RELATIONSHIP BETWEEN DISTURBANCES OF AXOPLASM TRANSPORT AND CHANGES IN FUNCTION OF SINGLE MEDULLATED NERVE FIBERS IN TETANUS

V. V. Mikhailov, O. G. Astaf'eva, and I. L. Shvarts

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Disturbance of the function of single medullated nerve fibers in tetanus is preceded by slowing of axoplasm transport from the cell body into its axon. The authors consider that a change in the functional properties of peripheral nerve fibers is connected with disturbance of the activity of neurons as trophic centers.

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We have previously shown that in the paralytic stage of tetanus phasic neural influences on the skeletal muscles are lost because of disturbances of the trophic influences of the spinal cord motor centers on their peripheral nerve fibers [7].

In face of data showing changes in nucleoprotein and protein metabolism in the motoneuron in tetanus [1, 10] and of the important role of the axoplasm in nutrition of nerve fibers [2], we decided to investigate whether the functional changes in nerve fibers during tetanus are associated with disturbance of the flow of axoplasm from the nerve cell body into the axon.

EXPERIMENTAL METHOD

Experiments were carried out on frogs (Rana ridibunda) poisoned with tetanus toxin (1 M. L. D. for the mouse is equivalent to 0.0000081 mg of dry toxin), strychnine, chlorpromazine, and phenol. The poisons were injected into the right gastrocnemius muscle in the following doses (per 100 g body weight): tetanus toxin 1.4 mg, strychnine 0.1 mg, phenol 10 mg. Chlorpromazine was injected into the dorsal lymph sac in a dose of 25 mg. The experiments were performed on special preparations of poisoned and nonpoisoned animals. To analyze the optical nonhomogeneity of the axoplasm, its movement was recorded by means of a type MKU-1 miniature motion picture camera. From 2 to 6 thick medullated fibers of the frog's sciatic nerve were dissected by Tasaki's method. The preparation was placed on the stage of the microfilming apparatus and photographs were taken with a magnification of 630 x for 2-3 h at a speed of 2 frames/min. The processed film was examined with a projector at a film speed of 24 frames/sec. The velocity of flow of the axoplasm was calculated with the use of a scale grid, one division of which was equivalent to 10 μ the magnification being the same as that of the single nerve fibers. Altogether 241 single nerve fibers were examined in the experiments, 41 of them being controls.

To study the function of single nerve fibers of the sciatic nerve, one thick medullated nerve fiber was dissected Tasaki's method. The tests used to reflect the functional state of the single nerve fiber were the duration of the absolute and relative refractory periods and the velocity of conduction of the nervous impulse. The method by which there parameters were determined was described previously [6]. Altogether 97 single medullated nerve fibers, including 35 controls, were investigated.

EXPERIMENTAL RESULTS

Despite intramuscular injection of toxin, as a rule the frogs showed no signs of development of local tetanus. However, when the velocity of flow of the axoplasm was investigated, the changes of the "poisoned" side were greater than on the control side.

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TABLE 1. Changes in Functional Properties of Ventral and Dorsal Roots in Cats and Frogs Poisoned with Tetanus Toxin

Stage of experiment	Species of animals	No. of expt.	Efferent portion			Afferent portion		
			Absolute refractory period	Relative refractory period	Velocity of conduction of impulse	Absolute refractory period	Relative refractory period	Velocity of conduction of impulse
Control	Cat	7	2,4± 0,54	5,1± 0,98	100± 3,24	1,9± 0,31	5,1± 0,76	102,5± 3,6
	Frog	10	2,12± 0,07	4,5± 0,16	46,7± 1,6	2,32± 0,29	4,8± 0,20	48,8± 1,6
Spastic stage	Frog	10	2,16± 0,1 $P > 0,5$	4,54± 0,15 $P < 0,5$	49,5± 1,4 $P < 0,5$	2,23± 0,12 $P < 0,5$	4,6± 0,18 $P < 0,1$	50,4± 1,8 $P > 0,1$
Paralytic stage	Cat	7	6,6± 0,82 $P < 0,01$	14,2± 1,06 $P < 0,001$	85± 3,15 $P < 0,01$	4,2± 0,32 $P < 0,05$	10,6± 1,22 $P < 0,001$	82,5± 2,25 $P < 0,01$
	Frog	8	7,06± 0,3 $P < 0,01$	15,1± 0,8 $P < 0,001$	18,8± 0,99 $P < 0,001$	7,2± 0,3 $P < 0,01$	14,8± 0,7 $P < 0,001$	21,8± 1,2 $P < 0,01$

Note. Recording electrodes applied to ventral and dorsal roots of segments S_1-L_7 of cat's spinal cord or segments L_9-L_7 of frog's spinal cord. Stimulating electrodes applied to peroneal and tibial nerves of cat and to sciatic nerve of frog.

The results showed that in the early stages of poisoning the rate of flow of the axoplasm on the poisoned side increased slightly. At later stages (on the 4th-5th day after injection of the toxin), when permanent muscular rigidity or frequent spontaneous spasms were observed, the rate of flow of the axoplasm on the "poisoned" side was reduced, while on the "nonpoisoned" side it was increased. Later still (6th-9th day after poisoning), when the spastic stage in the animals had been followed by the paralytic stage, the flow of axoplasm on the "poisoned" side was seen to have stopped, while it still continued on the control side.

The spastic and paralytic stages of tetanus poisoning can thus be distinguished by the rate of flow of the axoplasm. The changes described above evidently arose because of the specific action of tetanus toxin on the neural elements, because the asphyxia associated with paralysis of the muscles of respiration did not significantly modify the rate of flow of the axoplasm [6].

In the next series of experiments strychnine or phenol—substances inducing convulsions—was injected into frogs. Phenol damages the spinal motoneurons, while strychnine affects the system of internuncial neurons [3, 8]. In other experiments the velocity of axoplasm flow was investigated while the activity of the motoneurons was inhibited because of weakening (by chlorpromazine) or strengthening (reproduction of Sechenov inhibition) of supraspinal influences on them [4]. It will be seen in Table 1 that strychnine, by blocking inhibitory processes in the spinal cord, acts in approximately the same way as tetanus toxin in the spastic stage; the rate of flow of the axoplasm diminished slightly. The possible initial acceleration of axoplasm flow could not be recorded. These results may be explained by assuming that in the experiments with strychnine, in contrast to the comparatively slowly developing tetanus poisoning, severe poisoning developed rapidly with well marked muscular rigidity. During poisoning with phenol, on the other hand, against the background of development of spasms we observed a marked increase in the velocity of flow of the axoplasm, i.e., the stimulant effect of phenol was similar to the action of another cell poison—the toxin of botulism [6]. After administration of chlorpromazine the velocity of flow of the axoplasm increased slightly. Sechenov inhibition caused a similar effect.

The study of functional properties of the nerve fibers showed that in tetanus only two stages can be distinguished instead of the three stages observed by studying movement of the axoplasm: preservation of the properties of single nerve fibers at the normal level in the early stages and their modification in the paralytic stage, i.e., only in cases when we recorded cessation of flow of the axoplasm. Under these conditions the velocity of spread of the nervous impulse was sharply reduced and the duration of the refractory periods increased. We

may conclude from a comparison of the results of our function tests with those obtained by Erlanger and Gasser [9] for nerve fibers of types A, B, and C that in tetanus poisoning the thick medullated nerve fibers lose their ability to conduct the nervous impulse rapidly and in their functional properties they come to resemble tonic nerve fibers. In poisoning with strychnine, phenol, or chlorpromazine, no changes in the functional properties of the thick medullated nerve fibers were observed.

The fact that the functional properties of single nerve fibers are dependent on the amount of axoplasm entering the axon from the nerve cell body shows that in tetanus poisoning these changes are evidently the result of interruption of the flow into the nerve fiber of the trophic materials necessary for maintaining its ability to conduct nervous impulses rapidly.

Since it was impossible to determine to which type (sensory or motor) the thick medullated nerve fibers belonged when they were isolated from the whole nerve trunk, several additional series of experiments were performed. In them we attempted to discover what changes took place in the function of nerve fibers in the central or dorsal roots. As Table 1 shows, the values of the refractory periods and conduction velocity of the nervous impulse in the ventral and dorsal roots of frogs and cats* varied with the stage of poisoning. In the spastic stage no changes were found compared with the controls, while in the paralytic stage the refractory periods increased sharply and the velocity of conduction of the nervous impulse fell in fibers belonging to both the ventral and dorsal roots.

The results show that in paralytic tetanus all the thick medullated nerve fibers may be affected regardless of whether they belong to the sensory or motor division of the nervous system. This points to the "central" pathogenic action of tetanus toxin on the nerve cells from which the thick medullated fibers emerge. In this respect the mechanism of action of tetanus toxin is close to that of the exotoxins of other microorganisms of the genus Clostridium—the agents of botulism and gas gangrene [5].

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*Dose of tetanus toxin 0.2 mg/kg (intramuscularly).