was not found between BP and changes in the diameter of the vessels (experiments of series II, $r = +0.32$, $P > 0.05$), although vasoconstriction amounted to about 4%. It can be concluded from these results that microcirculatory changes observed in the mesentery in the initial phase of ganglion stimulation are due, on the one hand, to the vasoconstriction of resistive vessels and, on the other hand, to an increase in CO. The increase in CO in this case is evidently nonspecific in character and may be due to gradual narrowing of the lumen of the mesenteric arteries bringing blood into the mesentery and exclusion of the corresponding regions of the mesentery and intestine from the circulatory system. A direct or indirect influence at the same time on the contractile characteristics of the heart muscle likewise cannot be ruled out. Subsequent changes in CO may have been due to a response of the hemodynamic system as a whole to changes in TPR. Definite correlation was found between the changes in BP in the phase of maximal hypertension, expressed as a percentage, and ΔD ($r = -0.46$, $P < 0.01$). This suggests that the changes in BP and the corresponding microhemodynamic changes in the microcirculatory network of the mesentery at this stage were due mainly to vasoconstriction of the resistive vessels of the mesentery.

Comparison of the results of the two series of experiments shows that vasoconstriction of the mesenteric vessels in response to stimulation of the celiac ganglion took place against the background of a fall in TPR and, conversely, the initial hypotension was associated with a very small rise in TPR. This suggests that the vessels of other organs, such as the liver or spleen, must dilate in response to electrical stimulation of the celiac ganglion.

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EFFECT OF TETANUS TOXIN ON MECHANISMS OF REGULATION OF TRANSMITTER SECRETION IN NEUROMUSCULAR JUNCTIONS BY CALCIUM IONS

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The peripheral synaptic defect caused by tetanus toxin (TT) is due to a disturbance of transmitter secretion in the neuromuscular junction: spontaneous liberation of transmitter and, in particular, that induced by a nervous impulse, are inhibited [1, 4, 9, 12]. The most important factor regulating the secretion process is calcium ions [10, 11]. The stages of this type of regulation can be investigated by using various procedures: 1) changes in the calcium ion concentration gradient on the membrane whose permeability is unchanged, i.e., by changing the extracellular calcium concentration; 2) increasing the quantity of free cytoplasmic calcium on account of its liberation from intracellular depots, from mitochondria for example; 3) by changing membrane permeability for calcium ions by activation of endogenous ionophores of calcium channels, as is the case during depolarization, or by addition of exogenous ionophores; 4) by influencing the calcium-dependent process of secretion of quanta of transmitter through specialized regions of the presynaptic membrane ("active zones") directly.

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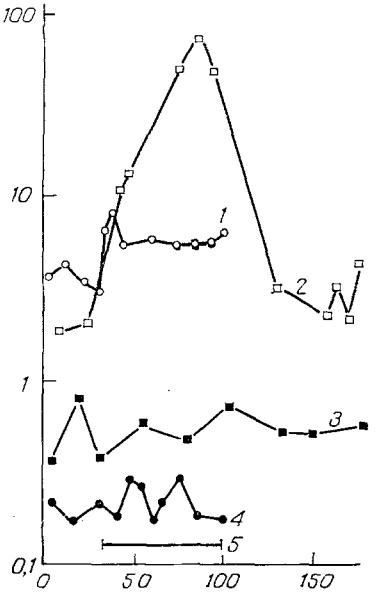

Fig. 1

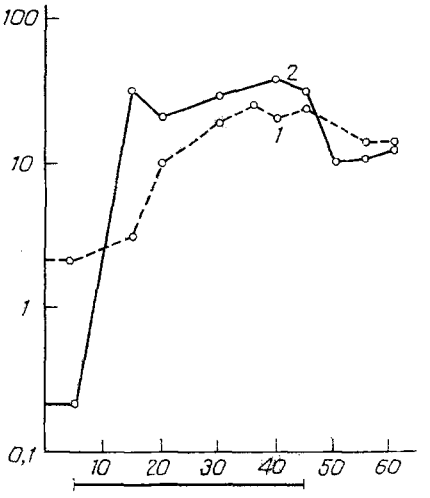

Fig. 2

Fig. 1. Effect of factors increasing inflow of calcium ions on spontaneous transmitter secretion in single neuromuscular junctions. Abscissa, time of observation (in min); ordinate, frequency of MEPPs (in Hz); 1) Ca^{++} concentration in external medium increased to 20 mM; 2) A 23,187; 3) TT + A 23,187; 4) TT + Ca^{++}; 5) time of action of each factor.

Fig. 2. Effect of ouabain on spontaneous secretion of transmitter in single neuromuscular junctions. 1) Ouabain (0.1 mM); 2) TT + ouabain. Line below abscissa represents time of action of ouabain. Remainder of legend as to Fig. 1.

TABLE 1. Effect of Various Methods of Increasing Calcium Ion Concentration in Cytoplasm on Frequency of MEPPs (M ± m)

Experimental conditions	Frequency of MEPPs, Hz					
	Ca_0^{2+}			A 23 187	ouabain	
	0	2 mM	20 mM	2 μM	0,1 mM	1 mM
Normal	0,61±0,18	2,90±0,19	11,6±1,2	48,3±0,1	21,6±3,2	75,4±1,3
Injection of TT	0,56±0,13	0,52±0,07	0,42±0,15	0,55±0,17	0,6±7,4	125,0±17,1

Legend. Ca_0^{2+}) Calcium concentration in external medium.

The effects of procedures of this type were evaluated in the present investigation, the aim of which was to study the state of the calcium component of the mechanism of transmitter secretion during inhibition of that process by TT. In the course of the experiments the calcium ion concentration in the incubation medium was changed and pharmacological agents such as the calcium A 23,187, ouabain, and 4-aminopyridine, also were used.

EXPERIMENTAL METHODS

August rats weighing 100-120 g were used. The test objects were isolated preparations of the diaphragm and phrenic nerve, kept in constant-temperature (34-35°C) chambers through which carbogenized (95% O_2 + 5% CO_2) Tyrode's solution or a modification of it depending on the experimental conditions, was passed. To avoid mechanical displacement of the muscle, in all experiments involving indirect stimulation of the muscle and in some experiments to record spontaneous activity, glycerinized preparations were used, in which electromechanical connection was severed [13]. The nerve was stimulated electrically by square pulses (0.5 msec) with a frequency of 50 Hz for 1 sec. Secretion of transmitter evoked by stimulation

of the nerve and spontaneous secretion were evaluated by recording end-plate potentials (EPPs) and miniature EPPs (MEPPs) intracellularly by means of glass microelectrodes filled with 2.5 M KCl solution in the usual way. The processes studied were recorded on an SDR-41 tape recorder. Purified TT, in a volume of 0.1-0.3 ml ($0.5 \cdot 10^6$ MLD for mice in 1 ml) was injected into the diaphragm 3-3.5 h before isolation of the preparation. The $CaCl_2$ in the external medium was increased to 20 mM by equiosmolar replacement of $NaHCO_3$ and part of the NaCl, thus ensuring adequately complete ionization. Calcium-free solution was prepared by adding EDTA (5 mM). The substances were used in the following concentrations: calcium ionophore A 23,187 — $2 \cdot 10^{-6}$ M, ouabain 10^{-4}-10^{-3} M, 4-aminopyridine 10^{-6}-10^{-4} M.

EXPERIMENTAL RESULTS

Data on changes in the calcium ion concentration in the external medium on the frequency of MEPPs are given in Table 1. In a single fiber an increase in the Ca^{2+} concentration in the external medium under normal conditions led to a marked rise in the MEPP frequency, with a marked peak after the change of solution, followed by flattening out at a steady level (Fig. 1). In preparations poisoned with TT, in which the level of secretion was substantially lower, after replacement of the solutions the changes in MEPP frequency described above were not observed (Fig. 1).

Ionophore A 23,187 normally activated mediator secretion considerably, although the result of its action in a single fiber was manifested rather slowly; no significant changes in MEPP frequency could be detected, however, in preparations treated with TT (Table 1, Fig. 1).

Ouabain also caused dose-dependent activation of transmitter liberation, but its action not only continued after poisoning with TT, but in many fibers it was actually stronger under these conditions (Table 1). Characteristically, in the case of long exposures a high level of spontaneous activity fell rather slowly after rinsing the preparation (Fig. 2), but in high doses (1 mM) it sometimes remained virtually unchanged for a long period of observation (over 1 h).

4-Aminopyridine caused no appreciable changes in MEPP frequency either under normal conditions or after poisoning with TT, but it had a significant effect on the character of distribution of amplitudes of MEPP: In the case of poisoning the initial, slightly skew, curve was transformed into a polymodal curve. The specific feature of the effects of 4-aminopyridine — the appearance of "giant" MEPPs [5] — still remained when its action was preceded by poisoning. In response to indirect excitation of the poisoned preparation, the addition of 4-aminopyridine potentiated the known [4] activating effect of repetitive stimulation on secretion when inhibited by TT: The total number of quanta liberated (during the period of stimulation, namely 1 sec) increased from 29.8 ± 10.3 to 485.1 ± 17.1. The contributions of induced secretion increased much more: The total number of quantum components of EPPs arising during stimulation changed from 17.1 ± 2.7 to 339.8 ± 15.4, i.e., the relative proportion of synchronously discharged quanta increased considerably. In many cases the effect of 4-aminopyridine actually led to restoration of conduction: to the appearance of action potentials in the muscle fiber.

The character of reactions of the terminal, when poisoned by TT, to factors affecting different stages of the process of calcium regulation thus was found to be very varied. The activating effect of an increase in the external Ca^{2+} concentration was absent in the poisoned terminals, in which transmitter secretion after a change in the Ca^{2+} concentration in the external medium was the same as in calcium-free medium under normal conditions. Addition of ionophore A 23,187, which increases the permeability of the outer membrane for calcium ions and, consequently, under normal conditions considerably activates transmitter secretion, also was ineffective when acting on the poisoned terminal. It can be concluded from these facts that the final stage of the secretion process — interaction of calcium ions with complementary structures of the terminal, leading to exocytosis, is disturbed in the injured terminals. However, the effects of ouabain, an inhibitor of the sodium pump, do not fit in completely with this explanation. Ouabain leads to considerable activation of transmitter secretion by both normal and poisoned terminals. In the case of poisoning, the level of secretion was actually higher than that under normal conditions and estimation of the number of quanta of transmitter secreted indicates preservation of the operative fraction of transmitter, or even an increase in it, in poisoning, in good agreement with the results of electron-microscopic observations, indicating the accumulation of vesicles in the poisoned terminal [2]. It can be tentatively suggested that in the case of poisoning ouabain activates secretion,

not through the abolition of pump electrogenesis and the development of depolarization, but chiefly through the outflow of calcium, as a result of the inflow of sodium, from the mitochondrial depots, a rise in its concentration in the cytoplasm, and its asynchronous supply to the transmitter secretion sites [7, 14]. This method of increasing the quantity of free calcium in the terminal, brought about through the sodium pump, is effective to a certain extent in restoring exocytosis of the transmitter, when inhibited by the toxin, and it probably determines the preservation of the activating effect of repetitive stimulation and of other modulating factors (biogenic amines, K^+, etc.) after poisoning [6].

The effect of nondepolarizing doses of 4-aminopyridine — transformation of the curve of distribution of MEPP amplitudes and the appearance of "giant" MEPPs, reflecting spontaneous desynchronization of the liberation of quanta of transmitter [3, 5], evidently develops in the last stage of the secretion process and persists after poisoning, when there is a change in the distribution of MEPP amplitudes reflecting disturbance of the mechanism of quantum standardization [3]. It is curious that the reactivating effect of 4-aminopyridine on the poisoned ending is particularly effective as regards the synchronous liberation of quanta of transmitter due to repetitive stimulation. These unique features of the reactivating effects of ouabain and 4-aminopyridine are in agreement not only with modern views on different sources of free calcium in the terminals [10], but also with data on the ultrastructural localization of calcium in two zones of the terminal: one — intracytoplasmic, the other — above the crest of the synaptic fold, reflecting evidently a calcium channel located in the "active zone" of the presynaptic membrane [8]. It can be tentatively suggested that TT, although not injuring intracellular sources of Ca^{2+}, disturbs the local inflow of Ca^{2+} in the "active zones," i.e., its specific fixation by presynaptic membrane receptors (gangliosides) [15] prevents the functioning of both endogenous and exogenous ionophores.

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