiments by methods described previously [4]. The effect of mediators on the process of regeneration was investigated in frogs in which a deficiency of adrenalin or acetylcholine synthesis was induced by extirpation of the adrenals and pancreas respectively by A. V. Kibyakov's method. The metabolic disturbances arising in the medullectomized and depancreatized animals were compensated by injection of adrenalin or acetylcholine in a dose of 1 ml/50 g body weight (concentration $1 \cdot 10^{-4}$ g/ml) into the ventral vein 45 min before the acute experiment began [2].

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TABLE 1. Velocity of Movement of Axoplasm during Regeneration of Thick Medullated Nerve Fibers under Normal Conditions and in Botulism

Group of animals	No. of fibers	Time after division of nerve (in days)	Time after injection of toxin	Velocity, R	P
Intact	24			287 ± 2.1	<0.001
Division of nerve:					
early stage of regeneration	19	1-7	–	533 ± 4.2	<0.001
late	17	12-29	–	268 ± 3.3	<0.001
Division of nerve + botulinus toxin	14	12-24	18-24 h	146 ± 7	<0.001
Paralysis due to injection of botulinus toxin plus division of nerve	75	14-27	2-3 days	No movement	

TABLE 2. Changes in Functional Properties of Regenerating Thick Nerve Fibers against the Background of Acetylcholine and Adrenalin Deficiency in Animals with Botulinus Poisoning

Group of animals	Time after division of nerves (in days)	No. of expts.	Conduction velocity of nervous impulse (in msec)		Late stage of regeneration			
					absolute		relative	
			M	P	M	P	M	P
Control		10	57.7		1.5		4.1	
Early stage of regeneration	2	10	55.7	<0.1	1.7	<0.1	4.3	<0.1
Late stage of regeneration	8-20	20	47.2	<0.001	2.9	<0.001	6.6	<0.002
Demedullation	2	9	44	<0.01	2	<0.001	6.1	<0.001
Demedullation + adrenalin	2	8	48.1	<0.001	2.4	<0.01	6.3	<0.001
Depancreatization	2	13	49.5	<0.001	2.2	<0.001	6.0	<0.001
Depancreatization + acetylcholine	2	7	48.3	<0.01	2.4	<0.001	5.6	<0.001
Paralysis due to botulinus toxin	2	8	46.3	<0.001	2.5	<0.002	5.9	<0.002
Late stage of regeneration + botulinus toxin (pareses of skeletal muscles)	8-20	5	45.8	<0.001	2.7	<0.001	5.8	<0.001

EXPERIMENTAL RESULTS

Results indicating movement of labeled amino acids, radioactive isotope, and enzymes in the axons of the central end of the divided nerve [8, 10, 13] demonstrated the need for direct measurement of the velocity of movement of the axoplasm in the regenerating thick medullated axon.

As Table 1 shows, the flow of axoplasm was considerably increased in the fibers of the central end of the divided nerve in the first week after operation, but later (after 1-14 days) movement of the axoplasm began to slow down and its velocity was below normal. This confirms the fact that increased formation and displacement of the neuroplasm in the axon evidently coincides with the development of chromatolysis and activation of nucleoprotein metabolism in the body of the nerve cell [6, 10].

However, the functional state of the regenerating nerve fiber showed changes which were not strictly dependent on the velocity of axoplasm transport. In the early stages of regeneration, for instance, despite an increase in the velocity of axoplasm transport, the thick fibers of the central end of the divided nerve conducted nervous impulses at the same speed, and possessed the same values of absolute and relative re-

fractoriness as intact nerve fibers (Table 2). During the period of weakening of the axoplasm flow the functional properties of thick medullated nerve fibers composing the central end of the divided nerve were appreciably changed; the conduction velocity of the nervous impulses was reduced while the duration of the phases of absolute and relative refractoriness increased (Table 2).

The combination of slowing of the axoplasm flow and a decrease in the velocity of conduction of the nervous impulse and increase in refractory period thus led to the conclusion that the late stage of regeneration is characterized by a deficiency of trophic materials in the nerve fibers essential for their normal functional activity.

Since the most important trophic substances, namely adrenalin and acetylcholine, enter the nerve cells mainly in the region of the body [2, 13], where they activate nucleoprotein metabolism [1], it was necessary to determine the functional properties of the regenerating nerve fibers in the presence of a deficiency of mediator formation in the body.

As Table 2 shows, in the presence of a reversible disturbance of acetylcholine and adrenalin synthesis in the body, the spread of the nervous impulse in the regenerating axons is slowed, while the refractory period is increased compared with the control. Injection of compensatory doses of adrenalin or acetycholine did not restore the normal functional state of the regenerating axon by comparison with the intact nerve fibers [5].

Hence, when mediator synthesis is disturbed in the body the functional properties of the regenerating medullated nerve fibers are changed soon after division of the nerve trunk and are not restored after saturation of the body with the corresponding mediator.

Having discovered these properties of the change in velocity of axoplasm transport and in the functional properties of the regenerating thick medullated nerve fibers in intact animals, the next step was to discover whether botulinus toxin exerts its usual stimulant effect on neuroplasm synthesis [4] at the stage of regeneration of the thick medullated nerve fibers in frogs at which the axoplasm flow is retarded, and their refractory periods increase. On the other hand, it was also interesting to discover whether nerve cells damaged by botulism, in which axoplasm synthesis is depressed [4], respond by activation of this process soon after division of the test nerve trunk. As Tables 1 and 2 show, in the late stages of regeneration botulinus toxin slowed the axoplasm flow still more, but had no appreciable effect under these conditions on the conduction velocity of the nervous impulse or on the absolute and relative refractory period. In late botulism, at times when movement of the axoplasm had ceased, division of the nerves did not restore its flow in the thick medullated nerve fibers of the central end of the damaged nerve and had no effect on their functional state.

These results suggest that the long recovery period of the motor function of the affected muscles in convalescence after botulism may be due to deep inhibition of cytoplasm synthesis in nerve cells damaged by the toxin.

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