ACTION OF TETANUS TOXIN AND COLCHICINE ON SYNAPTIC MEMBRANES OF THE RAT CEREBRAL CORTEX

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Purified tetanus toxin (TT), in experiments in vitro, was shown to affect neither the Na,K-ATPase activity of the synaptic membrane fraction of the rat cerebral cortex nor the inhibition of Na,K-ATPase activity produced by electrical stimulation of a suspension of synaptic membranes, nor the binding of GABA-^{3}H by synaptosomes. TT and colchicine (1 mM) reduced the osmotic sensitivity of the nerve endings. Colchicine, in low concentrations (10^{-5} to 10^{-3} M), does not affect Mg- and Na,K-ATPase but, in higher concentrations (10^{-2} M), it inhibits the activity of both ATPases considerably.

KEY WORDS: *synaptosomes; electrical stimulation; GABA binding; colchicine; osmotic sensitivity; contractile proteins; tetanus toxin.*

Tetanus toxin (TT) disturbs the secretion of mediators by nerve endings [5, 6, 10, 17, 19]. TT is bound selectively with the presynaptic membranes (PSM) [1, 21]; interacting with the ganglioside–cerebroside complex [11, 23], it causes changes in the rate of renewal of the proteins of nerve endings [12], and inhibits contractile activity of the actomyosin-like protein of synaptic origin [7], the function of which is linked with the mechanism of secretion of neuromediators [2, 3]. An important role in the mechanism of the structural changes in PSM responsible for coupling depolarization and mediator secretion is played by transport Na,K-ATPase and contractile proteins. The plant alkaloid colchicine, which disturbs the structure of contractile proteins and, in particular, of tubulin, is known [2, 3] to block the secretion of mediators in various synapses. These facts may also point to a contractile mechanism of mediator secretion.

The object of this investigation was to study the effect of TT and colchicine, in experiments in vitro, on Na,K-ATPase activity of the synaptic membrane fraction (SM), the reassimilation of the inhibitory mediator GABA-^{3}H by the synaptosomes, and on the osmotic sensitivity of the synaptosomes.

EXPERIMENTAL METHOD

Experiments were carried out on brain tissue of noninbred rats weighing 180–200 g. The fractions of enriched SM and mixtures of light and heavy synaptosomes were obtained as described previously [15]. Active uptake of GABA-^{3}H in buffered salt medium at pH 7.4 was measured by using freshly isolated synaptosomes by the method described previously [14]. Activity of Na,K-ATPase was determined by the accumulation of orthophosphate set free during the reaction [20]. Electrical stimulation of the SM suspension was carried out for 30 min at 37°C after free incubation for 5 min in Krebs–Ringer medium [4]; the protein concentration in the suspension was 1-3 mg/ml. Samples containing 100-150 µg protein were taken after electrical stimulation for determination of ATPase activity. Protein was estimated by Lowry's method. The osmotic sensitivity of the freshly isolated synaptosomes was recorded as a change in optical density of the suspension at 520 nm (20°C) in Krebs–Ringer medium with pH 7.4 in the presence of TT, colchicine (Sigma, USA) or human serum albumin (Reanal, Hungary), which was

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TABLE 1. Na,K-ATPase Activity of Intact Synaptic Membranes and after Electrical Stimulation, Binding of GABA-^{3}H by Synaptosomes, and Osmotic Sensitivity of Synaptosomes under the Influence of TT and Colchicine (mean results of 5-6 experiments, each with 2 or 3 repetitions)

Experimental conditions	Na,K-ATPase		Binding of GABA-^{3}H, nmoles/mg protein/20 min at 37°C*	Change in optical density, %†
	activity of enzyme, μmoles/mg protein/h	effect of elec. stim., % of corresponding control		
control (nothing added)	8,1±0,4	68	4,0±0,036	100±2,0
TT (400 MLD, 5 μg protein)	8,5±0,5	—	3,90±0,06	—
TT (4000 MLD, 50μg protein)	7,95±0,4	65	3,8±0,08	115±2,2 ‡
Colchicine 1 mM	—	—	—	112±4,9‡
Albumin 50 μg	—	—	—	99,5±8,2

*Data given after subtraction of absorption control.
†Mean optical density in control 0.15.
‡P < 0.05.

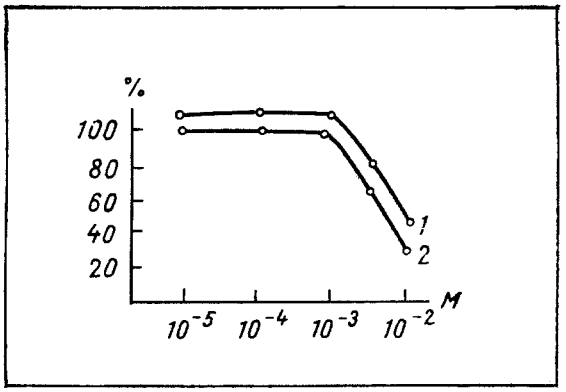

Fig. 1. Change in activity of Na,K-ATPase and Mg-ATPase of synaptic membrane fraction of rat cerebral cortex under influence of colchicine: 1) Na,K-ATPases; 2) Mg-ATPase. Ordinate, activity of ATPases (in %); activity of ATPases in control (without colchicine) taken as 100%; abscissa, concentration of colchicine (molarity of solution).

used as the control in the experiments with TT. TT was purified by the method described previously [11]. The activity of the purified TT was $5 \cdot 10^5$ MLD/mg protein. For the electrical stimulation experiments a suspension of freshly isolated SM was used; for the experiments with colchicine the SM were kept for a week at −10°C in 30 mM Tris-HCl, pH 7.4, and 0.1 mM ethyleneglycol tetraacetate (EGTA). The colchicine was dissolved in 50% ethanol, the concentration of which in the final sample did not exceed 3-5%. In the study of ATPase activity and osmotic sensitivity of the synaptic structures an additional control for the possible action of the ethanol was used. The results were subjected to statistical analysis with the aid of the Student–Fisher criterion.

EXPERIMENTAL RESULTS AND DISCUSSION

As Table 1 shows, even in large doses TT did not affect the Na,K-ATPase of the SM fraction of rat brain. These results are in agreement with those of the writers' previous investigations into the action of TT on the Na,K-ATPase of unpurified synaptosomes [8]. Electrical stimulation of the SM suspension inhibited Na,K-ATPase activity and TT did not change this effect.

This effect of inhibition was described by the writers previously [4] as evidence that depolarization of electrically excitable synaptic membranes causes structural changes in the membrane, expressed as labilization of protein–lipid bonds and an increase in cationic permeability.

Even in large doses TT did not affect the binding of GABA-^{3}H by the rat brain synaptosomes. Tetanus toxin, like colchicine (1 mM), caused a statistically significant change in the osmotic sensitivity of the synaptosomes, consisting of an increase in the optical density of the suspension at 520 nm which, in turn, indicated a decrease in the volume of the synaptosomes. Albumin, taken in the same concentration as TT, caused no change in the optical density of the synaptosomal suspension.

It will be clear from Fig. 1 that colchicine, in low concentrations (10^{-5}-10^{-3} M) did not affect the activity of Na,K- or Mg-ATPase of the SM fraction of the cerebral cortex, in agreement with data in the literature on the action of colchicine on Na,K-ATPase of brain membranes[22]. However, in high concentrations (10^{-2} M) considerable inhibition of the ATPases tested was observed (by 60-80%). The inhibitory action of high concentrations of colchicine is nonspecific, for together with Na,K-ATPase activity , activity of Mg-ATPase also was inhibited.

During the discussion of these results it must be remembered that structural and functional changes in PSM responsible for triggering the secretory mechanism must of necessity be reduced to two main processes: an increased entry of Ca^{2+} ions inside the terminals and structural changes in PSM preparing them for specific contact with synaptic vesicles [2]. The first of these processes is largely bound with the state of Na,K-ATPase activity which, during excitation of nerve endings, is inhibited [2-4]. An important role in the second process is played by the contractile protein of synaptic structures. The suggestion has been made that the Ca-dependent mechanism of exocytosis consists of interaction between the myosin-like protein of the membranes of the synaptic vesicles and the actin-like protein of the PSM [2, 3, 16].

It follows from these experiments that the Na,K-ATPase of the PSM is not the target for TT. Presumably TT does not affect the passive transport of Ca^{2+} through the PSM. The high-affinity system of reassimilation of mediators and choline by the terminals, with high transfer affinity and a low Michaelis constant, is known to be coupled with the function of Na, K-ATPase. Substances blocking uptake of mediators can also inhibit Na,K-ATPase activity [13]. From this standpoint the results indicating no effect of TT on the system for reassimilation of GABA by synaptosomes are in agreement with data showing that TT has no effect on Na,K-ATPase. It has been shown [9] that TT substantially reduces the effectiveness of osmotic regulation of the process of acetylcholine secretion in neuromuscular synapses. These, and also the effects of TT as regards changes in osmotic sensitivity of the synaptosomes demonstrated by these experiments, can be explained by the binding of TT with the glycolipids of PSM [1, 11, 21, 23] and by the structural changes arising thereupon in the membranes. The action of colchicine, however, may be directed both toward the actin components of PSM and toward the fragments of microtubules composing PSM (adjacent to the active zone of PSM) or the cytoplasm of the nerve endings. It is interesting to note that tubulin, a component of the microtubules, in the brain [18] and the actomyosin-like protein of synaptic origin can associate with glycolipids.

It can tentatively be suggested that the action of TT is directed mainly at those regions of PSM which, during excitation of the nerve endings, are responsible for contact between the synaptic vesicles and the corresponding regions of PSM, i.e., which carry out the final stage of the mechanism of exocytosis. Interaction between TT and PSM causes structural changes in the membrane coupled with a disturbance of the function of the contractile proteins of the synaptic structures, which ultimately leads to the blocking of secretion of mediators. The change in the properties of the contractile proteins of the synaptic structures may be due to penetration of the toxophore group of TT inside the nerve endings [19].

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CHANGES IN THE RATE OF METABOLIC CLEARANCE OF CORTISOL IN DOGS AFTER TERMINAL STATES

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In the early period of resuscitation after circulatory arrest for 15 min in dogs the rate of metabolic clearance of 17-hydroxycorticosteroids (17-HCS) was found to be reduced, more especially in animals which subsequently died. This decrease was due mainly to a decrease in the clearance of plasma 17-HCS by organs in the splanchnic region and was evidently connected with the circulatory disturbances.

KEY WORDS: *circulatory arrest; postresuscitation period; rate of metabolic clearance of 17-hydroxycorticosteroids.*

Increased secretion of certain adaptive hormones in various types of stress and terminal states has recently been conclusively proved, but the problem of coexisting changes in hormone utilization in the body still remains unexplained. The most complete picture of the uptake of hormones by effector tissues is given by the rate of metabolic clearance of the hormone, reflecting the volume of plasma irreversibly freed from hormone in unit time [11]. Its value, according to various workers [4, 9, 12, 13], correlates directly with the strength of the hormonal effect.

Changes in the rate of metabolic clearance of cortisol in the postresuscitation period after circulatory arrest for 15 min were studied.

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