EFFECT OF INORGANIC IODINE ON OXIDATIVE PHOSPHORYLATION IN BRAIN AND KIDNEY MITOCHONDRIA OF NORMAL AND HYPERTHYROID RABBITS

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It has been proven that an excess of thyroid hormones in the organism results in a serious pathological disturbance of the energy balance in cells because uncoupling is produced in the oxidative phosphorylation process in the mitochondria. Iodine functions as part of the thyroid hormone molecule, but it also acts as an effective medicinal in the treatment of Basedow's disease [1-5]. It has been suggested that the favorable therapeutic effect of iodine may be due to its ability to block the mechanisms of the thyroid gland involved in the accumulation of iodine into the granular parenchyma from the blood plasma [6, 8, 10-12].

The present work deals with the interrelationship of thyroid hormones and iodine in the mitochondria of peripheral tissues and in particular it has been designed to study the effect of iodine on the uncoupling action of the thyroid hormone in brain and kidney mitochondria from rabbits in various conditions of experimental thyrotoxicosis.

EXPERIMENTAL METHODS

The rabbits in these trials weighed 1½-2 kg. Experimental thyrotoxicosis was produced by administering for 2 months a thyroid gland extract at the daily rate of 70-80 mg/kg of body weight. The test animals were divided into four groups: group 1) normal controls; group 2) hyperthyroid; group 3) animals receiving magnesium chloride at the rate of 170-180 mg/kg body weight daily in addition to the thyroid hormones; group 4) rabbits receiving the thyroid hormones and also the antithyroid preparation methimazole (1-methyl-2-mercapto-imidazole) at a 15 mg per day dose level.

TABLE 1. Effect of Inorganic Iodine on Oxidative Phosphorylation in Rabbit Brain Mitochondria

Group of animals	Concentration of iodine in test sample (M)	No. of exp.	O_2 absorp.		$P_{inorg.}$ incorp.		P:O
			μ atoms	%	μM	%	
1st (normal)	0	9	6,6	100	15,9	100	2,41 ± 0,07
	10^{-7}	5	11,3	170	20,4	122	1,80 ± 0,14
2nd (hyperthyroid)	0	9	7,1	100	12,8	100	1,80 ± 0,18
	10^{-7}	3	11,9	168	16,4	128	1,38 ± 0,13
3rd (hyperthyroid + $MgCl_2$)	0	8	8,1	100	15,1	100	1,86 ± 0,20
	10^{-7}	4	11,6	143	16,0	106	1,38 ± 0,14
4th (hyperthyroid + methimazole)	0	6	7,0	100	18,2	100	2,60 ± 0,09
	10^{-7}	5	8,3	118	16,5	82	2,00 ± 0,20

TABLE 2. Effect of Inorganic Iodine on Oxidative Phosphorylation in Rabbit Kidney Mitochondria

Group of animals	Concentration of iodine in test sample (M)	No. of exp.	O_2 absrop. μ atoms	O_2 absrop. %	$P_{inorg.}$ incorp. μM	$P_{inorg.}$ incorp. %	P:O
1st (normal)	0	10	9,7	100	20,2	100	2,08 ± 0,19
	10^{-7}	5	11,9	123	20,2	100	1,70 ± 0,14
	10^{-8}	4	14,2	147	23,4	116	1,65 ± 0,15
2nd (hyperthyroid)	0	7	14,2	100	18,1	100	1,27 ± 0,21
	10^{-7}	4	18,0	126	12,0	60	0,66 ± 0,08
3rd (hyperthyroid + $MgCl_2$)	0	9	13,5	100	18,8	100	1,39 ± 0,11
	10^{-7}	4	15,4	113	22,6	130	1,46 ± 0,14
4th (hyperthyroid + methimazole)	0	8	11,5	100	19,4	100	1,69 ± 0,20
	10^{-7}	6	12,3	107	17,8	92	1,45 ± 0,16

Mitochondria were separated from brain and kidney in isotonic sucrose medium (0.25 M) by differential centrifugation first at 700 g and then at 13,000 g. Respiration of the mitochondria was measured manometrically in the Warburg apparatus and expressed as μatoms of oxygen absorbed by the sample during the incubation time. Esterification of inorganic phosphate was determined by the Fiske and Subbarow method and expressed as micromoles of phosphate per sample.

The reaction medium in each vessel contained 30 μM DL-glutamic acid, 3.5 μM ADP, 2.5 μM NaF, 10 μM $MgCl_2$, 35 μM glucose, 0.160 mg hexokinase, 0.022 μM cytochrome C, 40 μM phosphate buffer (pH 7.4); inorganic iodine was added in the concentrations of 0.1 and 1.0 μM and the calcium ion at 0.1 μM concentration. The total volume was made up to 2 ml with 0.18 M sucrose solution. Samples were incubated for 21 min at 28°. Each sample contained the mitochondria from 500 mg of tissue.

The results, presented in Tables 1 and 2, represent average values and are calculated for the whole volume of the sample (2 ml) and the entire time of incubation (21 min).

EXPERIMENTAL RESULTS

Several series of experiments were designed to determine whether iodine shows any favorable effect directly on the mitochondria from hyperthyroid tissue. The iodine was added to the standard incubation medium at a 10^{-7} and 10^{-8} M concentration (see Table 1).

It is shown in Table 1 that the rate of oxygen uptake by the brain mitochondria in the hyperthyroid condition is at the same level in all groups of experimental animals. However, the incorporation of inorganic phosphate by the mitochondria from hyperthyroid rabbits, not under other treatment (2nd group), was decreased and as a consequence the P : O ratio fell. A similar change in the phosphorylation coefficient was found in the experiments with mitochondria from animals receiving magnesium chloride in addition to the thyroid hormones (3rd group). In the 4th group the methimazole in vivo completely eliminated the uncoupling effect of the thyroid hormones as demonstrated by the increased incorporation of inorganic phosphate and elevation in the P : O value.

It is interesting that the respiratory apparatus of the brain mitochondria, which evidences a well-established resistance to thyroid hormones, increases sharply in activity under the influence of iodine in vitro showing a 70% rise in oxygen uptake (group 1). A similar reaction was noted also in brain mitochondria obtained from rabbits in other experimental groups (groups 2 and 4).

Analogous results were obtained with kidney mitochondria, in which the increased oxygen uptake during the hyperthyroid state was accompanied by a fall in the esterification of inorganic phosphate (see Table 2). This P : O ratio in the kidney mitochondria from normal animals showed a decrease under the influence of iodine of approximately 20% compared with the test in the absence of iodine.

Addition of inorganic iodine in vitro to kidney mitochondria obtained from hyperthyroid animals, not treated with other substances, intensified the toxic action of the thyroid hormones; there was a marked fall in phosphate esterification and in the P : O coefficient.

Experiments on kidney mitochondria [7] have shown that iodine in high concentrations ($5 \cdot 10^{-4}$ M) is capable of uncoupling oxidative phosphorylation. This finding was confirmed by others [9] under similar conditions (iodine concentration in the sample was 10^{-3} M).

When experiments were made with various substrates and substances that block the electron transport in the respiratory chain, it was demonstrated [10] that in order to manifest the "swelling" effect of I_2 (as well as of ICN and triiodothyronine) in mitochondria, it is essential for electron transfer to occur through the cytochrome b link.

From our data it is evident that iodine at a 10^{-7} M concentration, when added to brain and kidney mitochondria, increases the oxygen uptake to a greater extent than it does the phosphate esterification, and therefore the P : O ratio decreases. Apparently iodine at this concentration does not depress the binding of inorganic phosphate. However, in its action on kidney mitochondria from untreated hyperthyroid rabbits, iodine intensifies the uncoupling action of the thyroid hormones. Similar results were obtained also in experiments with heart mitochondria [2]. It is of interest that the respiratory apparatus in brain mitochondria (which do not react to thyroid intoxication) under the influence of inorganic iodine increases oxygen uptake to practically double the control level.

SUMMARY

A study was made on the influence of inorganic iodine in vitro on the process of oxidative phosphorization in the mitochondria of the brain and kidneys in normal and hyperthyroid rabbits. Thyrotoxicosis was induced by daily per os administration during two months of a thyroid hormone preparation in a dose of 70-80 mg per kg of body weight. It was found that iodine added in vitro to mitochondria of the brain and kidneys aggravates the toxic influence of thyroid hormones, increasing the degree of their disconnecting influence.

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