OXIDATIVE PHOSPHORYLATION IN SPINAL CORD MITOCHONDRIA OF RATS WITH EXPERIMENTAL TETANUS

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Spinal cord mitochondria of rats with local experimental tetanus utilize oxygen and inorganic phosphate more intensively than spinal cord mitochondria of healthy animals. The values of the P/O ratio are the same in normal animals and animals with tetanus. Purified preparations of tetanus toxin in doses of $5 \cdot 10^2$–$1 \cdot 10^5$ MLD (for mice) did not affect respiration or phosphorylation of intact mitochondria.

KEY WORDS: tetanus toxin; mitochondria; spinal cord; oxidative phosphorylation.

Experiments have shown [6] that tetanus toxin (TT) uncouples respiration and phosphorylation in mitochondria and that this effect exhibits species (tetanus-susceptible species) and tissue (mitochondria of the cerebral cortex) specificity. However, the clinical picture of tetanus is caused by disturbance of the activity not of the brain, but of the medulla and spinal cord, in which the energy processes have not been investigated. Moreover, the uncoupling effect could be due not to tetanospasmin itself, but to impurities [7], for the authors concerned [6] used an unpurified preparation of TT. It will be clear from what has been said above that further research is needed in order to explain the state of intracellular energy metabolism in tetanus.

The object of this investigation was to study oxidative phosphorylation in the spinal cord mitochondria of animals with local tetanus and to analyze the effects of purified TT on intact mitochondria.

EXPERIMENTAL METHOD

A preparation of TT from batch No. 21 of Leningrad Institute of Vaccines and Sera, purified on Sephadex G-100, followed by elution of the toxin with 0.1 M phosphate buffer [1], was used in the experiments. The eluate was concentrated by freeze-drying or with the aid of Ficoll. Tetanospasmin T_6, obtained by Bizzini and co-workers [3],* was used in some of the experiments. Both preparations were transferred in 0.01 M tris-buffer to a column filled with Sephadex G-50. The toxicity of the preparations was determined by titration in mice.

Experiments were carried out on mitochondria isolated from the spinal cord of rats weighing 250-350 g. Local tetanus of both hind limbs was produced by injecting TT into the thigh and leg muscles in a dose of 0.1 MLD on each side. The animals were decapitated 3 days later, the lumbar enlargement of the spinal cord was removed, and the specimen was washed with cold 0.25 M sucrose. The spinal cord tissue from 7-10 animals was homogenized

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TABLE 1. Oxidative Phosphorylation in Spinal Cord Mitochondria of Rats after Treatment with TT *in vivo* and *in vitro* (M ± m)

Toxin			Substances added	No. of experiments	Decrease in inorganic P in μatoms/mg protein/10 min		O_2 absorption, in μatoms/mg protein/10 min		P/O	
in sample		method of concentration			toxin	normal	toxin	normal	toxin	normal
MLD × 10^3	mg protein									
Animals with tetanus			—	7 (15)†	1,20± 0,10	0,93± 0,06	0,76± 0,05	0,60± 0,03	1,58± 0,04	1,55± 0,05
0,5	0,075	—	Ca^{2+}	3	0,94± 0,14	0,95± 0,18	0,56± 0,05	0,56± 0,07	1,68± 0,11	1,70± 0,12
0,5	0,075	—	EDTA	5	0,99± 0,17	1,02± 0,17	0,56± 0,11	0,59± 0,10	1,77± 0,08	1,73± 0,08
2	0,130	—	EDTA	1‡	1,93± 0,02	1,91± 0,03	0,95± 0,03	0,89± 0,03	2,03± 0,07	2,15± 0,06
6	0,390	—	EDTA	2	1,84± 0,08	1,70± 0,03	0,81± 0,00	0,84± 0,04	2,27± 0,24	2,02± 0,04
10	0,650	Freeze-drying	—	2	0,71± 0,13	1,07± 0,14*	0,52± 0,08	0,68± 0,11	1,37± 0,04	1,57± 0,00
15	0,975	»	—	3	0,40± 0,08	1,00± 0,21	0,42± 0,05	0,68± 0,09	0,95± 0,10	1,47± 0,12
15*	0,040	—	—	1‡	0,84± 0,04	0,72± 0,02	0,60± 0,06	0,60± 0,05	1,40± 0,07	1,20± 0,14
40	0,600	Ficoll	—	3	0,83± 0,09	0,77± 0,08	0,59± 0,09	0,49± 0,02	1,41± 0,21	1,57± 0,16
40*	0,109	—	—	2	0,79± 0,02	0,76± 0,04	0,59± 0,07	0,59± 0,14	1,34± 0,04	1,29± 0,08
100*	0,272	—	—	3	0,94± 0,09	0,93± 0,18	0,66± 0,06	0,65± 0,06	1,42± 0,06	1,43± 0,17

*Bizzini's preparation.
†Number of experiments on healthy animals shown in parentheses.
‡Calculated from results obtained in parallel tests.

in 10 volumes of medium A of the following composition (in mM): mannitol 250, tris-HCl (pH 7.4) 10, KCl 10, EDTA 0.2. The suspension was centrifuged at 700 g (0-2°C) for 5 min. The supernatant was then centrifuged at 10,000 g for 10 min. The residue was resuspended in the isolation medium and used in the experiments as an unpurified fraction of mitochondria (3-6 mg mitochondrial protein per sample).

The intensity of respiration was measured manometrically in a Warburg's apparatus. The flask contained 1 ml of incubation medium of the following composition (in mM): medium A, K_2HPO_4 25, glucose 22.5, ATP—Na_2 2, $MgCl_2$ 4, glutamate 10, and hexokinase 1.5 mg per sample. After preincubation for 5 min to equalize the temperature, the O_2 absorption was measured in the course of 10 min. The contents of the samples were then precipitated with 10% TCA (1:1). The control samples were of the same composition but TCA was added to them before the mitochondria. From 2 to 4 parallel samples were used in each experiment. Protein was determined by Lowry's method [5], inorganic phosphorus after Lowry and Lopez [4] in modification [2].

EXPERIMENTAL RESULTS AND DISCUSSION

The investigations showed that the spinal cord mitochondria of the animals with tetanus utilized oxygen (by 27%; $P < 0.02$) and inorganic phosphate (by 29%; $P < 0.05$) more intensively than the spinal cord mitochondria of healthy rats. The difference observed evidently reflects the increased functional load on the neurons in tetanus. No significant difference could be found in the P/O ratio.

Experiments with the addition of TT to the incubation medium were started with a dose of 500 MLD (for mice). Comparison of the mean values obtained in 5 experiments shows that the absorption of oxygen, the decrease in phosphorus, and the value of the P/O ratio were the same in the control and experimental series (Table 1). It is known [2] that in certain cases an uncoupling effect can be found after the addition of Ca^{++} ions to the medium. In experiments with a dose of 500 MLD, and also after the addition of TT in doses of 2000 and 6000 MLD, EDTA was added to the medium to obtain higher values of the P/O ratio. As the experiments showed, even in the case when EDTA was not added and the medium contained 0.1 mM Ca^{++}, no uncoupling effect likewise was observed.

When larger doses of TT were used (15,000 MLD) respiration was inhibited by 38% and phosphorylation by 60% (P/O was reduced by 75%; $P < 0.05$), and as control experiments with the addition of albumin showed, the result could not be attributed to an increased protein load. It was postulated that this effect was due to impurities

that were concentrated by freeze-drying. To remove them, freeze-drying was replaced by dialysis against Ficoll. The uncoupling effect, and also the inhibition and phosphorylation were completely abolished by this operation.

In the next series of experiments the effect of highly purified tetanospasmin [3] was investigated. Even in very large doses (15,000, 40,000, and 100,000 MLD) this preparation did not affect respiration or phosphorylation in the spinal cord mitochondria.

It can be concluded that in experimental tetanus oxidative phosphorylation in the neurons is not significantly affected and that, consequently, the effect of TT on the energy metabolism of the cell is of no pathogenetic importance.

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