ACYLATION OF GLYCEROL-3-PHOSPHATE BY RABBIT HEART MITOCHONDRIA AND MICROSOMES: TRIIODOTHYRONINE-INDUCED INCREASE IN ITS ACTIVITY

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1. Introduction

Thyroid hormone raises the metabolic rate of the cell [1]. A demand for increased oxidative energy in the myocardium is met partially by an increased availability of plasma FFA [2, 3], and partially by the increased carnitine palmitoyltransferase (EC 2.3.1.23) activity [4]. Fritz proposed that the latter enzyme is not only the rate-limiting step of fatty acid (FA) oxidation, but also it determines whether the fatty acyl moiety is oxidized or esterified [5]. In the hearts of hyperthyroid guinea pigs, however, the myocardial triglyceride (TG) content and FA oxidation were simultaneously increased [4]. This is difficult to explain by evidence presently available, because i) the precursor of cardiac TG, plasma FFA, is increased in the hyperthyroid state [2, 3], and the incorporation of [^{14}C]palmitate or [^{14}C]tripalmitin into tissue glycerides can increase by the action of catecholamines [2, 6], which may be potentiated by the hormone [3, 7], ii) however, there are results showing that a supply of a second substrate for esterification, glycerophosphate (sn-glycerol-3-phosphate), is restricted, since the activity of phosphofructokinase, the rate-controlling step of glycolysis, does not increase in hyperthyroidism [8–10].

For these reasons, it was thought that the activity of glycerophosphate acylating enzymes in the myocardium likely increases in hyperthyroid animals, causing the TG accumulation. These enzymes have hitherto been scarcely studied in the heart [11] and there has been no work, to the author's knowledge, dealing with hormonal effects thereupon. Our study to be reported herein demonstrates that the formation of diacyl-glycerophosphate by both cardiac mitochondria and microsomes was raised approximately four-fold in triiodothyronine-treated rabbits. The biosynthesis of monoacyl-glycerophosphate by mitochondria was also significantly increased. The disproportionate increase in the former substance implicates that triiodothyronine stimulates particularly the acyl-CoA:monoacyl-glycerophosphate acyltransferase reaction.

2. Materials and methods

2.1. *Materials*

Disodium ATP, CoA, rac-glycerol-3-phosphate, 3,3′,5-triiodothyronine-sodium and bovine serum albumin, fraction V, were obtained from Sigma Chemical Co. Diacyl-glycerophosphate and [U-^{14}C]glycerophosphate were supplied by Pierce Chemical Co., and New England Nuclear Corp. respectively. Monoacyl-glycerophosphate was donated by Dr. M. Kates. Thin-layer chromatographic (t.l.c) plates coated with silica gel G were purchased from Brinkmann Co. All other reagents were of analytical grade.

2.2. *Induction of hyperthyroid state*

A group of albino male rabbits with body weights ranging from 1.9 to 2.5 kg was rendered hyperthyroid by a daily intramuscular injection of 125 μg triiodothyronine per kg body weight for a period of 6–10 days. This dose induced the typical hyperthyroid state as judged from growth rate and EKG. Some control animals were fed with laboratory chow for the same period as hyperthyroid rabbits, and others were

Table 1

The effect of triiodothyronine (T3) treatment on the acylation of glycerol-3-phosphate (P) by rabbit heart mitochondria and microsomes*.

Condition	Monoacyl-glycerol 3-P formation -nano moles/mg min	Diacyl-glycerol 3-P formation	n
	Mitochondria		
1. Fed control	0.150 ± 0.017	0.120 ± 0.022	3
2. Fasted control	0.147 ± 0.010	0.105 ± 0.008	6
3. T3 treated	0.270 ± 0.029	0.400 ± 0.043	8
	Microsomes		
4. Fed control	1.43 ± 0.20	0.75 ± 0.11	3
5. Fasted control	1.80 ± 0.13	0.10 ± 0.08	6
6. T3 treated	1.79 ± 0.12	5.02 ± 0.33	8

* The effect of T3 is highly significant ($p < 0.005$), except in the monoacyl-glycerol-3-P formation where values of $p < 0.025$ (1 vs. 3) and $0.2 < p < 0.5$ (4 vs. 6 and 5 vs. 6) were obtained.

starved for 3–6 days. These two types of controls were prepared because hyperthyroid animals lost weight despite their consumption of more than their usual daily ration. Thus, the fed controls gained 3.9 ± 1.2% (mean ± SEM) of their initial body weight during a period of 6–10 days, whereas the starved controls lost 14.0 ± 0.9% and the triiodothyronine-treated animals, 27.4 ± 2.0% of their initial weight during the period specified above.

The heart rates were recorded by an electrocardiograph. This was performed under anesthesia with an intraperitoneal injection of 2.5 ml of 2% chloralose in 20% urethane per kg body weight. The heart rate of fed and fasted controls as well as triiodothyronine-treated animals before start of injection was 256 ± 9 beats/min. This was increased after the injections to 417 ± 11 beats/min, representing a 162% increase.

2.3. *Subcellular fractionation*

This and following procedures have been described elsewhere in greater detail [11]. Heart tissues were excised from anesthetized rabbits and were homogenized with 0.25 M sucrose containing 0.02 M Tris–HCl buffer, pH 7.4. The homogenate was centrifuged at 800 *g* for 15 min, followed by further centrifugation of its supernatant at 10 000 *g* for 15 min. The resulting supernatant was then spun at 100 000 *g* for 60 min and the precipitate used as a microsomal fraction. The precipitate from 10 000 *g* centrifugation was resuspended and centrifuged at 8000 *g* for 15 min, yielding a mitochondrial pellet. The protein content was determined by the method of Lowry et al. [12].

2.4. *Enzyme assay*

The reaction mixture contained, in a final volume of 2.0 ml, 2.0 μmoles potassium palmitate, 0.8 μmole CoA, 12.0 μmoles ATP, 6.0 μmoles $MgCl_2$, 6.0 μmoles [U-^{14}C]glycerophosphate with a specific activity of approximately 360 000 dpm/μmole, 20 mg bovine serum albumin and 100 μmoles Tris–phosphate buffer, pH 7.4. The reaction was started with the addition of enzyme preparations and the incubation was carried out at 37°C in room air for either 5 min (microsomes) or 10 min (mitochondria). The reaction was terminated and lipids extracted by butanol [11]. An aliquot of butanol extract was dissolved in Bray's [13] solution for the counting of radioactivity.

The remainder of the extract was dried under N_2 gas, dissolved in chloroform–methanol (1:1, v/v) and chromatographed on t.l.c. plates. The two major reaction products, monoacyl- and diacyl-glycerophosphate, were separated by developing the plates with chloroform–methanol–acetic acid–water (65:25:8:4, by vol) [11]. The bands were visualized by iodine vapour and compared with authentic standards; the appropriate bands were subsequently scraped off and suspended

in Aquasol (New England Nuclear Corp.) for radioactivity determination. Quenching was corrected by the channels ratio method.

3. Results and discussion

The biosynthesis of diacyl-glycerophosphate by the mitochondrial and microsomal fractions was increased about four-fold in the hearts of hyperthyroid rabbits (table 1). In addition, mitochondrial monoacyl-glycerophosphate formation was also augmented two-fold, whereas microsomal biosynthesis was unchanged. The same conclusion was drawn regardless of whether the fed or fasted controls were used in comparison with the results obtained from hyperthyroid animals (table 1). These data suggest that the activity of glycerophosphate acyltransferase (acyl-CoA:sn-glycerol-3-phosphate-*O*-acyltransferase, EC 2.3.1.15) and acyl-CoA:monoacylglycerol-3-phosphate acyltransferase are under hormonal control and that these increases in activities are the possible cause of the accumulation of TG in the hyperthyroid heart. Although triiodothyronine has been known to cause an increase in protein synthesis as well as in some enzyme activities [1,9,14], there has been no report dealing with FA esterifying systems, in spite of the fact that the glycerophosphate acyltransferase has been postulated to be the rate-determining step in the biosynthesis of complex lipids [15]. Earlier, we observed an increased myocardial TG content following ethanol administration [16], which may be explained by the depressed FA oxidation under the condition of the unchanged FA uptake by the heart. A similar mechanism has been proposed for hypoxic hearts [2].

Since the plasma FFA is known to increase in the hyperthyroid state [2], and this, in turn, could cause changes in enzyme activity, we examined the formation of mono- and diacyl-glycerophosphate in starved and fed euthyroid rabbits. The acylation reaction in starved rabbits, in which plasma FFA concentration is higher than in fed animals [2], is similar to the reaction observed in fed rabbits (table 1).

Our findings that increases in mono- and diacyl-glycerophosphate were of different magnitudes indicate two underlying mechanisms: i) the first and second acylation are catalyzed by the two different enzymes in the heart, as shown in the liver [15,17–20], and ii) the hormone does not act on these enzymes indiscriminately, i.e. the microsomal acyl-CoA:monoacyl-glycerophosphate acyltransferase activity was stimulated to a greater extent than glycerophosphate acyltransferase activity. That the mitochondrial enzyme possesses characteristics different from those of the microsomal enzyme in euthyroid rabbit hearts has been reported elsewhere [11].

Although diacyl-glycerophosphate serves as the substrate for further reactions leading to the formation of glycero- and phospholipids [15,20], it is unlikely that an increased rate of diacyl-glycerophosphate formation is solely a result of inhibition of phosphatidate phosphohydrolase (EC 3.1.3.4) or phospholipid biosynthesis. On the contrary, an activity of phosphohydrolase was increased after subtotal hepatectomy [21], in which rapid TG accumulation was observed in the liver. Whether or not the latter enzyme, rather than glycerophosphate acyltransferase, could be another rate-controlling stage of the cardiac TG synthesis is as yet to be investigated.

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