EFFECT OF TETANUS TOXIN ON NORADRENALIN LIBERATION FROM RAT BRAIN SYNAPTOSOMES

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A purified preparation of tetanus toxin (TT) (80-800 MLD/mg protein) was shown to induce liberation of both endogenous and exogenous (labeled with ^{14}C) noradrenalin (NA) from isolated nerve endings (synaptosomes) of the rat brain. Within the range of concentrations studied TT does not inhibit secretion of NA evoked by depolarization of synaptosomes by different methods in vitro.

KEY WORDS: synaptosomes; secretion of noradrenalin; tetanus toxin.

There is electrophysiological and biochemical evidence that tetanus toxin (TT) can disturb the function of cholinergic, dopaminergic, and amino-acidergic synapses [1, 2, 8, 9]; this disturbance, moreover, is associated with injury to presynaptic processes and, in particular, disturbance of the process of mediator secretion. It was shown previously that depolarization of synaptosomes in vitro is an adequate model with which to study the effect of various factors on noradrenalin (NA) secretion [5].

The object of the present investigation was to study the action of purified TT on liberation of both endogenous and exogenous (labeled with ^{14}C) NA from brain synaptosomes and also the effect of TT on liberation of NA during depolarization of synaptosomes by various methods.

EXPERIMENTAL METHOD

Brain tissue of noninbred albino rats weighing 180-250 g was used in the experiments. Synaptosomes were isolated from the brain without the cerebellum [10]. The residue of synaptosomes was suspended in Krebs–Ringer solution of the following composition (in mM): NaCl 104, KCl 5, $MgCl_2$ 1.3, NaH_2PO_4 1.2, glucose 10, $CaCl_2$ 1.2, Tris-HCl 20; pH 7.6, at 25°C (solution No. 1).

Unpurified TT (batch 23 from Leningrad Institute of Vaccines and Sera) was purified by gel-filtration on Sephadex G-100. The titer of the toxin was 80,000 MLD for rats/mg protein.

To study the liberation of endogenous NA a suspension of synaptosomes (2-4 mg protein/ml) was preincubated before the experiment (5 min, 37°C). The TT preparation was added to the suspension before the beginning of preincubation. Synaptosomes were depolarized by means of ouabain (0.7 mM, 20 min) and electrical stimulation (ES) with square pulses of alternating polarity (100 Hz, 0.4 msec, 5 V, 12 mA) for 5 min [5]. To prevent breakdown of NA, 0.2 mM Pargyline, a monoamine oxidase inhibitor, was added to the incubation medium. After the end of incubation the synaptosomes were separated by centrifugation (12,000g, 10 min, 0-4°C) and the concentration of endogenous NA was determined in the supernatant after precipitation of the proteins with 1N $HClO_4$. The trihydroxyindole fluorescence method of determining catecholamines was used. Adsorption of NA and its elution from Al_2O_3 were carried out by the method of Anton and Sayre [7], and oxidation and measurement of the concentration of NA on a Hitachi-203 spectrofluorometer (Japan) [5].

To study the action of TT on uptake and liberation of labeled NA the synaptosomes (0.25 mg protein/ml) were preincubated during mixing for 15 min at 37°C in solution No. 1. After addition of 6.1 μmoles NA-^{14}C (Radiochemical Centre, Amersham, England, 35 mCi/mmole) in the presence of 0.2 mM Parygline, incubation was continued for 10 min. The synaptosomes were separated by centrifugation, washed twice with physiological

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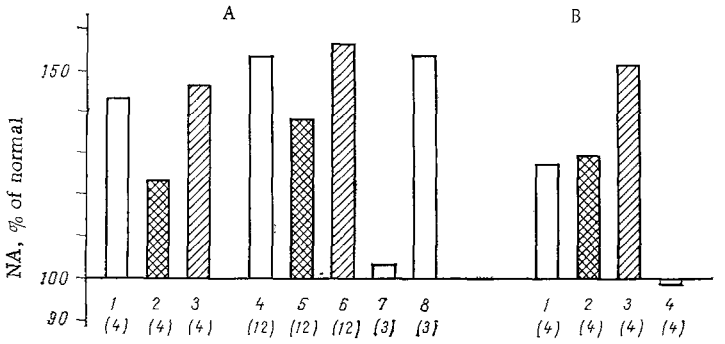

Fig. 1. Effect of tetanus toxin on liberation of endogenous (A) and exogenous (labeled with ^{14}C (B) NA from synaptosomes under normal conditions and after depolarization by various methods. A: 1) Electrical stimulation (ES); 2) tetanus toxin (TT), 500 MLD; 3) ES against the background of TT; 4) ouabain (0.7 mM); 5) TT (800 MLD); 6) ouabain against the background of TT; 7) TT inactivated by antitetanus serum; 8) ouabain against the background of inactivated TT. B: 1) K^+-depolarization (+35 mM KCl); 2) TT (800 MLD); 3) K^+-depolarization against the background of TT; 4) TT inactivated by antitetanus serum. Number of experiments given in parentheses. NA concentration in incubation medium in control taken as 100% (base line).

saline, and at this stage part of the residue was dissolved in 1N NaOH and used for counting radioactivity of assimilated NA, while the rest was resuspended either in solution No. 1 (control) or in solution No. 2 of the following composition (in mM): NaCl 74, KCl 35, $MgCl_2$ 1.3, NaH_2PO_4 1.2, glucose 10, $CaCl_2$ 2.4 Tris-HCl 20; pH 7.6, at 25°C. The synaptosomes thus prepared were again incubated with mixing (37°C) for 15 min, the synaptosomes were again separated by centrifugation after which radioactivity was determined in 0.1 ml of supernatant (2400 cpm/mg protein in the control). Radioactivity was counted with the aid of toluene scintillator with the addition of ethanol, and the measurements were made on an Intertechnique SL-30 (France) scintillation spectrometer. Liberation of mediator was expressed as percentages of the total radioactivity of the supernatant and residue. The TT was inactivated with "Diaferm-3 ÍÉM" (USSR) antitetanus serum in the ratio of 1 i.u. to 400 MLD for rats. Protein was determined by Lowry's method. The nonparametric U test was used for statistical analysis of the results.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of TT on liberation of endogenous NA from synaptosomes under the influence of ES and of depolarization by ouabain was studied. As Fig. 1A shows, ES for 5 min led to an increase ($P < 0.05$) in the NA concentration in the incubation medium on average by 43% (25-52%). However, TT itself in a dose of 500 MLD/ml suspension also caused an increase ($P < 0.05$) in the NA concentration in the incubation medium by 23% (6-50%) compared with the control, i.e., with the normal "leakage" of mediator. TT did not change the action of ES: Against the background of added toxin ES caused liberation of the same quantity of NA – by 46% (16-75%) above the control.

Treatment with 0.7 mM ouabain (Fig. 1A) also led to an increase ($P < 0.01$) in liberation of NA by 53% (34-67%). The TT preparation in a dose of 800 MLD/ml suspension of synaptosomes also increased ($P < 0.01$) liberation of endogenous NA by 38% (10-68%). Just as in the case of ES, TT did not change the action of ouabain: After addition of TT ouabain led to an increase ($P < 0.01$) in NA liberation by 56% (36-72%) compared with the control.

The results given in Fig. 2 show that the action of TT on secretion of endogenous NA is dose-dependent. With an increase in the dose the quantity of NA liberated rises proportionally. Inactivation of TT with antitetanus serum led to complete blocking of its effect on NA liberation.

The study of the action of TT on uptake of labeled NA by synaptosomes showed (results of seven experiments) that the rate of active uptake of the mediator in the presence of TT (800 MLD/ml suspension) was 95.5%

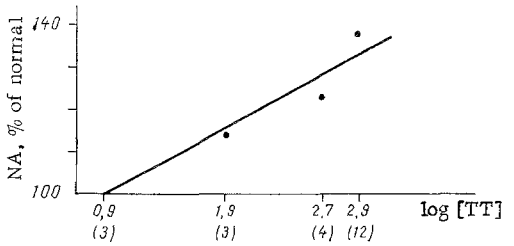

Fig. 2. Quantity of endogenous NA liberated as a function of dose of tetanus toxin (concentrations of TT used: 8, 80, 500, and 800 MLD). Legend as in Fig. 1.

(84-110%) of the control, i.e., it was not significantly changed. This is in agreement with data showing that TT has no effect on uptake of GABA-^{3}H by synaptosomes or on the activity of synaptosomal Na, K-ATPase [4], an enzyme which also determines the function of the system for active mediator uptake.

K^+-depolarization (35 mM KCl) of synaptosomes loaded with NA-^{14}C led to an increase ($P < 0.05$) in liberation of the mediator (Fig. 1B) by 27% (20-31%). Incubation of synaptosomes with TT (800 MLD/ml), leading to an increase ($P < 0.05$) in the content of labeled NA in the incubation medium by 29 (25-33%) compared with the control, had a similar effect. However, by contrast with the experiments with endogenous NA, the toxin evidently potentiated the action of K^+-depolarization (Fig. 1B), increasing ($P < 0.01$) the liberation of labeled NA to 51% (31-75%). Antitetanus serum completely abolished this effect of TT.

Two conclusions can thus be drawn from the results of these investigations: 1) TT, by its action on brain synaptosomes, increases the liberation of endogenous and exogenous NA mediator; 2) TT does not inhibit NA secretion from synaptosomes induced by depolarizing agents. These two effects have not been described before.

Reserves of nonvesicular, i.e., cytoplasmic NA are known to be small and to amount to 5-10% of its total content in nerve endings. It is therefore very probable that the liberation of NA observed in the present experiments under the influence of TT (about 20% of the content of NA in the synaptosomes) reflects liberation of mediator stored in vesicles.

The fact that the effects of TT and depolarizing agents in these experiments are not additive is also evidence in support of the view that depolarizing agents and TT liberate NA from the same stock. Further research is required to obtain final proof of this hypothesis.

According to data obtained by electron microscopy, TT does not change the ultrastructure of synaptosomes. The toxin evidently has no harmful action on synaptosomes, although it can change their osmotic sensitivity [4].

It was shown previously in the writers' laboratory [3] that TT in vitro suppresses superprecipitation of partially purified actomyosin-like protein from rat brain, i.e., the action of TT in this experimental model is comparable with the action of certain mitotic alkaloids. A mitotic poison such as vinblastine (0.25 mM) is known to cause spontaneous liberation of NA, GABA, dopamine [14], and serotonin [15] from rat brain synaptosomes. If isolated nerve endings were saturated with labeled mediators before the addition of this mitotic alkaloid (as was the case in the present experiments also), addition of the latter did not change the rate of liberation of mediator evoked by KCl. If, however, the vinblastine was present before addition of the labeled mediator, considerable suppression of uptake of mediators by isolated nerve endings and also a distinct decrease in liberation of transmitter induced by KCl were observed [14].

It was suggested previously that TT disturbs the liberation of mediators by the presynaptic terminal regardless of the nature of the mediator [12, 13]. In fact, as has already been pointed out, TT blocks the liberation of glycine [9], GABA [1, 8], acetylcholine [2], and dopamine [1]. The results of the present investigation suggest that there is an exception to this rule: Spontaneous liberation of NA by synaptosomes, which not only is not blocked but, on the contrary, is actually increased under the influence of TT. The mechanism of this phenomenon is not yet sufficiently clear, nor is it clear whether it takes place in vivo. It was shown sometime ago that if TT is introduced into the anterior chamber of the eye the effects of adrenergic innervation of the iris are unchanged [6]. At the same time, in severe generalized tetanus, sympathetic hyperactivity is known to be present [2, 11]. It is important to consider this fact in relation to the data now obtained showing potentiation of spontaneous NA secretion by central adrenergic terminals (synaptosomes) under the influence of TT.

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LITERATURE CITED

1. M. I. Aliev and G. N. Kryzhanovskii, Byull. Éksp. Biol. Med., No. 4, 314 (1979).
2. G. N. Kryzhanovskii, Tetanus [in Russian], Moscow (1966).
3. G. N. Kryzhanovskii, R. N. Glebov, N. M. Dmitrieva, et al., Byull. Éksp. Biol. Med., No. 12, 24 (1974).
4. G. N. Kryzhanovskii, Yu. G. Sandalov, V. I. Rodina, et al., Byull. Éksp. Biol. Med., No. 2, 139 (1977).
5. V. I. Rodina, V. V. Rozhanets, G. N. Kryzhanovskii, et al., Dokl. Akad. Nauk SSSR, 234, 235 (1977).
6. M. Ambache, R. S. Morgan, and G. P. Wright, J. Physiol. (London), 107, 45 (1948).
7. A. H. Anton and D. F. Sayre, J. Pharmacol. Exp. Ther., 138, 360 (1962).
8. H. F. Bradford, in: Handbook of Psychopharmacology, Vol. 1, New York (1975), p. 191.
9. D. R. Curtis and W. C. De Groat, Brain Res., 10, 208 (1968).
10. F. Hajos, Brain Res., 93, 485 (1975).
11. J. H. Kerr, J. L. Corbett, C. Prys-Roberts, et al., Lancet, 1, 236 (1968).
12. G. N. Kryzhanovsky (G. N. Kryzhanovskii), Arch. Exp. Path. Pharmak., 276, 247 (1973).
13. G. N. Kryzhanovsky (G. N. Kryzhanovskii), R. N. Glebov, R. M. Kulyguina, et al., in: Proceedings of the 4th International Conference on Tetanus, Vol. 1, Dakar (1975), p. 205.
14. W. J. Nicklas, S. Puszkin, and S. Berl, J. Neurochem., 20, 109 (1973).
15. T. Segawa, H. Murakami, A. Inouye, et al., J. Neurochem., 30, 175 (1978).

ENERGY METABOLISM AND CONTRACTILE ACTIVITY OF THE MYOCARDIUM AFTER CARDIOCYTOTOXIC INJURY

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Experiments on anesthetized dogs with a closed chest showed that injection of anticardiac cytotoxic serum into one of the main branches of the left coronary artery was followed by zonal disturbances of energy metabolism (a decrease in the ATP, ADP, AMP, and glycogen concentrations and contractility of the affected area of the left ventricle). Compensatory hyperfunction of the left and right ventricles and an increase in their noradrenalin concentration were found. The indices of energy metabolism in the unaffected area of the heart showed no significant change.

KEY WORDS: cardiodynamics; myocardial metabolism; injury to the heart.

It was shown previously that injection of anticardiac cytotoxic serum (ACS) into one branch of the left coronary artery led to sharp and prolonged disturbances of the hemodynamics similar to the picture of cardiogenic shock [1] and due to disturbances of the contractile activity of the myocardium [3] and to deposition of blood [5]. Focal injury of the heart muscle developed in the region of direct action of ACS [7].

To shed light on the mechanisms of disturbance of cardiac activity after cytotoxic injury, it was considered important to compare changes in energy metabolism in different parts of the heart with zonal changes in contractile activity of the myocardium.

Department of Experimental Cardiology, A. A. Bogomolets Institute of Physiology, Academy of Sciences of the Ukrainian SSR, Kiev. Central Research Laboratory, A. A. Bogomolets Kiev Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Gorev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 2, pp. 151-153, February, 1980. Original article submitted January, 19, 1979.

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