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EFFECT OF TRIIODOTHYRONINE ON THE CONTENT OF PHOSPHOLIPIDS IN THE RAT LIVER NUCLEI

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The aim of the present study was to examine the effect of triiodothyronine (T_3) on the content of phospholipids and on the incorporation of blood-borne palmitic acid into the phospholipid moieties in the nuclei of the rat liver. T_3 was administered daily for 7 days, $10 \mu\text{g} \times 100 \text{g}^{-1}$. The control rats were treated with saline. Each rat received ^{14}C -palmitic acid, intravenously suspended in serum. 30 min after administration of the label, samples of the liver were taken. The nuclei were isolated in sucrose gradient. Phospholipids were extracted from the nuclei fraction and from the liver homogenate. They were separated into the following fractions: sphingomyelin, phosphatidylcholine, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine and cardiolipin. The content and radioactivity of each fraction was measured. It was found that treatment with T_3 reduced the content of phosphatidylinositol and increased the content of cardiolipin in the nuclear fraction. In the liver homogenate, the content of phosphatidylinositol decreased and the content of phosphatidylethanolamine and cardiolipin increased after treatment with T_3 . The total content of phospholipids after treatment with T_3 remained unchanged, both in the nuclear fraction and in the liver homogenate. T_3 reduced the specific activity of phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine and cardiolipin and had no effect on the specific activity of sphingomyelin and phosphatidylinositol both in the fraction of the nuclei and the liver homogenate. It is concluded that excess of triiodothyronine affects the content of phospholipids in the nuclei. The changes in the content of phospholipids in the nuclei largely reflect changes in their content in the liver.

Key words: *phospholipids, liver nuclei, ^{14}C -palmitic acid, rat.*

INTRODUCTION

Phospholipids located in the nuclei play an important role in the regulation of the function of the organelle. They affect the metabolism of DNA (1, 2) and RNA (3–6) and are involved in signal transduction into the nucleus (7, 8). However, our present knowledge concerning the metabolism of nuclear phospholipids is still limited. The content of phospholipids in the nuclei depends on the phase of DNA replication (9, 10). The nuclear phospholipid

fatty acid metabolism has been shown to depend on diet (11, 12). Insulin and nerve growth factor increase transport of phosphatidylinositol 3-kinase into the nuclei (13, 14). The incorporation of radioactive phosphorus into the nuclear phosphatidylinositol is inhibited by insulin-like growth factor (15). Hypothyroidism results in changes in the composition of phospholipids, in reduction in the incorporation of ^{14}C -acetate into phospholipid moieties and in elevation of the activity of phospholipase A_1 and A_2 in the nuclei (16). The data obtained in hypothyroid rats indicate that thyroid hormones are involved in regulation of the metabolism of the nuclear phospholipids. The aim of the present study was to examine the effect of excess triiodothyronine on the content and composition of phospholipids and on the incorporation of blood-borne ^{14}C -palmitic acid into phospholipid moieties in rat liver nuclei.

METHODS

The experiments were carried out on male Wistar rats, 291 ± 12 grams of body weight, fed *ad libitum* a commercial pellet diet for rodents. The rats were divided into two groups- 1-control, 2-treated with triiodothyronine (T_3). T_3 (Sigma) was administered subcutaneously in a dose of $10 \mu\text{g} \cdot 100 \text{g}^{-1}$ of body weight, daily for 7 days. Two days after the last dose of T_3 , the rats were anaesthetised with pentobarbital sodium ($80 \text{mg} \cdot \text{kg}^{-1}$, i.p.). After anaesthesia, the rats were kept on a heating pad so that their body temperature was stable. Each rat received ^{14}C -palmitic acid (Du Pont, S.A. $57 \text{mCi} \cdot \text{mmol}^{-1}$), $5 \mu\text{Ci} \times 100 \text{g}^{-1}$, intravenously, suspended in serum. The serum was obtained from blood of an untreated rat. 30 min later samples of the liver were taken. The control rats were treated with saline and were run handled with the T_3 -treated ones. The liver nuclei were isolated according to the method of Lamar (17). The purity of the nuclei preparation was evaluated by determination of the activity of 5'-nucleotidase and lactic dehydrogenase and by examination under the electron microscope as described previously (18). The dry weight of nuclei and liver sample was determined after keeping the samples for 48 hr at a temperature of 70°C . Liver samples and the nuclei were homogenised in chloroform/methanol (2:1) and lipids were extracted according to Folch *et al.* (19). Phospholipids were separated into different fractions by means of thin-layer chromatography on silica plates (0.25 mm, Merck). The developing solvent was composed of chloroform:methanol:acetic acid:water 50:37.5:3.5:2 v/v/v/v (20). The following phospholipid fractions were obtained: sphingomyelin, phosphatidylcholine, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine and cardiolipin. The phospholipids were identified according to appropriate standards (Sigma). The gel bands containing individual phospholipids were scraped off the plates. The phospholipids were quantified by determination of phosphorus (21). Radioactivity of each fraction was counted in Tricard 1900 T4 (Packard) using Ultima Gold Packard scintillation cocktail. The results obtained were evaluated statistically by means of the Student t-test for unpaired data. The results presented are means \pm standard deviations. $N = 10$.

RESULTS

Treatment with triiodothyronine (T_3) reduced the content of phosphatidylinositol and increased the content of cardiolipin both in the liver homogenate and in the nuclear fraction. The content of other phospholipid

classes and the content of total phospholipids remained stable (Table 1). The specific activity of the total phospholipids and the specific activity of each phospholipid fraction, with the exception of sphingomyelin and phosphatidylinositol, was reduced in the fraction of the nuclei and in the liver homogenate of T₃-treated rats (Table 2).

Table 1. Effect of triiodothyronine (T₃) on the content of different classes of phospholipids (μmol of phospholipid phosphorus $\times \text{g}^{-1}$ of dry weight) in the fraction of the liver nuclei and in the liver homogenate.

Phospholipid	Nuclear fraction		Liver homogenate	
	Control	T3	Control	T3
Sphingomyelin	4.5 \pm 1.7	3.3 \pm 1.2	6.6 \pm 1.3	6.0 \pm 1.3
Phosphatidylcholine	25.2 \pm 2.1	26.0 \pm 3.2	38.6 \pm 6.7	42.5 \pm 4.1
Phosphatidylserine	4.6 \pm 1.1	4.0 \pm 1.4	2.3 \pm 0.3	2.4 \pm 0.7
Phosphatidylinositol	3.0 \pm 1.3	1 \pm 0.7 ^a	5.4 \pm 0.6	4.1 \pm 1.0 ^b
Phosphatidylethanolamine	11.6 \pm 1.0	12.7 \pm 3.8	23.3 \pm 3.4	28.9 \pm 3.1 ^b
Cardiolipin	0.9 \pm 0.1	1.2 \pm 0.2 ^c	3.0 \pm 0.4	4.6 \pm 1.3 ^b
T	49.1 \pm 7.5	50.3 \pm 6.1	75.4 \pm 9.9 ^d	77.9 \pm 5.6

T — the sum of individual phospholipids

^a $p < 0.05$

^b $p < 0.02$ vs. the respective control value

^c $p < 0.01$

^d $p < 0.001$

Table 2. Effect of triiodothyronine (T₃) on the specific activity ($\text{dpm} \times \mu\text{mol P}^{-1}$) of individual phospholipids.

Phospholipid	Nuclear fraction		Liver homogenate	
	Control	T3	Control	T3
Sphingomyelin	29.1 \pm 7.0	23.3 \pm 4.4	23.9 \pm 5.3	21.0 \pm 6.1
Phosphatidylcholine	125.5 \pm 11.6	90.7 \pm 10.3 ^d	157.1 \pm 36.5	98.4 \pm 13.2 ^d
Phosphatidylserine	106.9 \pm 23.3	67.9 \pm 8.3 ^d	92.8 \pm 15.3	52.4 \pm 7.3 ^d
Phosphatidylinositol	137.0 \pm 10.6	136.3 \pm 17.8	98.5 \pm 25.9	89.8 \pm 12.4
Phosphatidylethanolamine	88.8 \pm 13.5	49.3 \pm 6.9 ^d	129.5 \pm 33.9	44.1 \pm 5.7 ^d
Cardiolipin	37.9 \pm 9.6	27.4 \pm 7.6 ^d	12.6 \pm 2.9	7.3 \pm 2.4 ^d
T	110.3 \pm 13.2	72.6 \pm 8.1 ^d	132.9 \pm 30.5	93.6 \pm 5.8 ^c

T — the specific activity of total phospholipids

^c $p < 0.01$ vs. the respective control value

^d $p < 0.001$

DISCUSSION

The activity of enzymes involved in the metabolism of phosphoinositides in the nucleus is high enough for the phosphatidylinositol cycle to operate in the organelle (7, 8). The activity of enzymes involved in the metabolism of other phospholipids is very low (22) and, in consequence, the phospholipids must be imported from the maternal cell. There are controversies regarding the presence of cardiolipin in the nucleus. Some authors have found it to be present, whereas others did not detect it in healthy tissues (23—25). Cardiolipin was found in the nuclei of some pathological cells, namely in hepatoma (24) and in Daudi lymphoma cells (26). In our preparation, cardiolipin constituted ~1.8% of the total nuclear phospholipids which is in the range reported by others (23—25). Treatment with T_3 did not change the total content of phospholipids either in the nuclear fraction or in the liver homogenate. It did, however, influence the content of certain classes of phospholipids. This was manifested in a reduction in the content of phosphatidylinositol and elevation in the content of cardiolipin both in the fraction of the nuclei and in the liver homogenate. The content of phosphatidylethanolamine was elevated only in the liver of T_3 -treated rats. There are no data available on the relationship between the content of phospholipids in the nuclei and in the maternal cells. Our results suggest that the content of different phospholipids in the nuclear fraction, with the exception of phosphatidylethanolamine, depends on the content in the maternal cells. The transport of phosphatidylethanolamine seems to be impaired when T_3 is in excess.

Further insight into the effect of treatment with T_3 on the content of phospholipids in the nuclei was obtained with the use of labelled palmitic acid which was administered intravenously. It has previously been shown that blood borne labelled palmitic acid rapidly enters the nuclear lipids. Most of the radioactivity was found in the fraction of phospholipids (18). This means that there is a constant, fast exchange between the nuclear and the extranuclear pool of phospholipids. There is a certain level of activity of phospholipases A_1 and A_2 in the nuclei and thus some deacylation of glycerophospholipids may take here (16). However, no data on the presence of activity of glycerophosphate acyltransferase in the nuclei are available. This enzyme is responsible for acylation of glycerophospholipids at the sn1 position. Therefore, labelled palmitic acid must have been incorporated into the phospholipid moieties outside the nucleus. Treatment with T_3 reduced the specific activity of the total phospholipids in the nuclei and in the liver to a similar degree. Regarding individual phospholipid fractions, the specific activity of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine and cardiolipin was reduced and the specific activity of sphingomyelin and phosphatidylinositol remained unchanged both in the

nuclear liver homogenate fractions. This strongly suggests that the newly labelled phospholipids are transported to the nuclei in the amounts roughly proportional to the amounts labelled in the liver. In other words, the content of phospholipids in the nuclei is very closely related to the content of phospholipids in the maternal cell.

Summarising the data obtained, we have shown that treatment with triiodothyronine affects the content of phospholipids in the nucleus. The changes observed in the nucleus primarily reflect changes in the liver.

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