BINDING OF TETANUS TOXIN BY MUSCLE SARCOSOMES IN THE PRESENCE OF ANTITOXINS

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UDC 615.373.39:576.851.551.097.29].033:612.74

Binding of labeled tetanus toxin by muscle mitochondria was investigated in the presence of antitetanus, antidiphtheria, and normal sera and of animal albumin. The intensity of binding of the neutralized tetanus toxin was shown to be of the same order as that of the active toxin.

As previous investigations showed, tetanus toxin is fixed for a long time and in large quantities in the skeletal muscles on the side of injection [2-4], and neutralization of tetanus toxin by antitetanus serum has no marked effect on its binding by the brain tissues [1, 5]. Considering the character of the toxic effects and, in particular, the severe functional changes arising in the skeletal muscles in tetanus and also the disturbance of energy production under these circumstances, the study of the specific features of tetanus toxin binding by the muscle sarcosomes is extremely interesting.

The writer studied the action of antitetanus serum on binding of purified labeled tetanospasmin ($PT\text{-}I^{131}$) by the mitochondria of skeletal muscles. The specificity of this action was checked by control experiments to study the effect of normal and antidiphtheria sera and also of a protein with low molecular weight (animal albumin) on the binding of tetanus toxin by these organelles.

EXPERIMENTAL METHOD

Mitochondria were obtained from muscle sarcosomes by the method of Chappell and Perry [6] in 0.25 M sucrose containing the following components: 0.05 M Tris-buffer, pH 7.4, 0.1 M KCl, 5 mM $MgSO_4$. Each sample contained 4-5 mg mitochondrial protein.

Tetanus toxin (in a dose of 1 μg/mg mitochondrial protein), purified by Pillemer's method [9] and labeled by the method developed in the author's laboratory [2-4], containing 0.005 μg protein per LD_{50} for albino mice, was added to the sarcosomes thus obtained. The antitoxin ("Diaferm-3 IÉM" antitetanus serum) was diluted in 0.25 M sucrose and added to the sample in doses of 1.5, 15, 50, and 150 i.u. "Diaferm-3 IÉM" antidiphtheria horse serum in a dose of 150 i.u., normal guinea pig blood serum in a dose of 6 mg protein, and animal albumin in doses of 0.2, 2, and 20 mg per sample were used in the control tests. In some of the experiments neutralization with $PT\text{-}I^{131}$ was carried out by preliminary incubation of the preparation with with antitoxin for 1 h at 37°C.

Incubation of the sarcosomes with $PT\text{-}I^{131}$ was carried out in an isotonic medium [7] containing the following components: 0.5 ml phosphate buffer 0.066 M, pH 7.4, 0.1 ml 0.2 M $MgSO_4$, 0.1 ml 0.2 M EDTA, 0.1 ml 0.02 M ATP, 0.1 ml 0.2 M sodium pyruvate, and 1.7 ml 0.25 M sucrose solution, for 10 min at 37°C and washed twice to remove the label by centrifugation at 12,000 g for 10 min each time at 0-2°C. The radioactivity of the sarcosomes was determined by the SBT-13 counter of the low-background UMF-1500 instrument. Protein was determined by Lowry's method [8].

Department of Biochemistry, I. I. Mechnikov Odessa University. (Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 76, No. 11, pp. 88-90, November, 1973. Original article submitted January 3, 1973.

TABLE 1. Effect of Various Doses of Antitetanus Serum and Conditions of Neutralization of PI-I^{131} on Its Fixation by Muscle Sarcosomes during Incubation for 10 min at 37°C

Experimental conditions	n	RSA		Change (in %)
		M±m	P	
PT-I^{131} (control)	37	22,1±1,6		100
PT-I^{131} neutralized by antitetanus serum	26	19,6±2,2	0,33	89
PT-I^{131} + 1.5 a.u. antitetanus serum	7	28,2±4,7	0,26	121
PT-I^{131} + 15 a.u. antitetanus serum	7	28,0±4,7	0,26	126
PT-I^{131} + 50 a.u. antitetanus serum	7	19,6±2,2	0,33	89
PT-I^{131} + 150 a.u. antitetanus serum	16	16,2±1,8	0,02	73
PT-I^{131} + 150 a.u. antidiphtheria serum	10	19,0±1,1	0,14	86
PT-I^{131} + 6 mg normal serum	7	20,3±1,1	0,3	91,8
PT-I^{131} + 200 μg animal albumin	7	30,1±3,6	0,06	136
PT-I^{131} + 2 mg animal albumin	7	23,1±2,8	0,87	105
PT-I^{131} + 20 mg animal albumin	7	21,7±2,6	0,96	99

Note. Relative specific activity (RSA) represents ratio between number of pulses/min of PT-I^{131} fixed on 1 mg protein of washed sarcosomes and number of pulses/min from PT-I^{131} added to 1 mg protein of original sarcosomes; n) number of experiments; P) criterion of significance determined from Student–Fisher table; M, m) mean and error of the mean.

EXPERIMENTAL RESULTS

The experimental results show that guinea pig muscle sarcosomes have considerable ability to fix labeled tetanus toxin (Table 1). Even when previously neutralized by specific serum, the tetanus toxin also was intensively fixed by the muscle sarcosomes. The observed decrease in fixation (by 11%) of the neutralized tetanus toxin was not statistically significant.

Fixation of labeled tetanus toxin by sarcosomes also was found to take place in the presence of an excess of antitetanus serum. Different doses of antitetanus serum acted differently on the fixation of tetanus toxin by the mitochondria. As a control of the specificity of action of each dose of antitoxin, the effect of animal albumin, in doses equivalent in protein to the doses of antitetanus serum, on fixation of PT-I^{131} was studied. The results showed that small doses of antitetanus serum actually increased the fixation of PT-I^{131} by the muscle sarcosomes, but the same increase was also observed in the presence of the corresponding doses of animal albumin; this can evidently be explained by the stimulant action of these proteins on the mitochondria. Only large doses of antitetanus serum reduced the fixation of tetanus toxin by the muscle sarcosomes. A dose of 150 i.u. serum reduced the fixation of tetanus toxin by the sarcosomes by 27%. No such decrease was observed when equivalent doses of animal albumin (a decrease of 1%) or of normal serum (a decrease of 8%), or of antidiphtheria serum (a decrease of 14%) could be observed.

The results showing the effect of antitoxins on the fixation of tetanus toxin by muscle sarcosomes do not provide unequivocal evidence of the specificity of this process. The results which indicate that the intensity of fixation of neutralized tetanus toxin is of the same order as the fixation of active toxin by the muscle sarcosomes confirm the previous hypothesis [2, 3] that the site of the tetanus toxin molecule responsible for reception of the toxin is independent of the active center responsible for binding with antitoxin.

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