EFFECT OF BACTERIAL ENDOTOXINS ON OXIDATIVE PHOSPHORYLATION IN THE LIVER

L. I. Niselovskaya and L. P. Kopytovskaya

UDC 612.35:[612.262:612.398.145.1].014.46:576.8.097.29

Endotoxins of the Enterobacteriaceae and of Haemophilus pertussis have no effect on the level of oxidative phosphorylation in the mitochondria of the rat liver. Injection of these toxins into animals with increased sensitivity to them (adrenalectomy) likewise did not lower the level of oxidative phosphorylation in the liver.

* * *

Poisoning of animals with diphtheria and staphylococcal exotoxins causes dissociation of respiration and phosphorylation in mitochondria isolated from the liver of these animals [1, 2], but this is not observed during poisoning with dysentery endotoxin [3].

The object of the present investigation was to determine: 1) whether the absence of effect on oxidative phosphorylation in the liver is also characteristic of other endotoxins, 2) whether the level of oxidative phosphorylation in the liver is modified during poisoning by endotoxins under conditions of increased sensitivity of the animals to these toxins, and 3) whether the "classical" endotoxins differ in any way from the endotoxin of Haemophilus pertussis in their action on oxidative phosphorylation in the liver.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 80-100 g. Poisoning was caused by injection of bacterial toxins in near-lethal or smaller doses. The products tested were prepared by Boivin's method from Escherichia coli (in a dose of 1 mg or less), Salmonella paratyphi B (0.06 mg or less), Shigella flexneri (1 mg or less), and H. pertussis (0.025 mg; prepared by Teyssier's method). An increase in sensitivity to the endotoxins was brought about by adrenalectomy, reducing the minimal lethal doses for the rats: for endotoxines of the Enterobacteriaceae by 170-200 times and for H. pertussis by 20 times. The products were injected 48 h after the operation. At various times after injection of the toxins, the animals with obvious signs of poisoning were decapitated. Isolation of the mitochondria and the conditions of their incubation were as described previously [3]. Glutamate was used as substrate. The absorption of oxygen and decrease in inorganic phosphate were measured and the oxidative phosphorylation ratio P/O calculated. Protein of the mitochondria was determined by the biuret method [5].

TABLE 1. Respiration, Phosphorylation, and P/O Ratio in Liver Mitochondria of Control Rats and Rats Poisoned with S. paratyphi and E. coli Toxins

State of animal	Time after injection of toxin	No. of expts.	ΔP	ΔO	P/O
Control	—	18	0,43±0,05	0.22±0,02	1.94±0.13
Paratyphoid poisoning	1 day	12	0,48±0.10	0,23±0,03	2.03±0.27 $P>0.05$
	2—4 h	6	0.94±0.22	0,38±0,07	2.47±0,28 $P=0,1$
Control	—	16	0,73±0,11	0,37±0.04	1.94±0.20
E. coli poisoning	3 h	12	0,88±0,18	0,39±0.05	2.09±0.24 $P>0,05$
	1½ h	5	1.06±0.15	0,51±0,07	2.10±0.27 $P>0.05$

Note. In all tables ΔP and ΔO are given in μatoms/ mg protein.

Department of Biochemistry and Department of Microbiology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad (Presented by Academician of the AMN SSSR V. S. Il'in). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 4, pp. 51-53, April, 1969. Original article submitted April 15, 1968.

TABLE 2. Respiration, Phosphorylation, and P/O Ratio in Liver Mitochondria of Adrenalectomized Rats and of Adrenalectomized Rats Poisoned with Paratyphoid and Dysentery Endotoxins

State of animal	No. of expts.	ΔP	ΔO	P/O	
Adrenalectomy	8	0.62 ± 0.07	0.24 ± 0.01	2.54 ± 0.25	
Adrenalectomy + paratyphoid toxin	8	0.51 ± 0.06	0.17 ± 0.01	2.96 ± 0.21	P< 0.2
Adrenalectomy	11	0.41 ± 0.05	0.29 ± 0.03	1.51 ± 0.24	
Adrenalectomy + dysentery toxin	11	0.52 ± 0.05	0.25 ± 0.01	2.04 ± 0.19	P = 0.1

TABLE 3. Respiration, Phosphorylation, and P/O Ratio in Liver Mitochondria of Intact and Adrenalectomized Control Rats and Rats Poisoned with H. pertussis Endotoxin

Group of animals	No. of expts.	ΔP	ΔO	P/O
Control	12	0.67 ± 0.04	0.30 ± 0.02	2.25 ± 0.08
Rats receiving sublethal dose of H. pertussis endotoxin.	12	0.84 ± 0.04	0.34 ± 0.03	2.62 ± 0.17 P > 0.1
Adrenalectomized rats.	6	0.59 ± 0.09	0.22 ± 0.02	2.68 ± 0.32 P = 0.3
Adrenalectomized rats receiving sublethal dose of H. pertussis toxin. . .	7	0.65 ± 0.07	0.18 ± 0.02	3.66 ± 0.36 P < 0.01

the operation. At various times after injection of the toxins, the animals with obvious signs of poisoning were decapitated. Isolation of the mitochondria and the conditions of their incubation were as described previously [3]. Glutamate was used as substrate. The absorption of oxygen and decrease in inorganic phosphate were measured and the oxidative phosphorylation ratio (P/O) calculated. Protein of the mitochondria was determined by the biuret method [5].

EXPERIMENTAL RESULTS

Injection of endotoxins of S. paratyphi and E. coli did not dissociate phosphorylation from respiration in mitochondria isolated from the liver of the poisoned animals (Table 1). Both phosphorylation and respiration in the mitochondria were higher than the control level; the value of P/O was not lowered.

Adrenalectomy by itself did not lower the level of oxidative phosphorylation in the liver mitochondria. Injection of paratyphoid and dysentery endotoxins into the adrenalectomized rats 24 and 48 h after the operation likewise did not reduce the P/O ratio (Table 2). The intensity of oxidative phosphorylation was not reduced in mitochondria isolated from the liver of the animals after injection of H. pertussis endotoxin, or in experiments on adrenalectomized rats receiving H. pertussis toxin (Table 3).

Poisoning of the animals with exotoxins and endotoxins had different effects on the energy metabolism of their liver. Diphtheria and staphylococcal exotoxins caused dissociation of oxidative phosphorylation in the liver [1, 2]. Similar results were obtained when the effect of O-streptolysin was studied [4]. In contrast to this, endotoxins (of S. paratyphi and E. coli) had no effect on oxidative phosphorylation in the liver. Poisoning with dysentery endotoxin likewise had no dissociating action on oxidative phosphorylation [3].

The effect of H. pertussis endotoxin, which in its physical, chemical, and immunologic properties occupies an intermediate position between exotoxins and endotoxins, on energy metabolism in the liver was found to be similar to the action of endotoxins of the Enterobacteriaceae. It is a noteworthy fact that adrenalectomy did not reduce the intensity of oxidative phosphorylation in the liver of poisoned animals.

The mechanisms of the disturbance of metabolism in cases of poisoning by bacterial endotoxins and exotoxins thus differ in relation to their action on oxidative phosphorylation in the liver: exotoxins cause a sharp decrease in the level of oxidative phosphorylation, while endotoxins do not reduce it even if the sensitivity of the animals to the action of these bacterial toxins is increased.

LITERATURE CITED

1. L. I. Niselovskaya, in: Phosphorylation and Function [in Russian], Leningrad (1960), p. 363.
2. L. I. Niselovskaya and E. M. Paderina, Vopr. Med. Khimii, No. 3, 256 (1963).
3. L. I. Niselovskaya, in: Annual Report of the Institute of Experimental Medicine, Academy of Medical Sciences of the USSR (for 1961/62) [in Russian], Leningrad (1963), p. 320.
4. V. I. Sisenko and L. S. Kositskaya, in: Acute and Chronic Streptococcal Infection [in Russian], Leningrad (1967), p. 84.
5. P. Baudet and C. Giddey, Helv. Chim. Acta, 31, 1879 (1948).