ROLE OF THYROID HORMONES IN THE MECHANISM OF THE UNCOUPLING ACTION OF DIPHTHERIA TOXIN

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UDC 615.372:576.852.23/.015.2:615.357.441

The action of diphtheria toxin injected into rabbits on oxidative phosphorylation in the liver was studied during blocking of thyroid function. The agent used to block thyroid hormonal function was mercazolyl (1-methyl-2-mercaptoimidazole), which has high antithyroid activity. Whether in conjunction with or without thyroidectomy mercazolyl had a protective action against diphtheria toxin in the rabbits, as shown by the absence of disturbance of oxidative phosphorylation in the liver mitochondria. No connection was found between the changes in the oxygen consumption by the rabbits and the disturbance of energy metabolism in the liver through the effects of diphteria toxin.

Previous investigations demonstrated that changes in responses of the mitochondria to the uncoupling action of 2,4-dinitrophenol (DNP) on oxidative phosphorylation in the liver are dependent on the supply of thyroid hormones in the body [7]. Because of the great importance of the internal secretary function of the thyroid gland in the mechanism of this uncoupling action of DNP, it was essential to study the role of the thyroid gland in the mechanism of the uncoupling action of a bacterial toxin on oxidative phosphorylation in the liver; diphtheria toxin (DT) was chosen for this purpose, for its action on metabolism in the liver is probably mediated through neurohumoral regulatory mechanisms [3].

This paper describes the results of a study of the level of oxidative phosphorylation in the liver after intravenous injection of DT into animals whose thyroid function was blocked by administration of the compound mercazolyl (1-methyl-2-mercaptoimidazole), which possesses high antithyroid activity [1, 4-6, 11].

EXPERIMENTAL METHOD

Experiments were carried out on 78 male rabbits weighing 2.2-2.7 kg. To begin with, for 3-5 days the oxygen consumption of the animals was measured daily each morning over a period of 20 min [2]. Hypothyroidism was produced in 3 ways: by thyroidectomy, by daily administration of mercazolyl (5 mg/kg) for 16-18 days, and by a combination of daily feeding with mercazolyl (5 mg/kg) for 8-9 days and thyroidectomy, followed by a further period of feeding on mercazolyl in the same dose for 6-7 days. The animals were sacrificed on the 3rd day after intravenous injection of DT (1.5 MLD in a volume of 1.5 ml) and on the 5th-8th day after thyroidectomy. To determine the effect of operative trauma, a mock thyroidectomy was performed on a group of rabbits, in which the whole course of the operation was reproduced except actual removal of the gland. Energy metabolism was studied in mitochondria isolated from the liver [8]. To measure oxidation and phosphorylation, the mitochondria were incubated in a reaction mixture at pH 7.4 for 20 min at 30°C in an atmosphere of air. The rate of oxidation was measured in a Warburg apparatus and the rate of phosphorylation was determined from the decrease in concentration of inorganic phosphate [10]. The conposition 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

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 G. N. Veselkin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 7, pp. 34-36, July, 1972. Original article submitted January 25, 1972.

TABLE 1. Respiration and Phosphorylation of Liver Mitochondria (Calculated per Milligram Mitochondrial Protein) and Oxygen Consumption (in ml/kg-min) in Normal and Hypothyroid Rabbits Receiving Diphteria Toxin by Intravenous Injection

Experimental conditions and number of animals in group (n)	in μ atoms						Oxygen consumption (in ml/kg body weight per min)		
	succinic acid			glutamic acid			before experiment	at end of experiment	change in percent
	ΔP	ΔO	P/O	ΔP	ΔO	P/O			
Control (n = 13)	0,65±0,08	0,45±0,05	1,44±0,06	0,45±0,05	0,19±0,02	2,46±0,14	11,2±0,32	10,7±0,71	—4
Thyroidectomy (n = 10)	0,64±0,07	0,42±0,05	1,62±0,17	0,36±0,04	0,16±0,02	2,34±0,23	11,6±0,62	9,9±0,781	—15
Mock thyroidectomy (n = 6)	0,82±0,11	0,53±0,04	1,58±0,17	0,46±0,05 *	0,21±0,009	2,20±0,32 *	—	—	—
DT (n = 16)	0,38±0,05†	0,48±0,03	0,79±0,09†	0,27±0,06 *	0,19±0,02	1,24±0,16 *	12,1±0,401	10,1±0,67 *	17—
Thyroidectomy + DT (n = 13)	0,14±0,03†	0,46±0,04	0,37±0,11†	0,10±0,05†	0,14±0,01	0,71±0,38†	12,3±0,270	9,6±0,36†	—22
Mercazolyl + thyroidectomy + DT (n = 9)	0,70±0,05	0,46±0,03	1,55±0,15	0,47±0,06	0,18±0,004	2,57±0,25	10,9±0,33	8,6±0,26†	—21
Mercazolyl + DT (n = 11)	0,55±0,08	0,44±0,03	1,31±0,19	0,36±0,05	0,16±0,01	2,25±0,31	10,6±0,23	9,1±0,47†	—15

Note: P relative to the control for each series of experiments.

*P ≤ 0.05, †OP > .01

mitochondria in 0.25 M sucrose. The quantity of oxygen absorbed and of phosphate esterified was calculated per milligram mitochondrial protein [9].

EXPERIMENTAL RESULTS AND DISCUSSION

Only very slight changes in the state of the rabbits were observed after thyroidectomy, together with a gain in weight and a decrease in oxygen consumption by 15%. The energy metabolism in the liver of these rabbits and of those undergoing the mock thryoidectomy was unchanged (Table 1). Injection of diphtheria toxin reduced the oxygen consumption on the average by 17%. Disturbance in energy metabolism also were observed: when both substrates were used the level of oxidative phosphorylation (P/O) was almost halved by comparison with the control. Similar results were obtained by one of the writers after injection of 1 MLD of the toxin [3]. Injection of DT on the third day after thyroidectomy reduced the oxygen consumption of the rabbits on the average by 22% and caused a sharp decrease in the rectal temperature. The energy metabolism in the mitochondria of the liver of these animals was disturbed to an even greater degree than in rabbits with an intact thyroid but injected with DT. A different picture was found in the study of energy metabolism in the liver of rabbits receiving mercazolyl before and after thyroidectomy: the level of oxidative phosphorylation in the liver mitochondria of these animals was unchanged. In rabbits not undergoing thyroidectomy but receiving mercazolyl, injection of diphtheria toxin likewise produced no disturbances of coupling of respiration with phosphorylation in the liver mitochondria. The oxygen consumption of these rabbits was reduced on the average by 16%.

The first noteworthy fact to appear during analysis of the experimental results was that mercazolyl, whether in conjunction with thyroidectomy or alone, had a protective action against DT, as shown by the absence of disturbance of oxidative phosphorylation in the liver mitochondria of rabbits poisoned with the toxin. This points to a role of thyroid hormone in the mechanism of action of DT on tissue metabolism. Similar results were to be expected in the thyroidectomized animals receiving mercazolyl. It was found, however, that thyroidectomy instead potentiated the action of DT. This may have been due to administration of the DT too early after the operation (2nd-3rd day), giving insufficient time for the action of the endogenous thyroxine to be terminated. In addition, an important pathway in the mechanism of the regulatory function of the thyroid gland is its interaction with the pituitary. In the early periods after thyroidectomy the pituitary possibly begins to respond to a change in the concentration of thyroxine and the main source of its production in the body is no

longer present, so that certain changes occur in the response of the tissue to the toxin. However, these hypotheses require special verification.

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