ROLE OF THYROID HORMONES IN THE MECHANISM OF ACTION OF DIPHTHERIA TOXIN ON OXIDATIVE PHOSPHORYLATION IN RABBIT LIVER MITOCHONDRIA

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In rabbits with experimental hypothyroidism the ability of the liver mitochondria to carry out oxidative phosphorylation was undisturbed after intravenous injection of diphtheria toxin. In rabbits with experimental hyperthyroidism, the sensitivity of the liver mitochondria to the uncoupling action of diphtheria toxin was sharply increased. It is postulated that the endocrine function of the thyroid gland plays an essential role in the uncoupling action of diphtheria toxin on oxidative phosphorylation.

KEY WORDS: thyrotoxicosis; hypothyroidism; diphtheria toxin; oxidative phosphorylation.

One of the writers [2] showed previously that if a certain dose of 2,4-α-dinitrophenol (DNP) is added in vitro to the incubation medium of mitochondria from the liver of animals with hypo- and hyperthyroidism, its action on oxidative phosphorylation depends on the level of thyroid activity in the animal. On the basis of these results, and in accordance with the views of Hoch [5], the essential role of the endocrine function of the thyroid gland was postulated in the mechanism of the uncoupling action of DNP. Accordingly a possible role of thyroid hormone can be assumed also in the mechanism of the uncoupling action of certain bacterial toxins, notably diphtheria toxin [1].

In previous investigations [3, 4] the effect of diphtheria toxin (administered by different methods) on the energy metabolism in the liver was studied in animals after extirpation of the thyroid gland or after depression of its function with mercazolyl (1-methyl-2-mercaptoimidazole).

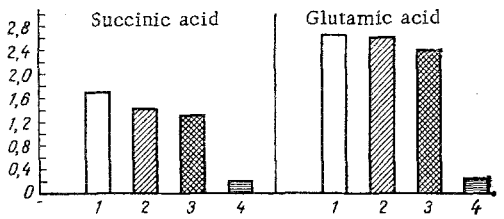

Fig. 1. Stability of level of oxidative phosphorylation in rabbit liver mitochondria: 1) control; 2) diphtheria toxin, 0.9 MLD i.v.; 3) thyrotoxicosis; 4) thyrotoxicosis +diphtheria toxin 0.9 MLD.

As a continuation of the investigations published previously, in the present study the reactivity of rabbits with hyperthyroidism to diphtheria toxin and the effect of hyperthyroidism on the sensitivity of oxidative phosphorylation in the liver mitochondria to the uncoupling action of diphtheria toxin were examined.

EXPERIMENTAL METHOD

Experiments were carried out on 74 male chinchilla rabbits weighing 2.5-3 kg. Thyrotoxicosis was induced by feeding

*Deceased

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TABLE 1. Effect of Diphtheria Toxin on P/O Ratio in Liver Mitochondria from Normal Rabbits and Rabbits with Hypo- and Hyperthyroidism (M ± m)

Group No.	Experimental conditions and number of animals in group (n)	P/O		Body weight (in kg)		Rectal temperature (in °C)	
		succinic acid	glutamic acid	initial	on day of experiment	initial	on day of experiment
1	Control (healthy rabbits) (n = 13)	1,44±0,06	2,46±0,14	2,50±0,53	2,60±0,55	39,0±0,253	38,8±0,133 $P_x>0,05$
2	Healthy rabbits + diphtheria toxin (1.5 MLD) (n = 16)	0,79±0,09 $P<0,01$	1,24±0,16 $P<0,01$	2,63±0,165	2,30±0,197 $P>0,05$	38,8±0,07	38,7±0,4 $P>0,05$
3	Hypothyroidism + diptheria toxin (1.5 MLD) (n = 11)	1,31±0,19 $P>0,05$	2,25±0,31 $P>0,05$	2,40±0,67	2,25±0,08 $P>0,05$	38,9±0,526	38,1±0,144 $P<0,01$
4	Control* (healthy rabbits) (n = 9)	1,67±0,099	2,70±0,127	2,52±0,51	2,63±0,53 $P>0,05$	39,1±0,25	39,0±0,13 $P_x>0,05$
5	Healthy rabbits + diphtheria toxin (0.9 MLD) (n = 9)	1,42±0,091 $P_x>0,05$	2,66±0,189 $P>0,05$	2,79±0,09	2,71±0,09 $P>0,05$	38,8±0,04	38,7±0,07 $P>0,05$
6	Thyrotoxicosis (n = 6)	1,31±0,509 $P_x>0,05$	2,38±0,508 $P>0,05$	2,92±0,08	2,54±0,10 $P<0,05$	38,9±0,05	39,0±0,02 $P>0,05$
7	Thyrotoxicosis + diphtheria toxin (0.9 MLD) (n = 10)	0,21±0,131 $P_x<0,001$	0,25±0,173 $P<0,001$	2,98±0,06	2,65±0,04 $P_x<0,05$	39,0±0,05	38,9±0,06 $P_x>0,05$

Legend. *) Control to experiments with rabbits with thyrotoxicosis (groups 5, 6, and 7). P_x) Comparison within the group; P) comparison of each group with control.

the animals with thyroid extract in tablet form (0.1 g) in a dose of 0.34-0.46 mg iodine/kg body weight daily for 12 days. Hypothyroidism was produced by feeding mercazolyl (5 mg/kg) daily for 16-18 days. The animals were killed on the first to third day after intravenous injection of diphtheria toxin (1.5 MLD in a volume of 1.5 ml, 1.2 MLD in a volume of 1.2 ml, and 0.9 MLD in a volume of 0.9 ml) (Fig. 1). To measure oxidation and phosphorylation, mitochondria isolated from the liver [3] were incubated in a reaction mixture, pH 7.4, for 20 min at 30°C, in an atmosphere of air. The rate of oxidation was measured in a Warburg apparatus. The rate of phosphorylation was estimated from the increase in the content of inorganic phosphate [3]. The composition of the reaction mixture was: $MgCl_2$ 0.004 M, ATP 0.002 M, phosphate buffer 0.008 M, glucose 0.045 M, succinic acid 0.008 M, glutamic acid 0.016 M, KF 0.02 M, hexokinase 0.3 mg, and 0.6 ml of a suspension of mitochondria in 0.25 M sucrose. The quantity of assimilated oxygen and of esterified phosphate was calculated per milligram mitochondrial protein [3].

EXPERIMENTAL RESULTS

A single intravenous injection of 1.5 MLD diphtheria toxin caused the body temperature of normal rabbits to rise on the average by 0.4°C. The animals became lethargic, refused their food, and their mean oxygen consumption fell by 15%. Diphtheria toxin in this dose sharply reduced phosphorylation in the liver mitochondria of normal animals. The P/O ratio was reduced almost by half (Table 1).

After injection of diphtheria toxin into rabbits with hypotheroidism only a very slight and not significant tendency was observed for the P/O ratio to fall in the liver mitochondria, and it remained virtually within the limits of normal variations (Table 1). The general condition of these rabbits showed little change and their oxygen consumption was reduced on average by 15%.

A different picture was seen in the rabbits with hyperthyroidism. To begin with, injection of the dose of diphtheria toxin used in the first two groups of experiments (1.5 MLD) and its reduction to 1.2 MLD resulted in 100% mortality of the animals within 24 h (Table 2).

TABLE 2. Action of Diphtheria Toxin, Injected Intravenously in Different Doses into Normal Rabbits and Rabbits with Thyrotoxicosis

Group No.	Experimental conditions	Number of Rabbits	Number Dying	Number Surviving
1	Diphtheria toxin, 1.5 MLD (normal rabbits)	16	0	16
2	Thyrotoxicosis +diphtheria toxin, 1.5 MLD	6	6	0
3	Diphtheria toxin, 1.2 MLD (normal rabbits)	9	0	9
4	Thyrotoxicosis +diphtheria toxin, 1.2 MLD	5	5	0
5	Diphtheria toxin, 0.9 MLD (normal rabbits)	9	0	9
6	Thyrotoxicosis +diphtheria toxin, 0.9 MLD	10	0	10

In the fifth and seventh groups of experiments (Table 1) diphtheria toxin was accordingly injected in a dose of 0.9 MLD, and the rabbits were killed 24 h after injection. Under the influence of this dose of toxin the decrease in mitochondrial phosphorylation in the normal rabbits was very slight and not significant, as also was the decrease in the P/O ratio. In the rabbits with hyperthyroidism, injection of diphtheria toxin in this dose, which had hardly any effect on the healthy rabbits, caused a sharp decrease in the oxidative phosphorylation in their liver mitochondria, and the P/O ratio became extremely small (Table 1), groups 4 and 7).

Oxidative phosphorylation in the group of rabbits with thyrotoxicosis (without injection of the toxin) was reduced only very slightly in these experiments: 1.67 in the control and 1.31 in the experimental group with succinic acid, and 2.70 and 2.38 in the group with glutamic acid, respectively.

When the results of these experiments are analyzed it should be noted that the resistance of the rabbits to diphtheria toxin varied with the state of their thyroid function; hyperthyroidism led to a sharp decrease in resistance. The reactivity of the mitochondria toward the uncoupling action of diphtheria exotoxin on phosphorylation and respiration also varied sharply depending on the level of supply of thyroid hormones to the tissues.

In hypothyroidism the ability of the liver mitochondria to carry out oxidative phosphorylation was virtually undisturbed after injection of diphtheria toxin.

The resistance of oxidative phosphorylation processes in the liver cells to the uncoupling action of diphtheria toxin was sharply reduced in experimental hyperthyroidism. The lowered resistance of energy metabolism in the liver to poisons and toxins with uncoupling action in thyrotoxicosis must evidently be taken into account also when patients with hyperthyroidism develop certain infectious diseases.

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