STIMULATION OF MITOCHONDRIAL RNA SYNTHESIS BY THYROID HORMONE

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1. Introduction

The fundamental experiments performed by Tata and his coworkers [1–3] have shown that the action of thyroid hormones is initiated by induction processes occurring in the cell nucleus. These changes at the transcriptional level are followed by effects at the translational level and an increased capacity of ribosomes for protein synthesis [4].

Since the increase in the metabolic rate is one of the most striking effects of thyroid hormones on mammals, changes of mitochondrial structure and function caused by thyroid hormones have for a long time attracted the interest of biochemists [5–7]. In the course of studies within the last decade it has been elucidated that neither uncoupling of oxidative phosphorylation nor swelling of mitochondria represent the mode of action of thyroid hormones.

The discovery of reactions leading to nucleic acid and protein syntheses within mitochondria has raised the question whether mitochondrial DNA and mitochondrial nucleic acid and protein syntheses can constitute a target for the action of physiological and pharmacological agents. Roodyn and coworkers [8, 9] have shown an increased rate of protein synthesis in isolated liver mitochondria following the injection of triiodothyronine.

In this paper we present the results of studies on the effects of thyroidectomy and subsequent treatment with triiodothyronine on mitochondrial RNA polymerase.

2. Materials and methods

2.1. *Treatment of experimental animals*

76 Male Wistar rats (300–350 g) were thyroidectomized surgically. An injection of radioactive iodine (Na ^{131}I, 1.5 mCi/kg) followed 2–3 weeks after surgery. The animals were fed Altromin-R *ad libitum*. Studies on RNA polymerase were performed 3–4 months after surgery. Animals of the same age and strain were used as controls. 3,3′,5-Triiodo-L-thyronine-Na was injected subcutaneously at a dose of 250 μg/kg at various times prior to cell fractionation.

2.2. *Preparation of cell fractions*

The animals were killed by decapitation, the livers quickly removed and four livers pooled for one preparation. After homogenizing in 0.32 M sucrose solution + 0.5 mM EDTA, nuclei were removed by centrifugation at 1000 *g* (10 min). The pellet was suspended in 0.32 M sucrose solution + 3 mM Mg^{2+} and the nuclear fractions were isolated using high-molar sucrose according to the method of Widnell and Tata [10] in the modification of Bass [11].

From the supernatant of the first centrifugation, *mitochondria* were isolated by differential centrifugation. The mitochondrial pellet was washed 3 times using 0.32 M sucrose solution + 0.5 mM EDTA. All preparations were performed at 0–4°.

A highly purified mitochondrial fraction was obtained by subsequent gradient flotation [12]. Our mitochondrial preparations contain less than 3% nuclear DNA in the mitochondrial DNA.

2.3. *Assay of RNA polymerases*

For measuring RNA polymerase, pretreated mitochondria must be used: mitochondria were suspended in 0.32 M sucrose solution and half the volume of 0.07 M phosphate buffer at pH was added. The suspension was incubated under aerobic conditions at 25° for 10 min, followed by fast cooling in ice-water The mitochondria, showing an intracristal swelling [13], were centrifuged at 16,000 *g* (10 min) and suspended in 0.32 M sucrose solution + 0.5 mM EDTA. From each cell preparation polymerase assays were run in triplicate besides two test samples terminated at 0 time. The deviation of such triplicate assays did not exceed ± 7%.

RNA polymerase reactions were measured using the following test systems (1 ml):

1) nuclei (+ Mg^{2+}, pH 8.5 – the product mainly being rRNA); 100 mM tris-HCl, pH 8.5; 0.25 mM EDTA; 6 mM NaF; 6 mM Mg^{2+}; 1 mg bovine albumin (BSA); 0.5 mM each of ATP, GTP, CTP, ^{3}H-UTP 14.8 μCi (0.125 mM); 0.2 ml nuclear suspensions (200–400 μg DNA).

2) nuclei (+ Mn^{2+}, pH 7.5, high-ionic strength – the product being "DNA-like" RNA); 100 mM tris-HCl, pH 7.5; 3 mM Mn^{2+}; 0.25 mM EDTA; 1 mg BSA; 0.1 ml at 25° saturated $(NH_4)_2SO_4$ solution; 0.5 mM each of ATP, GTP, CTP; ^{3}H-UTP 14.8 μCi (0.125 mM); 0.2 ml nuclear suspension (200–400 μg DNA).

3) Mitochondria: 100 mM tris-HCl, pH 8; 0.25 mM EDTA; 6 mM Mg^{2+}; 50 mM KCl; 1 mg BSA; 0.5 mM each of ATP, GTP, CTP; ^{3}H-UTP 14.8 μCi (0.04 mM) and 0.2 ml (pretreated) mitochondrial suspension (4 mg protein).

Incubation at 37°, nuclei for 3 min mitochondria for 5 min.

Incorporation of radioactivity was measured after precipitating acid insoluble material and washing with ice-cold 2% perchloric acid, extraction of lipids (3 times with alcohol-ether (3:1), followed by ether) and nucleic acid hydrolysis (0.5 N perchloric acid, 15 min, 75°). DNA was measured using the method of Burton [14]. Protein was estimated by the Biuret method. Radioactivity was measured using a TriCarb 3380 liquid scintillation system with external standardization.

3. Results

With both nuclei and mitochondria, DNA was used as reference for enzyme activity because of the high variability of RNA content. Thus a comparison of the nuclear and the mitochondrial processes is possible. But since the DNA content of mitochondria is rather variable, for comparative pharmacological studies data based on mitochondrial protein content are more reliable. Therefore, data based on this reference are also given in this paper.

The mitochondrial fractions isolated contained the following amounts of DNA (μg/ml protein; mean ± S.D.): control, 0.21 ± 0.06; thyroidectomized, 0.27 ± 0.09; triiodothyronine, 0.26 ± 0.1.

3.1. *RNA polymerases in thyroidectomized rats*

Table 1 shows that nuclear RNA polymerases decrease following thyroidectomy. These results are in complete agreement with the data of Tata and Widnell [1].

There is also a decrease when RNA polymerase is studied in mitochondria isolated from livers of thyroidectomized animals. This decrease is even more pronounced than that observed with the RNA polymerases of cell nuclei.

In contrast to nuclear preparations, mitochondrial RNA polymerase is completely resistant to RNAase and actinomycin before the pretreatment with inorganic phosphate solution. Thus nuclear polymerases do not contribute to the mitochondrial assay to an appreciable extent. The specificity of mitochondrial RNA polymerase has been published before in detail [13, 16–18].

3.2. *Effect of triiodothyronine on RNA polymerases of thyroidectomized rats*

An increase in the activity of nuclear RNA polymerase – measured in the presence of Mg^{2+} and at low-ionic strength – which is mainly responsible for the formation of ribosomal RNA, can be observed in the liver of thyroidectomized animals 24 hr or later following the injection of 250 μg/kg triiodothyronine [1, 2]. On the other hand, the activity of the RNA polymerase measured in the presence of Mn^{2+} and at high-ionic strength is found to increase only after an even longer lag period. This polymerase mainly forms RNA with a "DNA-like" composition. Our data have completely confirmed these findings obtained with isolated nuclei (table 1).

Table 1
Activity of RNA polymerases in isolated liver nuclei and mitochondria of hypothyroid rats and after injection of 3,3′,5-triiodothyronine into thyroidectomized animals.

Cell fraction	Conditions	Reference	UMP incorporated (moles × 10^{15}/min/μg DNA or mg protein)				
			Control	Thyroidectomy	+ Triiodothyronine		
					9 hr	18 hr	36 hr
Nuclei	Mg^{2+}, pH 8.5	per μg DNA	100.0% (10) 131 ± 15[c]	68.3% (9) 89 ± 23	73.8% (3) 97 ± 24	62.2% (3) 81 ± 22	129.9% (4) 170 ± 32[c]
Nuclei	Mn^{2+}, pH 7.5	per μg DNA	100.0% (11) 327 ± 44[c]	72.6% (9) 237 ± 52	78.3% (3) 256 ± 52	69.2% (3) 226 ± 53	110.7% (4) 362 ± 76[b]
Mitochondria	Mg^{2+}, pH 8	per μg DNA	100.0% (10) 497 ± 112[c]	46.3% (8) 230 ± 59	39.3% (3) 159 ± 67	73.6% (3) 365 ± 69[b]	105.9% (4) 526 ± 106[c]
		per mg protein	100.0% (10) 102 ± 14[c]	52.0% (8) 53 ± 11	41.9% (3) 43 ± 7	99.6% (3) 101 ± 19[c]	127.7% (4) 130 ± 23[c]

The figures in parenthesis give the number of preparations tested. Each preparation is equivalent to four livers.
[a] $p < 0.05$; [b] $p < 0.015$; [c] $p < 0.0027$. For calculating the significance the rates were compared with the thyroidectomized group.

Subsequent to an injection of triiodothyronine the activity of the mitochondrial RNA polymerase also increases. Up to 9 hr after the injection no significant increase can be measured. But 18 hr after subcutaneous injection of the hormone control values are obtained. A further increase is found within the next 24 hr. The increase in mitochondrial RNA polymerase activity seems to precede the corresponding increase measured with the nuclear enzymes. The increase in mitochondrial UTP incorporation roughly corresponds with the increased incorporation of orotic acid into "rapidly labelled" nuclear RNA – which so far represents one of the first events observed in the liver after application of thyroid hormone [1, 3].

4. Discussion

It is interesting that Roodyn and coworkers [8, 9], when measuring the incorporation of radioactively labelled amino acids into protein, could not find a difference in the rate of incorporation between the mitochondrial and the microsomal fraction. There was no striking effect on the rate of amino acid incorporation up to 35 hr following the injection of the hormone. It is of further significance that no increase in the RNA content of mitochondria could be detected. This agrees well with our results which indicate that except for a significant increase in the rate of labelling of mitochondrial RNA, no net increase in the RNA content of these cell particles could be measured.

Assuming that important actions of thyroid hormones result from a synthesis of mitochondrial structures [15] involved in respiration and oxidative phosphorylation, our data could indicate that both an increased nuclear RNA synthesis and a stimulated mitochondrial RNA synthesis are necessary for the complete formation of mitochondrial membrane structures. In this respect it is interesting to note that a phase of increased nucleic acid synthesis in these particles precedes an increased mitochondrial protein synthesis. Data on mitochondrial DNA polymerase to be presented elsewhere show that there is *no* increase in the activity of DNA polymerase when measured during the same time interval.

Although nothing is known so far of the capacity of RNA synthesized in mitochondria to act as a messenger in protein synthesis, some indications available today may point to such a function [16, 17].

From the data available it may be excluded that mitochondria contain the information for synthesizing

the major part of the mitochondrial enzymes. The synthesis of a few key components, however, would suffice to regulate the synthesis of mitochondrial membrane structure at the cytoplasmic level.

The stimulation of mitochondrial RNA polymerase by thyroid hormone gives us the possibility to further investigate the correlation between the genetic information given by the nucleus and that contributed by the mitochondria themselves in the regulation of mitochondrial biogenesis. If mitochondrial DNA is "naked", as most investigators have suggested, a direct action of the hormone on this DNA should involve an "induction" mechanism different from that believed to occur in the cell nucleus. But in previous studies a small amount of "repressor-like" material on mitochondrial DNA could not be excluded.

It has been shown [13, 16] that mitochondrial RNA polymerase *in vivo* is insensitive to actinomycin D – due to the impermeability of the mitochondrial membrane to this inhibitor – so it will be interesting to study the effect of actinomycin and other inhibitors on the increase of RNA polymerase seen after the application of thyroid hormones.

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