MECHANISM OF CHANGES IN THE MEMBRANE POTENTIAL IN VARIOUS TYPES OF MUSCLE FIBERS IN EXPERIMENTAL TETANUS

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In early tetanus the fall in membrane potential (MP) of skeletal muscle fibers is nonspecific in character: it is connected with the development of hyperactivity of the muscles, and when this is abolished by division of the nerve, the MP of the skeletal muscle fibers returns to normal. In the late stage of tetanus poisoning the functional properties of the tetanic and tonic muscle fibers are altered to a greater degree than in early tetanus. Administration of ATP, cysteine, and phenylephrine appreciably raises the MP of the poisoned muscle fibers.
KEY WORDS: tetanus; muscle fibers; membrane potential.

In the "paralytic" stage of tetanus direct repetitive stimulation of a striated muscle causes it to contract, followed by a prolonged tonic aftereffect. The refractory period is considerably increased, the conduction velocity along the motor nerve fibers is slowed, and often a partial block of the transmission of excitation from nerve to muscle appears and the ultrastructure of the synapses is altered. These changes are accompanied by a progressive disturbance of synthesis of the neuroplasm in the nerve cells and of its proximal-distal transport along thick nerve fibers [2, 4, 6].

It was accordingly decided to study whether the level of polarization of striated muscle fibers changes in the course of tetanus poisoning and whether this is connected with the selective depression of activity of the tetanic neuromuscular synapses while the activity of the tonic synapses remains unchanged.

EXPERIMENTAL METHOD

Experiments were carried out on frogs (Rana ridibunda) and albino rats (weight 170-150 g). Tetanus toxin (1 MLD for mice, 0.00005 mg) was injected into the frogs in a dose of 1 mg/100 g body weight intramuscularly and into rats in doses of 0.00001 mg/100 g body weight intramuscularly (to produce local tetanus) and 0.1 mg/100 g intravenously (to produce generalized tetanus). On the 4th-5th day after receiving the toxin the frogs developed a paroxysmal syndrome, which changed on the 10-12th day into "paralytic" tetanus. Local tetanus appeared in the rats 48 h after the injection and generalized tetanus 30-35 h after the injection.

The membrane potential (MP) of the muscle fibers was determined in the frogs in an isolated tetanic muscle (sartorius) and a tonic muscle (the tonic bundle of the ileofibrularis and the rectus abdominis), and in rats in the mixed gastrocnemius muscle. The MPs of the muscle fibers were recorded by the method described earlier [5].

ATP and cysteine in doses of 10 mg/kg and phenylephrine in a dose of 0.1 mg/kg were injected intraperitoneally; strychnine in a dose of 0.2 mg/100 g body weight was injected intramuscularly.

EXPERIMENTAL RESULTS AND DISCUSSION

The initial object was to study the functional properties of the various types of muscle fibers in frogs with generalized tetanus at a time before disturbances occurred in axoplasm transport, the conduction of

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TABLE 1. Changes in MP (in mV) of Striated Muscle Fibers of Frogs and Rats in the Course of Experimental Tetanus

Species of animals	Type of muscle	Series of experiments	n	M	$\pm m$	P_1	P_2
Frogs	Tetanic m. sartorius	Control	132	77,3	2,0		
		Control + adrenalin	103	85,6	0,86	<0,001	
		Tetanus:					
		spastic stage	106	70,0	1,44	<0,001	
		same + adrenalin	102	67,4	1,08		>0,1
		paralytic stage	121	51,2	0,71	<0,001	
		same + adrenalin	101	66,4	0,86		<0,001
		Strychnine	132	59,1	0,94	<0,001	
	Tonic: tonic bundle of ileofibrularis	Control	129	67,6	1,2		
		Control + adrenalin	122	70,0	0,84	>0,05	
		Tetanus:					
		spastic stage	110	65,3	0,88	>0,1	
		same + adrenalin	104	70,5	1,07		<0,001
		paralytic stage	135	61,5	0,6	<0,001	
		same + adrenalin	103	66,1	1,05		<0,001
	m. rectus abdominis	Control	103	67,5	1,07		
		Tetanus:					
		spastic stage	105	68,8	0,99	>0,5	
		paralytic stage	108	60,5	0,97	<0,001	
Rats	Mixed m. gastrocnemius	Control + ATP	110	70,0	1,0		
		Control + phenylephrine	89	70,9	1,13	>0,5	
		Control + cysteine	84	79,2	1,17	<0,001	
		Generalized tetanus	100	72,0	0,83	>0,5	
		Local tetanus:	105	66,9	1,05	<0,01	
		early stage					
		2 days	109	68,1	0,9	>0,1	
		4 days	103	56,8	1,03	<0,001	
		late stage					
		14 days	109	57,8	0,97	<0,001	
		late stage + ATP	92	66,7	0,96		<0,001
		same + cysteine	97	63,2	0,89		<0,001
		same + phenylephrine	97	76,2	0,95		<0,001
		Inactivated toxin	100	70,7	0,86	>0,5	
		Early stage of tetanus (4 days) + plus division of nerve	102	72,0	1,0	>0,5	<0,001

Legend: P_1) compared with the control; P_2) compared with the corresponding stage of poisoning.

excitation along the nerve fibers, neuromuscular transmission, and the parameters of excitability of the muscles themselves [1, 3, 4, 8]. Despite this, in frogs with the generalized form of tetanus a marked decrease was found in MP of the fibers chiefly of the tetanic muscles, whereas MP of the tonic muscle fibers usually showed no marked change. Meanwhile the tetanic fibers of the poisoned muscle, unlike the tonic, lost their ability to respond by a marked increase in MP after the addition of adrenalin in a concentration of $1 \cdot 10^{-6}$ g/ml to the Ringer's solution surrounding the muscle. A lowering of the polarization level of the skeletal muscle fibers in generalized tetanus also was observed in the rats.

These changes can be explained by the onset of prolonged hyperactivity of the tetanic muscle fibers during the spasms, leading to their fatigue and to a fall in MP, as is observed during prolonged tetanic stimulation of a skeletal muscle [12]. The results of experiments in which the sciatic nerve of rats was divided in the early stage of tetanus and of poisoning of the frogs with strychnine confirmed this hypothesis: In the first case depression of the muscular spasticity in the rats restored the normal MP of the fibers, whereas the appearance of spasticity after injection of strychnine into the frogs lowered the MP of the tetanic muscle fibers by about the same degree as in the poisoned animals with an intact innervation of their skeletal muscles.

It can be concluded from these results that changes in the level of polarization of the muscle fibers in frogs and rats on the appearance of muscular spasticity in the stage of "spastic" tetanus are predominantly neurogenic in character.

As the damage to the tetanic neuromuscular synapses in tetanus poisoning progressed and their activity was blocked in the late "paralytic" stage in the animals the functional properties of the tetanic and tonic muscle fibers were changed by a much greater degree than in early tetanus. In this stage MP of the fibers of both tetanic and tonic muscles fell in the frogs. The ability of both types of affected muscles in the frogs to respond by hyperpolarization to the action of exogenous adrenalin (in a concentration of $1 \cdot 10^{-6}$ g/ml) was restored. A sharp fall in MP of the fibers of the mixed gastrocnemius muscle was observed in the zone of development of the paralytic stage of tetanus in the rats also.

It can be concluded from the results of these experiments as a whole that the most specific picture of damage to the peripheral neuromuscular junctions develops mainly in "paralytic" tetanus, when significant disturbance of the activity of the tetanic neuromuscular synapses appear. However, it was not clear how reversible the changes in the level of polarization of the muscle fibers are, or whether they were connected with a deficiency of high-energy compounds in the muscles in tetanus [10]. In the next series of experiments this problem was therefore studied in rats with experimental "paralytic" tetanus treated with injections of ATP and cysteine – the latter a reactivator of thiol enzymes participating in synthesis [7]. These substances were used in doses which, under normal conditions, had no effect on the level of polarization of healthy muscle fibers. As Table 1 shows, injections of these substances into poisoned animals appreciably increased the MP of the muscle fibers in the zone of damage by tetanus toxin. These results show that poisoned muscles can evidently assimilate ATP and cysteine from the circulatory system and incorporate them into their metabolism, when they determine the level of polarization of the muscle fibers.

The next problem to be studied was the extent to which the ability of the damaged rat muscle fibers to respond by hyperpolarization to the action of adrenergic substances on the muscle was modified. Since in warm-blooded animals adrenalin injected intravenously and intraperitoneally is quickly inactivated in the organs and tissues [9], the sympathomimetic agent phenylephrine was used for these purposes. The results of these experiments showed that, unlike ATP and cysteine, phenylephrine produced a hyperpolarizing effect in the fibers of normal muscles, and an even stronger effect in the fibers of the poisoned muscles. This indicates that in "paralytic" tetanus the changes in metabolism were not severe enough to cause the muscle fibers to completely lose their ability to respond by changes in the level of polarization of their membranes to pharmacological stimulation of the activity of the sodium pump [11].

To sum up, it can be concluded that in "spastic" tetanus the changes in polarization of the muscle fibers are predominantly secondary in character, associated with hyperactivity of the motor centers, whereas in "paralytic" tetanus the fall in MP arises probably as a result of profound disturbances of the activity of the tetanic neuromuscular synapses.

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