ACTIVITY OF TRANSFER ATPase IN SUBCELLULAR FRACTIONS OF THE SPINAL CORD TISSUE OF RATS POISONED WITH TETANUS TOXIN

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The Mg-ATPase activity in the anterior horn tissue of the rat spinal cord on the side of injection of the toxin in generalized tetanus is increased in the coarse mitochondrial (CM) fraction and unchanged in the microsomes. The activity of Na,K-ATPase is increased in local tetanus in the microsomes, and in generalized tetanus in CM and the microsomes. On the side opposite to injection of the toxin no changes in Mg-ATPase activity were found in the subfractions but Na,K-ATPase activity was increased in the microsomes. Tetanus toxin in vitro does not affect the activity of unpurified preparations of Na,K-ATPase in CM from the cerebral cortex or of Mg-ATPase from the brain and mitochondria of the heart.

Poisoning with tetanus toxin (TT) blocks the secretion of inhibitory mediators in the spinal cord, and if large doses are given, the secretion of mediators in the neuromuscular synapse also is blocked [3]. However, the primary stage of the biochemical disturbances in the synapses in TT poisoning has not yet been explained. TT is known to bind specifically with gangliosides, structural components of synaptic membranes [9].

Considering that transfer ATPase plays an important role in membrane processes, which can be modified in TT poisoning, it was decided to investigate the state of the various ATPases of spinal cord tissue in experimental tetanus.

EXPERIMENTAL METHOD

Noninbred albino rats weighing 200 ± 20 g were used. After decapitation the spinal cord was quickly removed and the region of the anterior horns of the lumbosacral enlargement was isolated on a freezing stage. The tissue was homogenized in 10 volumes of 0.32 M sucrose with 0.05 M Tris-HCl, pH 7.4, at 0-2°C. The fraction of coarse mitochondria (CM) was obtained at 10,000 g (10 min) and the fraction of microsomes at 105,000 g (60 min). The fractions were suspended in 0.32 M sucrose and protein was determined by Lowry's method. The yield of CM and microsomes was 15.7 and 6 mg protein/g fresh tissue, respectively. Tissue from eight to 10 rats was used in each experiment.

To study the action of TT on ATPase, the CM of the cerebral cortex and mitochondria of the heart also were isolated. These fractions were used after a single freezing and thawing. The CM fraction was treated immediately before the experiment with 0.1% Na deoxycholate (DC) for 30 min at 20°C (1.3 mg protein/ml). Activity of the ATPases was determined [1] from the degree of accumulation of inorganic phos-

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TABLE 1. Action of Purified Tetanus Toxin on ATPases in Vitro

Fraction	Type of ATPase	ATPase activity (in μmoles P_{in}/mg protein/h)	TT concentration (in μg protein/ml)	Effect of TT
CM from rat cerebral cortex	Mg-ATPase	14,7±0,5	0,05—2	No effect
	Na,K-ATPase	5,5±0,3	0,05—2	» »
CM+ 0,1% DC	Mg-ATPase	8,3±0,45	0,05—4	» »
	Na,K-ATPase	6,6±0,25	0,05—4	Increase of 10—15% ($P>0,05$)
Mitochondria of heart	Mg-ATPase	99,5±1,8	0,05—25	No effect
	Ca-ATPase	60,7±1,3	0,05—25	» »

Note: Mean values from five or six experiments are shown. When cardiac mitochondria were used incubation lasted 10 min at 37°C, otherwise 20 min at 37°C. The following concentrations of TT were used: 0.05, 0.1, 0.5, 1, 2, 4, 10, and 25 μg protein/ml.

TABLE 2. ATPase Activity in Subcellular Fractions of Anterior Horn Tissue of Rat Spinal Cord under Normal Conditions and in Tetanus (M ± m)

Fraction	Experimental conditions	Activity of ATPases (in μmoles P_{in}/mg protein/h)					
		Mg-ATPase	%	Na,K-ATPase	%	Ca-ATPase	%
Homogenate	Normal	6,0±0,48 (8)	—	3,84±0,51 (8)	—	3,17±0,28 (8)	—
	Local tetanus	6,43±0,46 (8)	+7	3,0±0,29 (8)	−21	2,81±0,12 (8)	−12
	General tetanus:						
	Left	8,49±0,66 (4)	+41*	4,4±0,76 (4)	+15	2,9±0,24 (4)	−9
	Right	7,33±0,66 (4)	+22	2,65±0,76 (4)	−31	2,19±0,37 (4)	−31*
CM	Normal	7,50±0,31 (6)	—	3,75±0,35 (6)	—	3,76±0,35 (7)	—
	Local tetanus	7,53±0,52 (8)	0	4,24±0,25 (6)	+13	3,40±0,36 (8)	−10
	General tetanus:						
	Left	9,14±0,47 (4)	+22*	5,33±0,46 (4)	+42*	3,28±0,71 (4)	−13
	Right	7,47±0,27 (4)	0	3,25±0,10 (4)	−13	2,87±0,30 (4)	−24
Microsomes	Normal	8,43±0,97 (8)	—	4,46±0,28 (7)	—	4,20±0,44 (8)	—
	Local tetanus	8,70±1,07 (6)	+3	6,51±0,19 (6)	+45,5*	4,19±0,33 (5)	0
	General tetanus:						
	Left	8,68±0,81 (4)	+3	11,21±1,56 (4)	+151*	4,09±0,30 (4)	−3
	Right	7,65±0,64 (4)	−9	7,34±1,18 (4)	+65*	3,97±0,44 (4)	−5

Note: The left side was the "tetanus" side of the spinal cord, the right side the opposite. Number of experiments given in parentheses. + or −) increase or decrease in activity (in %) respectively compared with normal. Asterisks mark statistically significant changes, $P < 0.05$.

phate in the course of the reaction. The composition of the incubation medium (1 ml) was as follows, in moles: ATP-Na_2 3, $MgCl_2$ ($CaCl_2$) 5, NaCl 100, KCl 20, Tris-HCl, pH 7.4, 50, sucrose 32; protein 100-500 g. After incubation (10 min, 37°C) cold 10% TCA (1:1) was added, and the inorganic phosphorus was determined photocolorimetrically at 700 nm [8]. Activity of transfer Na,K-ATPase was determined from the difference between total and Mg-ATPase activity. Activation of ATPase in the presence of Mg transplant or Ca transplant ions was attributed to Mg (Ca)-ATPase.

The TT was purified by gel-filtration on Sephadex G-100 an elution with 0.05 M Tris-HCl, pH 7.4. The activity of the purfied TT was 3×10^5 MLD/mg protein (the doses here and subsequently are for rats). Local and general tetanus was produced by injecting TT (0.2 and 1.0 MLD, respectively) at several points (0.1 ml at each injection) into the muscles of the leg and thigh (thus ensuring a uniform distribution of TT arriving at the spinal cord along regional nerve pathways [3]. Tissues of the anterior horns of the spinal cord were investigated at the stage of local tetanus after 48 h and at the stage of generalized ascending tetanus after 72 h. For comparison, tissues also were investigated on the side opposite to injection of the toxin (control for pathological side effects).

EXPERIMENTAL RESULTS AND DISCUSSION

The possibility of a direct action of TT on the various ATPases was first studied. It will be clear from Table 1 that purified TT (15-1200 MLD; 0.05-25 μg protein/ml) had virtually no effect on the activity of the various ATPases over a wide range of concentrations in the experiments in vitro.

The results of the study of the state of the ATPases under normal conditions and in poisoning by TT are given in Table 2. Clearly during poisoning no significant changes in Ca-ATPase activity were found in the fractions of spinal cord tissue studied. Activity of Mg-ATPase increased considerably only in generalized tetanus, in the homogenate (by 41% over normal) and in the CM fraction (by 22%), but it was unchanged in the microsomes in the various stages of TT poisoning. It is interesting to note that Mg-ATPase activity in the subfractions of the uninfected spinal cord tissue was unchanged in general tetanus. The protein content in the fractions was virtually indistinguishable from normal at the various stages of TT poisoning. It will also be clear from Table 2 that Na,K-ATPase activity of the spinal cord tissue was increased in the microsomes (by 45.5%; $P < 0.001$) and CM (by 13%; $p = 0.15$-0.2) in local tetanus. In general tetanus the increase in Na,K-ATPase activity progressed considerably in the microsomes (by 151% over normal) and in CM (by 42%). However, in local and general tetanus no statistically significant changes were found in the Na,K-ATPase activity of spinal cord tissue homogenate. It is not yet clear whether this unexpected fact means that an inhibitory component is present from the beginning in the homogenate of the anterior horns of the spinal cord or whether that component appears only in TT poisoning, and is separated from the membrane structures into the cytoplasmic fraction during subsequent fractionation of the tissue. During the investigation of subfractions of spinal cord tissue on the side opposite to the injection of TT it was found in general tetanus (Table 2) that the Na,K-ATPase activity in the CM fraction (as in local tetanus) was not significantly changed, but in the microsomes it was increased (by 65% over normal). These results suggest that the increase in activity of transfer ATPase of synaptic origin in the CM fraction is the result of the action of tetanus toxin and that the observed increase in activity of the microsomal ATPase in general tetanus may be the result of summation of specific and nonspecific processes in tetanus poisoning.

The increase in Mg-ATPase activity of the CM fraction of the spinal cord, responsible for bioenergetic processes, observed in generalized tetanus is in harmony both with observations showing a decrease in the content of high-energy compounds (ATP) in nerve tissue, particularly in the late stage of tetanus poisoning [3], and also with results showing an increase in the activity of Mg-ATPases in brain [5, 10] and skeletal muscle tissues [4, 6, 7] in generalized tetanus. No data on changes in Na,K-ATPase activity in the tissues in tetanus poisoning are to be found in the literature. All that is known is that in the hypoxia which accompanies the late stages of tetanus [12] and in chronic convulsions [1, 2, 11] changes may take place in membrane permeability and in transfer ATPase activity. It is reported in the literature that TT in vitro does not affect the Mg-ATPase activity of skeletal muscles or liver mitochondria but increases the activity of dinitrophenol-activated liver ATPase [5]. However, the question of a direct pathogenic or indirect action of TT on the activity of membrane Na,K-ATPase is still undecided.

It is possible that in vivo TT acts on the permeability of the synaptic membranes and causes conformational changes in the protein–lipid complexes, thereby causing secondary specific changes in the active transport of ions through the membrane, in Na,K-ATPase activity and, ultimately, blocking synaptic processes, especially in inhibitory synapses which are particularly sensitive to TT.

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