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THE EFFECT OF TETANUS TOXIN ON THE CHOLINE ESTERASE ACTIVITY OF THE MUSCLES OF RATS

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The theory of chemical transmission of motor impulses to the motor end-plates by acetylcholine has increased the interest in the choline esterase content of striated muscle. Changes in the choline esterase activity of striated muscles and other organs under different conditions have been reported by several authors. Nachmansohn [1938] and Torda [1938*a*, *b*] have studied the development of choline esterase activity in the striated muscles of different animals before and after birth. We have found [Martini & Torda, 1937, 1938*a*, *b*] in rats and dogs that the choline esterase activity of striated muscle diminishes greatly after degeneration of the motor nerves. Feng & Ting [1938] found a smaller decrease in denervated toad muscle. Marnay & Nachmansohn [1937] had found an increase in the choline esterase activity of denervated guinea-pig muscle. This increase could, however, be attributed to the decrease in muscle volume [Couteaux & Nachmansohn, 1938], and when this factor was taken into account a slight decrease was demonstrable. Von Brücke [1937] found a great reduction in the choline esterase activity of the superior cervical ganglion in cats after denervation. We have also shown that the normal difference in choline esterase activity between the tibialis anterior and gastrocnemius [Martini & Torda, 1938*c*] disappears after removal of the cerebellum, owing to a reduction in the choline esterase activity of the tibialis anterior, and further [1938*d*] that during the first days following transverse section of the dorsal spinal cord of rats the relaxation of the gastrocnemius is associated with a reduction in its choline esterase activity which later, with the appearance of reflex activity, rises to a level above the original.

It was desirable to ascertain whether other changes in activity of muscle would induce similar changes in the choline esterase equivalent. These experiments were undertaken to determine the choline esterase activity of muscles from rats poisoned by tetanus toxin.

Methods

The experiments were carried out on white rats. A sublethal dose of tetanus toxin in 2 c.c. was injected under the skin of one hind leg. The toxin used was prepared in the Istituto Sieroterapico Milanese. Groups of twenty animals were injected simultaneously and groups of two to four were killed at intervals during the following days for estimating the choline esterase activity of the gastrocnemius and tibialis anterior muscles of the hind leg. The animals were perfused with Ringer solution through the aorta, and the entire muscles were removed and finely minced. In several experiments the choline esterase activity of the spinal cord was also determined.

The choline esterase was estimated manometrically according to the method described by Ammon [1934]. 100 mg. of the tissue were placed in the main vessel of the Warburg apparatus. The side vessel contained 10 mg. of acetylcholine chloride in 0·5 c.c. The hydrolysis took place in an atmosphere containing 95% N and 5% CO_2 at a temperature of 37° C. Measurements were taken every 15 min. and the hydrolysis was found to follow a linear curve. The choline esterase equivalent is expressed by the amount of acetylcholine in mg. hydrolysed by 100 mg. tissue in 1 hr. ($Q_{CH.E.}$ of Nachmansohn). The displacement of 140 c.c. CO_2 corresponds to the destruction of 1 mg. of acetylcholine.

Experimental

One to two days after the injection of the toxin a characteristic condition of contraction developed in the hind leg on the injected side, whereas the other leg remained unaffected. The contraction in the gastrocnemius became fully developed within 4–6 days and remained so during the following weeks. In some instances the amount of tetanus toxin injected was too large and the contractions gradually spread over the whole body. These animals were not used.

In confirmation of our earlier experiments we found that the choline esterase equivalent of the right and left gastrocnemius and of the right and left tibialis anterior muscles does not differ by more than 10% and mostly by not more than 5%, but that the tibialis anterior always yields

a higher choline esterase equivalent than the gastrocnemius. In eight control experiments the choline esterase equivalent of the gastrocnemius varied between 0·615 and 0·689 (average 0·654), and that of the tibialis anterior between 0·839 and 1·090 (average 0·917). Further comparisons are given by the means and standard errors given in Table I. After the injection of tetanus toxin and the development of contraction in the gastrocnemius the difference in the choline esterase content of the antagonistic muscles diminishes and may disappear; that of the gastrocnemius increasing and that of the tibialis decreasing. These changes occur within the first 24 hours after the injection. The results are tabulated in Table I. The figures represent mean values on from three to fourteen rats, the number of animals being given in column 1. When more than four animals were examined they were from different batches of injected rats.

TABLE I. Choline esterase equivalent of rat's gastrocnemius and tibialis anterior muscle. The figures are for the means and their standard errors

		mg. of acetylcholine hydrolysed by 100 mg. muscle in 1 hr.			
		Normal side		Injected side	
No. of rats	Days after injection of tetanus toxin	Gastrocnemius	Tibialis	Gastrocnemius	Tibialis
8	—	0·654 ±0·010	0·917 ±0·009	—	—
4	1	0·677 ±0·011	0·922 ±0·015	0·748 ±0·013	0·833 ±0·013
14	2	0·670 ±0·012	0·927 ±0·019	0·729 ±0·019	0·792 ±0·012
12	3	0·664 ±0·010	0·915 ±0·006	0·752 ±0·022	0·800 ±0·018
8	4	0·646 ±0·015	0·905 ±0·015	0·765 ±0·022	0·779 ±0·026
2	5	0·648 ±0·004	0·917 ±0·009	0·794 ±0·050	0·800 ±0·040
6	6	0·676 ±0·013	0·913 ±0·011	0·772 ±0·010	0·810 ±0·019
6	7	0·618 ±0·013	0·883 ±0·019	0·739 ±0·024	0·789 ±0·027
6	8	0·661 ±0·007	0·937 ±0·030	0·815 ±0·028	0·862 ±0·042
4	9	0·651 ±0·005	0·882 ±0·009	0·800 ±0·019	0·798 ±0·007
3	10	0·607 ±0·011	0·918 ±0·027	0·725 ±0·040	0·809 ±0·024
3	14	0·602 ±0·008	0·857 ±0·012	0·677 ±0·016	0·749 ±0·008
3	19	0·690 ±0·026	0·904 ±0·019	0·801 ±0·034	0·804 ±0·015

TABLE II. Choline esterase equivalent of spinal cord of rats injected with tetanus toxin. The figures are for the means and their standard errors

		mg. of acetylcholine by 100 mg. tissue in 1 hr.	
No. of rats	Days after injection of tetanus toxin	Cervical	Lumbar
8	—	4·237 ±0·093	4·908 ±0·204
12	1–4	4·277 ±0·245	5·089 ±0·122
18	5–19	5·174 ±0·155	5·471 ±0·166

The choline esterase equivalent of the spinal cord is much higher and the individual variation is greater than that of muscle. For instance, in the eight control experiments (Table II) it varied between 3·72 and 4·54

for the cervical and between 4·29 and 5·66 for the lumbar cord. Despite these individual variations the results in Table II show that, at least in the later stages of the intoxication, there is no diminution in the choline esterase content of the spinal cord. The figures in Table II also represent the mean values and standard error of the means obtained from the number of rats examined.

SUMMARY

1. The choline esterase equivalents of the gastrocnemius and tibialis anterior muscles and also of the spinal cord of rats were determined manometrically before and after an injection of tetanus toxin.

2. The choline esterase content of the gastrocnemius increases, that of the tibialis anterior decreases. Thus the difference in the choline esterase content of these antagonistic muscles which is observed in untreated animals disappears. There is no diminution in the choline esterase content of the spinal cord.

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