ACTIVATION OF LIVER MITOCHONDRIAL PHOSPHOLIPASE IN HYPERTHYROID RABBITS

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Some of the metabolic effects of thyroid hormones are realized at the mitochondrial level in many mammalian tissues. Highly specific receptor proteins for these hormones, for which the binding constant of triiodothyronine or thyroxine is 1-1.5 order of magnitude higher than the characteristic constants for other cell proteins, have been found on the inner membranes of mitochondria. It is important to note that such receptors have been found in the mitochondria only of those tissues that are characterized by metabolic sensitivity to thyroid hormones [10]. Mitochondria isolated from the liver immediately after administration of thyroxine to the animal utilize oxygen in the ADP-controlled state and at a higher rate than preparations from control animals [8]. The addition of low (corresponding to physiological) doses of thyroxine *in vitro* leads to rapid swelling of the organelles in isotonic medium [4, 7], and this is associated with liberation of fatty acids [12]. This fact, together with the observed ability of calcium-binding complexones to inhibit the swelling of mitochondria and the distinct synergism of Ca^{2+} ions and thyroid hormones in the production of swelling of the mitochondria, are evidence that thyroid hormones activate mitochondrial phospholipase *in vitro* [2, 3, 5].

However, there is no information on whether activation of mitochondrial phospholipase takes place in response to a rise of the thyroid hormone levels *in vivo*. The investigation described below was undertaken to remedy this deficiency.

EXPERIMENTAL METHOD

Rabbits weighing from 2 to 3 kg were used. Hyperthyroidism was induced by daily intraperitoneal injection of L-thyroxine in 0.05 N KOH in a dose of 200 µg/kg body weight for 3-4 weeks. Control animals received the same volume of solvent. Mitochondria were isolated from the animals' liver in the usual way [9]. Mitochondrial phosphorylase activity was assayed by determining the increase in the concentration of free fatty acids (FFA) discovered in the mitochondrial system after incubation for 25 min. To determine the FFA concentration, 0.8-ml samples of mitochondrial suspension were withdrawn from the test specimens containing 4 mg protein. Phospholipids were then extracted from the samples by the method described in [6]. Lipid material in a volume of 15 µl was applied to a Silufol (Czechoslovakia) plate measuring 20 × 20 cm. Meanwhile two indicators (palmitic acid in doses of 15 and 60 nanomoles) were applied to the same plate. To fractionate the test specimens the plate was placed in a solution consisting of petroleum ether (bp 40-50°C), ethyl ether, and acetic acid in the ratio of 80:20:1 by volume [1]. The plate was then sprinkled with 0.1% solution of 1-anilinonaphthalene-8-sulfonate (ANS) solution, pH 4.0 [11]. The intensity of fluorescence of the FFA-ANS complex was measured on a "Chromoscan-200" (England) apparatus. The quantity of FFA was counted by comparing the light sums of fluorescence of complexes in the experimental specimens and indicators.

EXPERIMENTAL RESULTS

The initial body weight of the rabbits with hyperthyrodism fell by 22-32% (mean 27%). During the same period the body weight of the control animals increased by 10-25%. The considerable decrease in body weight of the experimental animals is evidence of the severity of the hyperthyroidism.

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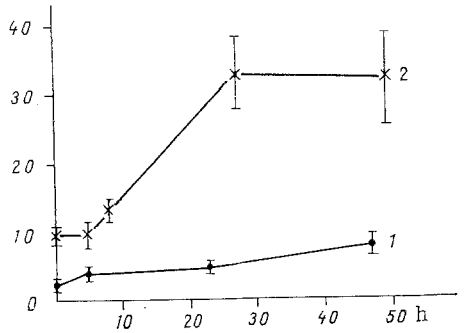

Fig. 1. FFA accumulation in rabbit liver mitochondria as a function of length of keeping at 3°C. 1) Control rabbits; 2) rabbits with hyperthyrodism. Abscissa, time (in h); ordinate, FFA concentration (nanomoles/mg protein).

TABLE 1. Dependence of Liver Mitochondrial FFA Level of Normal and Hyperthyroid Rabbits on Incubation Temperature (M ± m)

Temperature, °C	Incubation time, min	FFA concentration, nanomoles/mg protein	
		control (n = 5)	experiment (n = 6)
24	0	2,32±0,6	9,5±1,04
24	25	2,84±1,32	11,43±2,44
29	25	5,18±1,76	14,62±2,34
33	25	5,62±1,87	16,69±2,63
38	25	6,04±2,31	26,85±3,48

Legend. Mitochondria incubated in medium containing 120 mM KCl, 1 mM KH_2PO_4, 5 mM succinate, 100 µM $CaCl_2$, 5 mM Tris-HCl, pH 7.4.

Measurements showed that the liver mitochondria of hyperthyroid rabbits contained four times more FFA calculated per unit of protein than mitochondria of the control animals (9.54 and 2.32 nanomoles/mg protein respectively). This difference can be attributed to the greater activity of phospholipase A_2 in the hyperthyroid animals. It was shown previously that thyroxine *in vitro* activates this enzyme [2, 3]. To test this hypothesis, the temperature dependence of FFA accumulation in mitochondria of the two groups of animals was studied.

As Table 1 shows, during incubation the FFA concentration increased in mitochondria of both control and hyperthyroid animals, but by a greater degree in the latter case. The graph of logarithm of increase in FFA concentration as a function of reciprocal of temperature was a straight line over the temperature range 24-38°C for mitochondria of the experimental animals and 29-38°C for mitochondria of the controls. The activation energy calculated from these graphs was 29 kcal/mole for the experimental and 7 kcal/mole for the control rabbits. This difference in the activation energy values indicates a change in the physical state of the mitochondrial lipids during hyperthyroidism.

Keeping mitochondria at low temperature leads to hydrolysis of the membrane proteins. FFA accumulation in mitochondria of the control and hyperthyroid animals in the course of keeping at 3°C is illustrated in Fig. 1. It will be clear from Fig. 1 that the initial difference in FFA concentration between the experimental and control mitochondria increase with an increase in the keeping period. These results, like those given in Table 1, can be interpreted as the result of increased membrane phospholipase activity in hyperthyroidism.

A hyperthyroid state in rabbits thus leads to an increase in phospholipase activity of the liver mitochondria. This conclusion is supported by the presence of an original difference in the FFA concentration in experimental and control mitochondria and also by data on the kinetics of accumulation of hydrolysis products of membrane lipids at low and high temperatures. It may be that the preexisting difference in mitochondrial FFA levels is to

some extent the result of the raised level of thyroid hormones which activate the phospholipase molecules *in vivo* in a similar way to what is observed in isolated mitochondria on incubation with thyroxine.

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ACCUMULATION OF LIPID PEROXIDATION PRODUCTS AND DEPRESSION OF RETINAL ELECTRICAL ACTIVITY IN VITAMIN E-DEFICIENT RATS EXPOSED TO HIGH-INTENSITY LIGHT

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Stabilization of membrane structures of photoreceptor cells against induction of lipid peroxidation (LPO) is achieved mainly because of their high content of α-tocopherol (vitamin E) [1], an effective physical and chemical quencher of singlet oxygen [2], an acceptor of superoxide anion-radicals [2], and an antiradical agent [3]. A deficiency of the natural antioxidant, vitamin E, is one cause of degeneration of the photoreceptor layer of the retina [4, 5], accompanied by accumulation of LPO products *in vivo* [5, 6]. Meanwhile induction of LPO in the isolated retina leads to depression of its electrical activity [7]. A comparison of these facts suggests that the retina of vitamin E-deficient animals should be more sensitive to the harmful action of high-intensity light than the retina of animals kept on a diet with the standard concentration of vitamin E. Further evidence in support of the validity of this hypothesis would appear to be given by the fact that photoinduced accumulation of LPO products, preventable by antioxidants, has been demonstrated both *in vitro* [8] and *in vivo* [9].

Accordingly, in the investigation described below, the content of LPO products and the electroretinogram (ERG) were studied in vitamin E-deficient rats exposed to high-intensity light.

EXPERIMENTAL METHOD

Experiments were carried out on 20 male Wistar rats. Alimentary avitaminosis E was induced by keeping the rats on a synthetic diet deficient in α-tocopherol (the composition of the diet is given in Table 1 [10]). The concentration of α-tocopherol fell from 0.74 ± 0.08

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