PYREXIAL REACTION AND OXIDATIVE PHOSPHORYLATION IN THE LIVER FOLLOWING INTRAVENOUS AND INTRACEREBRAL INJECTION OF DIPHTHERIA TOXIN

G. I. Medvedeva and L. I. Niselovskaya

UDC 616.931-092.9.032 :]611.14 + 611 + 81]-07 : 616.36-07

Intravenous injection of diphtheria toxin into rabbits caused mild hyperthermia, a disturbance of the general condition, and a severe disturbance of coupled oxidative phosphorylation in the liver mitochondria. After injection of diphtheria toxin into the lateral ventricle the general condition of the rabbits showed little change, but the rectal temperature was considerably raised and the energy metabolism sharply disturbed in the liver mitochondria. This confirms the view that the action of diphtheria toxin on cell metabolism is mediated through the central neurohumoral regulatory mechanisms.

Diphtheria toxin which, in vivo, lowers the P : O ratio in the liver mitochondria has been shown not to affect mitochondrial energy metabolism in vitro [4]. A decrease in the density of phosphorylation in the liver mitochondria also takes place if diphtheria toxin is injected directly into the lateral ventricle [1]. It has accordingly been postulated that the action of diphtheria toxin on energy metabolism in the cells is mediated through central nervous mechanisms and is linked with its neurotropic properties.

The object of the present investigation was to compare the characteristics of the response to diphtheria toxin when injected intravenously and intracerebrally.

EXPERIMENTAL METHOD

Male chinchilla rabbits weighing 2.5-3 kg were used. Heat production was calculated from the oxygen consumption [2].

Diphtheria toxin of batch No. 6 (prepared at the Leningrad Institute of Vaccines and Sera) was injected intravenously in a dose of 1.5 MLD in a volume of 1.5 ml) and also into the right ventricle (0.2 MLD, 0.2 ml). The intraventricular injection was given by the method of Repin and Sorokin [6]. All solutions were made up under sterile conditions in bidistilled pyrogen-free water. The animals were decapitated on the second day after the injection of diphtheria toxin, the liver was quickly removed and washed in cold 0.25 M sucrose solution, and the mitochondria were isolated [4]. The mitochondria were incubated in a reaction mixture, pH 7.4 [5], for 20 min at 30°C, with air as the gaseous medium. The rate of oxidation was measured in a Warburg apparatus and the rate of phosphorylation was calculated from the decrease in the concentration of inorganic phosphorus [8]. The quantity of oxygen absorbed and of phosphorus esterified was calculated in microatoms (μA)/mg mitochondrial protein, which was determined by the biuret method. The rectal temperature was measured by an electric thermometer at a depth of 5 cm during the 5 h after injection of the toxin at 30-min intervals, again after 24 h, and before decapitation.

Department of General Pathology and Department of Biochemistry, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR P. N. Veselkin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 76, No. 8, pp. 42-44, August, 1973. Original article submitted November 27, 1972.

TABLE 1. Respiration and Phosphorylation of Liver Mitochondria (in μA/mg mitochondrial protein) and Oxygen Consumption (in ml/kg/min) in Rabbits after Injection of Diphtheria Toxin by Different Methods

Experimental conditions	No. of expts.		Statistical index	Succinic acid			Glutamic acid			Oxygen consumption by the animal		
	succinic acid	glutamic acid		Δ P	Δ O	P/O	Δ P	Δ O	P/O	initial (100%)	on day of expt.	difference
Control	23	16	$M \pm m$	0,49±0,05	0,36±0,04	1,51±0,04	0,39±0,03	0,16±0,02	2,55±0,10	11,1±0,32	10,8±0,71	−2%
			P								>0,05	
Intravenous injection of diphtheria toxin	25	19	$M \pm m$	0,26±0,04	0,42±0,03	0,61±0,04	0,19±0,04	0,17±0,01	1,12±0,16	11,3±0,38	9,6±0,75	−15%
			P	<0,001	>0,05	<0,0001	<0,0001	>0,1	<0,001		<0,05	
Intraventricular injection of diphtheria toxin	7	5	$M \pm m$	0,15±0,07	0,32±0,07	0,33±0,18	0,11±0,05	0,12±0,01	0,80±0,4	10,1±0,75	8,3±0,87	−18%
			P	<0,001	>0,2	<0,001	<0,001	>0,1	<0,001		<0,05	

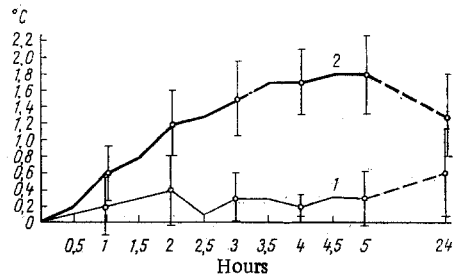

Fig. 1. Change in rectal temperature after intravenous (1) and intraventricular injection (2) of diphtheria toxin in 13 rabbits (mean data). Vertical lines show confidence limits. Ordinate, rise of temperature (in deg.); abscissa, time (in h).

EXPERIMENTAL RESULTS AND DISCUSSION

Intravenous injection of diphtheria toxin (series I) in a dose of 1.5 MLD produced mild hyperthermia in the rabbits. (The mean rise of temperature exceeded 0.4°C.) The animals were lethargic, they would not eat, they lost 230 g in weight on the average in 24 h, and their oxygen consumption fell by 15%. The energy metabolism of the mitochondria from the liver in all rabbits was severely disturbed after intravenous injection of the toxin (Table 1). The decrease in the P : O ratio when succinic or glutamic acid was used as the substrate was associated with a sharp decrease in phosphorylation while respiration was almost unchanged.

After injection of diphtheria toxin into the lateral ventricle (series II) the rabbits did not lose weight, they were alert, and they ate all their food. Their oxygen consumption was reduced slightly more than in series I (by 18%) but their rectal temperature was considerably raised. For example, 2 h after injection of the toxin the rectal temperature was 1.2°C higher than initially (Fig. 1), after 3.5-5 h it was 1.7-1.8° higher, and after 24 h it still remained 1.3° higher. In all the experiments there was a severe disturbance of the efficiency of oxidative phosphorylation (a decrease in P : O on account of inhibition of phosphorylation).

The mild pyrexial reaction to intravenous injection of diphtheria toxin in these experiments is in harmony with data in the literature [1, 3]. The sharp and prolonged pyrexial reaction to intracerebral injection of the toxin is analogous to that found after intraventricular injection of microdoses of bacterial lipopolysaccharides and of leukocytic pyrogens [7].

The mechanism of the central pyrogenic action of diphtheria exotoxin requires further study. However, the results of the present experiments confirm the view that the action of diphtheria toxin on cell metabolism is mediated through central neurohumoral regulatory mechanisms.

LITERATURE CITED

1. P. N. Veselkin, E. S. Gramenitskaya, and L. I. Niselovskaya, Byull. Éksperim. Biol. i Med., No. 11, 56 (1967).
2. P. N. Veselkin, Fiziol. Zh. SSSR, No. 10, 188 (1955).
3. N. A. Volokhova, The Effect of the Febrile Reaction of Bromine, Phenalin, and Alcohol on the Course of Experimental Diphtheria Toxemia, Candidate's Dissertation, Leningrad (1953).
4. L. I. Niselovskaya and E. M. Paderina, Vopr. Med. Khimii, No. 3, 256 (1963).
5. G. I. Medvedeva and L. I. Niselovskaya, Byull. Éksperim. Biol. i Med., No. 7, 34 (1972).
6. I. S. Repin and A. V. Sorokin, Pat. Fiziol., No. 4, 47 (1965).
7. I. S. Repin and N. A. Kalinina, Byull. Éksperim. Biol. i Med., No. 4, 64 (1967).
8. G. H. Fiske and V. Subbarow, J. Biol. Chem., 66, 375 (1925).