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THYROID HORMONES AND PHOSPHOLIPASE ACTIVITY OF RAT LIVER MITOCHONDRIA

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Endogenous mitochondrial phospholipase activity can be controlled by thyroid hormones for a number of reasons. First, thyroid hormones affect the rate of synthesis of phospholipids by the mitochondria [13] and also have a considerable influence on their fatty acid composition [12]. Modification of the phospholipase substrate molecules and, simultaneously, of the lipid environment of the enzyme under study is thus possible. Second, thyroid hormones facilitate transport of Ca^{++} ions through the mitochondrial membranes [11], and this is a matter of undoubted interest in the light of data showing the activating action of these ions on mitochondrial phospholipase [1]. Finally, as was shown previously in the writers' laboratory [3, 4], if thyroxine is added to isolated liver mitochondria it activates the phospholipase of these organelles.

The aim of the present investigation was to study phospholipase activity in liver mitochondria from rats receiving toxic doses of thyroid hormones.

EXPERIMENTAL METHOD

Male Wistar rats weighing 160-200 g were used. Hyperthyroidism was induced by intraperitoneal injection of L-thyroxine in a dose of 150-200 µg/100 g body weight daily for 7-8 days. Thyrotoxicosis was stimulated by injection of the hormone by the same route in a dose of 2 mg/100 g body weight for 5-6 days. Control animals received injections of the solvent (0.05 N KOH). Mitochondria were isolated by the standard method in medium containing 0.3M sucrose, 1 mM EDTA, and 10 mM Tris-HCl buffer, pH 7.4. The mitochondria were washed and kept in a solution of 125 mM KCl, 10 mM Tris-HCl buffer, pH 7.5. Mitochondrial phospholipase activity was estimated from the quantity of free fatty acids (FFA) present at the time of isolation of the mitochondria from the liver or accumulating during incubation, per unit of mitochondrial protein. FFA were determined quantitatively by the method in [9], modified by the writers for use with mitochondria. A calibration graph was plotted for standard solutions of oleic acid. Protein was determined by the biuret reaction.

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TABLE 1. Effect of Thryoid State on Initial FFA Level (in nanomoles/mg protein) in Liver Mitochondria and on Phospholipase Activity (M ± m)

Incubation time	Normal (27)	Hyperthyroidism (10)	Thyrotoxicosis (10)
0	6,5±0,53	17,0±1,6	6,9±0,71
15	14,5±1,6	26,5±1,3	14,3±1,4
30	18,9±1,3	33,9±2,3	26,2±1,4
60	28,4±1,9	42,2±2,3	42,9±5,0

Legend. Number of measurements given in parentheses.

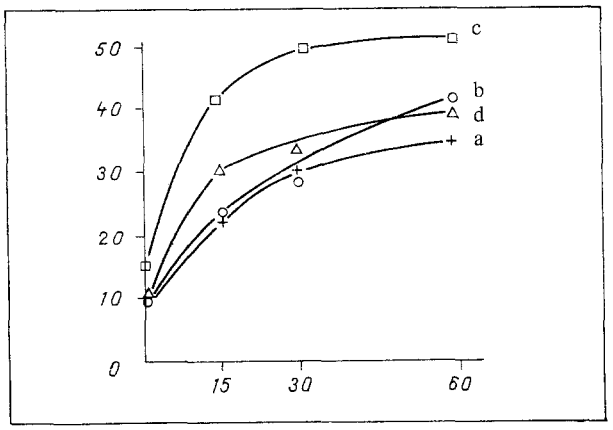

Fig. 1. Effect of a single injection of thyroxine on mitochondrial phospholipase activity depending on time after injection. a, b, c, d) Time course of FFA accumulation in mitochondria isolated from normal rats and rats receiving thyroxine (300 µg/100 g body weight), 24, 48, and 72 h before sacrifice respectively. Mitochondria were incubated in medium containing 125 mM KCl, 1 mM KH_2PO_4, 10 mM Tris-HCl, pH 7.5. The Ca^{++} concentration was 2 mM. Protein content in sample 5-6 mg, temperature of incubation medium 41-43°C. Abscissa, incubation time (in min); ordinate, FFA concentration (in nanomoles/mg mitochondrial protein).

EXPERIMENTAL RESULTS

Hyperthyroidism was accompanied by activation of rat liver mitochondrial phospholipase (Table 1). This agrees with the writers' previous data on activation of mitochondrial phospholipase in the liver of hyperthyroid rabbits [5]. As Table 1 shows, both the initial level of FFA and their accumulation in the course of incubation of the organelles with Ca^{++} ions were higher in preparations from hyperthyroid than from normal animals. As might be expected, addition of EDTA inhibited FFA accumulation practically completely, showing that this process is dependent on Ca^{++} ions. For instance, the rate of accumulation of FFA in mitochondria of normal animals in medium containing EDTA was approximately 5-10% of the rate of lipid hydrolysis in the presence of Ca^{++} (2 mM). A similar picture to that of hyperthyroidism also was observed when the rate of FFA accumulation was recorded in mitochondria from thyrotoxic animals (Table 1). The initial FFA level, however, was much lower in the latter case than in hyperthyroidism. This may perhaps be due to the fact that during poisoning with thyroid hormones, metabolic expenditure is increased [15]. As a result of the latter it can therefore be expected that the initial FFA level, which evidently reflects their endogenous level adequately, was not very high, even if phospholipase activity in the mitochondria was increased.

An increase in mitochondrial phosphorylase activity was observed not only as a result of prolonged administration of the hormone, but also after a single injection of thyroxine into normal rats. The results of the appropriate experiments are given in Fig. 1. About two days after injection of the hormone both the initial FFA level and the rate of hydrolysis of mitochondrial lipids were significantly increased.

A rise in the body level of thyroid hormones is thus accompanied by activation of endogenous phospholipase in liver mitochondria. The concrete mechanism of this phenomenon requires further study, although some preliminary suggestions can now be put forward. In hyperthyroidism the velocity of lipid peroxidation (LPO) reactions in the liver mitochondria is increased [7]. Activation of many phospholipases has been demonstrated with a decrease in hydrophobicity of the environment [10]. If this fact is extrapolated to mitochondrial phospholipase, it can be postulated that the acceleration of hydrolysis of mitochondrial lipids observed in the present experiments with thyroxine poisoning is based on an increase in hydration in the lipid regions, induced by LPO.

What is the functional role of increased phospholipase activity in the organelles of hyperthyroid and thyrotoxic rats? Mitochondrial phospholipase is known to be able to catalyze not only the hydrolysis of membrane lipids, but also transacylation [4, 8]. In other words, if necessary this enzyme can replace some fatty acids in mitochondrial lipids by others. In that way it can modify the properties of mitochondrial membranes and, in particular, their microviscosity and permeability, and this must have some effect on the levels of energy processes in the cells. Accumulation of lyosforms of phospholipids in the internal membranes of the mitochondria during activation of phospholipases may have an even stronger effect [14].

Activation of mitochondrial phospholipase caused by an increase in body levels of thyroid hormones can explain, at least partially, the decrease in resistance of the mitochondria observed in hyperthyroidism to various noxious factors and, in particular, to Ca^{++} ions, causing their swelling [2, 15] and injury to the system responsible for maintaining the transmembrane potential of these organelles [6].

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