ROLE OF ACETYLCHOLINE IN DISTURBANCES OF AXOPLASM TRANSPORT IN THICK MEDULLATED NERVE FIBERS IN EXPERIMENTAL BOTULISM AND TETANUS

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A disturbance of acetylcholine synthesis as a result of depancreatization caused an appreciable increase in the velocity of axoplasm transport in thick medullated nerve fibers of frogs, accompanied by a slight increase in the duration of the refractory periods and a decrease in the velocity of conduction of the nervous impulse. After administration of replacement doses of pharmacological acetylcholine, axoplasm transport, conduction velocity of the nervous impulse, and the duration of the refractory periods returned to normal. In botulism and tetanus, a deficiency of acetylcholine synthesis in the body does not prevent disturbances of axoplasm transport or loss of the ability for rapid conduction of the nervous impulse in thick medullated neurons.

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In botulism and tetanus a disturbance of the conduction of excitation in synapses of the central nervous system and in myoneural junctions is associated with the blocking of terminal divisions of the nerve fibers and inhibition of the release of acetylcholine in presynaptic endings [5]. Meanwhile, in the affected motor and sensory neurons, a disturbance of axon transport is observed, particularly in the late states of poisoning. Cessation of axoplasm transport is combined as a rule with slowing of conduction of the nervous impulse and a marked increase in duration of the phases of the refractory period [2, 3].

It was therefore decided to study the effect on the velocity of axoplasm transport of a temporary disturbance of acetylcholine synthesis, and to determine whether under these circumstances the character of development of botulinus and tetanus poisoning is modified and whether the affected neurons are capable of utilizing acetylcholine as a trophic substance.

EXPERIMENTAL METHOD

Experiments were carried out on frogs (Rana ridibunda) in the autumn and winter. The toxins were injected into the muscles of the posterior surface of the thigh in the following doses: botulinus toxin 1 mg/100 g body weight (1 MLD, equivalent to 0.00005 mg of dry toxin), tetanus toxin 1-4 mg/10 g body weight (1 MLD, equivalent to 0.00001 mg of dry toxin). To produce a reversible disturbance of acetylcholine metabolism, some animals underwent depancreatization. The operation was carried out under sterile conditions 6-8 days before the acute experiment. Tetanus toxin was injected 24 h before the operation and botulinus toxin 4-6 days after, i.e., to ensure that the picture of poisoning would develop on the 6th-8th day after the operation, at the time of maximal acetylcholine deficiency [1]. Compensation of the disturbed function of the nervous system in the depancreatized animals was produced by injecting 1 ml of pharmacological acetylcholine in a concentration of 1×10^{-4} g/ml into the abdominal vein 30-45 min before the acute experiments.

In the acute experiments, 5 or 6 thick medullated nerve fibers of the sciatic nerve, 10-20 μ in diameter, connected with the corresponding spinal nerve centers, were dissected in poisoned and nonpoisoned frogs by Tasaki's method [4], and the velocity of axoplasm transport in them was recorded by means of the

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TABLE 1. Changes in Velocity of Axoplasm Transport and in Physiological Properties of Thick Medullated Nerve Fibers in Botulism and Tetanus against a Background of Acetylcholine Deficiency

Series of experiments	Change in velocity of axoplasm transport (in μ/24 h)					Changes in physiological properties of thick medullated nerve fibers								
	no. of expts.	M	m	P_1	P_2	no. of expts.	vel. of cond. of nervous impulse (in m/sec)			absolute refractory period (in m/sec)			relative refractory period (in msec)		
							M	m	P	M	m	P	M	m	P
Control	10	312	7,6			10	53,5	0,5		1,3	0,07		5,5	0,12	
Injection of AC into intact frogs	10	200,4	7,1	<0,001		10	52,9	0,05	>0,1	1,28	0,06	>0,5	5,4	0,05	>0,5
6th-8th day after depancreatization	10	417,2	13,9	<0,001		10	49,6	0,61	<0,002	2,00	0,03	<0,001	6,7	0,1	<0,001
Injection of AC on 6th-8th day after depancreatization	10	305,4	12,0	>0,1	<0,001	10	52,8	0,16	>0,1	1,32	0,03	>0,5	5,6	0,08	>0,5
10th-12th day after depancreatization	10	300,0	8,0	>0,5	<0,001	10	52,8	0,08	>0,1	1,32	0,05	>0,5	5,8	0,07	>0,5
Injection of AC 24 h after poisoning animals with BT	10	198,7	11,2	<0,001	<0,001										
Botulinus poisoning (24 h after injection of BT) against background of AC deficiency	7	522,5	15,0	<0,001	<0,001										
Compensatory injection of AC in early stage of botulinus poisoning (24-48 h after injection of BT) against background of AC deficiency	9	226,2	6,3	<0,001	<0 001	10	53,6	0,04	>0,1	1,28	0,05	>0,5	5,4	0,05	>0,5
Botulinus poisoning against background of AC deficiency															
48 h after injection of BT	10	354,1	11,1	<0,05	<0,02	10	54,2	0,13	>0,1	1,25	0,05	>0,5	5,3	0,1	>0,25
72 h after injection of BT	10	159,1	2,9	<0,001	<0,001										
96 h after injection of BT	15	0	0	<0,001	<0,001	10	31,6	0,3	<0,001	3,5	0,15	<0,001	12,1	0,5	<0,001
Injection of AC in prespastic stage of tetanus poisoning	10	158,0	4,9	<0,001	<0,001										
Prespastic stage of tetanus poisoning against background of AC deficiency	10	168,4	8,0	<0,001	<0,001										
Compensatory injection of AC in prespastic stage of tetanus poisoning against background of disturbed AC synthesis	10	154,1	10,3	<0,001	<0,001										
Spastic stage of tetanus poisoning against background of AC deficiency	10	97,4	10,6	<0,001	<0,001	8	52,8	0,25	>0,5	1,32	0,07	>0,5	5,6	0,1	>0,5
Injection of AC in spastic stage of tetanus poisoning against background of AC deficiency						8	53,7	0,02	>0,5	1,3	0,05	>0,5	5,4	0,2	>0,5
Paralytic stage of tetanus poisoning against background of AC deficiency	13	0	0	<0,001	<0,001	9	28,5	0,5	<0,001	4,0	0,05	<0,001	15,5	0,04	<0,001

Legend: AC—acetylcholine; BT—botulinus toxin; P—significance of differences relative to control; P_1—significance relative to control; P_2—significance of differences relative to depancreatization experiments.

MKU-1 miniature motion picture camera. The velocity of conduction of the nervous impulse and duration of the absolute and relative phases of the refractory period were then studied by the method of Mikhailov and co-workers [2, 3]. Altogether 268 experiments, including 20 controls, were performed.

EXPERIMENTAL RESULTS

The disturbance of acetylcholine synthesis considerably modified the physiological state of the axoplasm and the velocity of its transport in thick medullated nerve fibers of poisoned and nonpoisoned frogs. As Table 1 shows, depancreatization at times of maximal mediator deficiency appreciably increased the axoplasm transport while reducing the velocity of conduction of the nervous impulse and increasing the duration of the phases of the refractory period. After administration of compensatory doses of pharmacological acetylcholine, the restored velocity of axoplasm transport and the parameters of function of the thick medullated nerve fibers were close to the control level. Similar results were obtained in the case of spontaneous restoration of acetylcholine synthesis at later periods after the operation.

A deficiency of acetylcholine in the body was thus associated with an increase in the velocity of axoplasm transport, but under these circumstances the thick medullated nerve fibers were evidently supplied with insufficient acetylcholine to maintain their normal function.

The acetylcholine deficiency produced in depancreatized animals modified the character of the changes in the thick medullated nerve fibers produced by botulinus and tetanus toxins. In botulism, in the preparalytic stage, the velocity of axoplasm transport was increased although, admittedly, to a somewhat lesser degree than in animals with an intact pancreas [2]. In the later stage of poisoning, however, the flow of axoplasm stopped completely. Injection of compensatory doses of pharmacological acetylcholine into animals poisoned with botulinus toxin slowed the velocity of axoplasm transport if the flow still continued, but had no effect if the flow of axoplasm had stopped. In tetanus poisoning developing in association with acetylcholine deficiency, the initial increase in velocity of axoplasm transport observed in nonpoisoned animals after the operation was not observed. In contrast to botulism poisoning, in tetanus the compensatory injection of pharmacological acetylcholine did not slow the flow of axoplasm. The study of the physiological parameters showed that in the early stage of botulinus and tetanus poisoning in depancreatized frogs the velocity of conduction of the nervous impulse and the duration of the phases of the refractory period were not significantly changed. In this case, injection of pharmacological acetylcholine had no significant effect on the physiological state of the thick medullated nerve fibers. If the flow of axoplasm had ceased in the late stages of botulinus and tetanus poisoning, the same decrease in the physiological parameters was found as in animals with an intact pancreas.

The results thus show that acetylcholine and botulinus and tetanus toxins can influence the transport of axoplasm and also, apparently, its synthesis in the nerve cell independently. It follows that changes in acetylcholine metabolism have no specific role in the mechanism of damage to motor and sensory neurons with thick medullated fibers in botulism and tetanus.

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