EFFECT OF NEURAMINIDASE ON THE PROTAGON – TETANUS TOXIN COMPLEX

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Experiments in vitro showed that by the action of neuraminidase on a complex of protagon, isolated from brain tissue with tetanus toxin the complex is destroyed and sialic acid and tetanus toxin appear in the supernatant. The effect is increased with an increase in the dose of neuraminidase. A direct relationship is observed between the quantity of sialic acid split off and of tetanus toxin liberated. The results give further evidence that tetanus toxin is linked with its receptor in the brain substance through the sialic acid residue of the gangliosides. The desirability of testing the effect of neuraminidase in conjunction with antitoxin to counteract the effects of tetanus toxin, with administration of neurominidase and antitoxin locally in the region of the affected nervous structures, is discussed.

Investigation [1, 2, 4-7] have shown that protagon, isolated from brain tissue, strongly binds tetanus toxin. This property of protagon is due to the gangliosides which it contains and which, together with cerebrosides, form a water-insoluble complex [6, 7, 10]. The degree of binding of the toxin depends on the sialic acid content in the gangliosides [8], and it is highest in gangliosides containing two sialic acid residues linked together [9, 10].

The effect of neuraminidase on the protagon–tetanus toxin complex was investigated. It could be postulated that the action of neuraminidase, by splitting sialic acid from the gangliosides, would be to liberate the toxin bound to the protagon. It was important to compare the quantity of liberated toxin with the quantity of sialic acid split off.

EXPERIMENTAL METHOD

Dry tetanus toxin (batch No. 21, strain No. 473 from Leningrad Research Institute of Vaccines and Sera), consisting of a filtrate of a culture of Clostridium tetani, precipitated with ammonium sulfate and vacuum-dried, was used in the experiments. The toxin was dissolved in 0.85% sodium chloride solution, centrifuged (2500 rpm, 10 min, 4°C), and the supernatant used after verification of its toxicity.

The neuraminidase preparation consisted of a lyophilized filtrate of cholera vibrios (N. V. Philips-Duphar, Amsterdam, The Netherlands). The neuraminidase was dissolved in 0.1 M phosphate buffer, pH 5.6.

Protagon was obtained by M. Sh. Promyslov's modification of Wilson and Cramer's method [12].

The dissolved tetanus toxin (1 mg) was added to the protagon (50 mg) homogenized in 0.85% NaCl solution; the total volume of the suspension was 2 ml. The suspension was incubated for 1 h at 37°C and centrifuged for 15 min at 1700 rpm (at 4°C). The residue was washed four times by suspension in 0.85% NaCl solution and centrifuged (15 min, 1700 rpm, 4°C). The residue obtained after the fourth washing,

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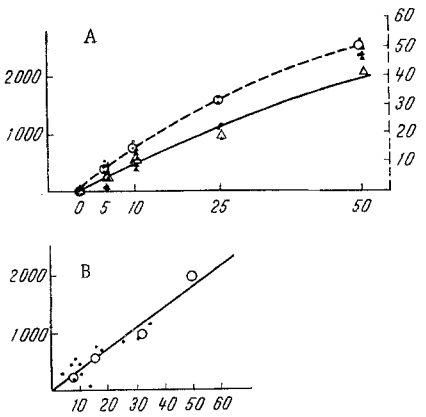

Fig. 1. Action of neuraminidase on protagon–tetanus toxin complex: A) liberation of sialic acid (circles, broken line) and of tetanus toxin (triangles, continuous line) by the action of neuraminidase in various doses; dark circles and triangles are the results of separate tests; unshaded circles and triangles represent mean data. Ordinate: on the left – toxin (in MLD/ml), on the right – sialic acid (in μg/ml); abscissa – neuraminidase (in mg/ml); B) ratio between amounts of sialic acid and tetanus toxin liberated from the protagon–tetanus toxin complex on admission of neuraminidase. Each point is the result of one test; circles denote mean data. Ordinate–toxin (in MLD/ml); abscissa, sialic acid (in μg/ml).

consisting of the protagon–tetanus toxin complex, was treated with an equal quantity of neuraminidase: 5, 10, 25, or 50 mg/ml. No neuraminidase was added to the control. The volume of all the samples was adjusted 2 ml with the same phosphate buffer (0.1 M, pH 5.6). The samples were incubated for 1.5 h at 37°C and centrifuged (1 h, 1700 rpm, 4°C). Tetanus toxin was estimated in the supernatant by biological tests on mice in the usual way, and sialic acid was determined by Warren's method [11]. Statistical analysis of the results was carried out by the correlation method [3].

The control samples (addition of phosphate buffer only, without neuraminidase) usually contained a small quantity of tetanus toxin and sialic acid in the supernatant after centrifugation. This quantity was taken as the zero level and deducted from the amount found in the experimental samples with added neuraminidase.

EXPERIMENTAL RESULTS

The experiment results are shown in Fig. 1. On addition of neuraminidase to the protagon–tetanus toxin complex, a definite increase in the sialic acid and tetanus toxin content was found in the supernatant. The intensity of this effect clearly depended on the quantity of neuraminidase added: with an increase in the content of neuraminidase in the supernatant the content of sialic acid and tetanus toxin also rose (Fig. 1A). Analysis showed a definite correlation (coefficient of correlation 0.7) between the quantity of tetanus toxin and of sialic acid isolated from the protagon–tetanus toxin complex; the quantity of sialic acid split off was directly proportional to the quantity of tetanus toxin liberated (Fig. 1B).

The results of these experiments thus showed that under the influence of neuraminidase the protagon–tetanus toxin complex breaks up and that tetanus toxin is liberated from it. This process is due to the spliting of sialic acid from the gangliosides. The results are additional evidence that tetanus toxin is linked with the gangliosides and, consequently, with its receptor in the brain substance through the sialic acid residue of the gangliosides. In view of these results it would be interesting to test the effect of neuraminidase, in conjunction with antitoxin, in the treatment of tetanus by administration of the neuraminidase and antitoxin locally in the region of the affected nervous structures.

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