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Acetyl-L-carnitine increases cytochrome oxidase subunit I mRNA content in hypothyroid rat liver

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The effect of acetyl-L-carnitine on the quantity of the messenger RNA for the subunit I of cytochrome oxidase in the liver mitochondria of hypothyroid rat was measured by Northern blot and solution hybridization. Three hours after pre-treatment of hypothyroid rat with acetyl-L-carnitine, the level of the transcript increased strongly. This effect was also obtained when acetyl-L-carnitine was administered to T_3 pre-treated hypothyroid rats. These results add further evidence to the suggestion that acetyl-L-carnitine is able to stimulate mitochondrial transcription under altered metabolic conditions.

Mitochondrial DNA transcription; Hypothyroid rat liver; Acetyl-L-carnitine

1. INTRODUCTION

Recent experiments in our laboratory [1] have shown that the steady-state concentration of the mRNA for the subunit I of cytochrome oxidase (CoI mRNA) undergoes an age-dependent decrease in the rat brain and heart. The reduction was reversed by an in vivo pre-treatment with acetyl-L-carnitine. The fact that in adult rats a similar treatment did not produce any significant variation in the mtRNA level supported the hypothesis that acetyl-L-carnitine acts by removing some age-induced alterations of mitochondrial metabolism which affect mtDNA expression. Here, we investigated the effect of acetyl-L-carnitine on the steady-state level of CoI mRNA in hypothyroidism, a pathological condition where, as well as in aging, impairment of energetic metabolism and reduction of the mitochondrial transcript level take place [2-9]. Pre-treatment of hypothyroid rats with acetyl-L-carnitine raised the liver CoI mRNA concentration to that of euthyroid individual. Moreover, acetyl-L-carnitine potentiates the effect of T_3 when both substances are administered in vivo.

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Abbreviations: mt, mitochondrial; CoI mRNA, messenger RNA for the subunit I of cytochrome oxidase; PTU, propylthiouracyl; T_3, 3,3'-5-triiodo-L-thyronine

2. MATERIALS AND METHODS

2.1. *Isolation of mitochondria and nucleic acid extraction*

Male Fisher 3-month-old rats, housed at a temperature of 22°C and fed ad libitum with a standard diet, were used for these studies. Rats were made hypothyroid by adding 0.05% (w/v) of propylthiouracyl (PTU) to drinking water for 20 days. T_3 treatment was performed by injecting intraperitoneally 30 μg/100 g body weight of 3,3',5-triiodo-L-thyronine in 0.9% NaCl/propylenglycol (40:60, v/v). The treatment was repeated after 24 h and the animals were killed 24 h after the second treatment. Acetyl-L-carnitine was administered by injecting into the rat 300 mg/kg body weight of acetyl-L-carnitine and killing the animals 3 h later. When T_3 and acetyl-L-carnitine treatments were combined, T_3 preceded acetyl-L-carnitine subministration. Rat liver mitochondria and mitochondrial nucleic acids were isolated as previously reported [1]. DNA and RNA concentrations were determined by diphenylamine and orcinol methods, respectively. The content of mtDNA co-extracted with mtRNA was determined by a quantitative hybridization procedure where the DNA from an aliquot of total mt nucleic acids was digested with the restriction enzyme *Bam*HI, run on 1% agarose gel and blot-hydridized to a nick-translated fragment of rat mtDNA. Hybridized radioactivity was compared with that measured in a series of standard hybridizations involving known quantities of purified mtDNA. Details of this procedure are described in [1].

2.2. *Hybridization*

An mtDNA restriction fragment containing part of the CoI gene was cloned in the vector Bluescribe (Stratagene) and labelled with T7 RNA polymerase as previously described [1]. The probe was a fragment of 855 bp derived from the digestion of *Eco*RI C with *Tag*I [10]. Solution hybridization and RNase treatment were carried out as previously reported [1]. The amount of hybrid was determined by the difference between the amount of thrichloroacetic-acid-insoluble radioactivity of the samples and that of the controls. The latter contained the same components of the hybridization mixture except the mtRNA. In all experiments, at least two different amounts of total mtRNA (10-20 ng) were used; each of them was hybridized to increasing quantities of radioactive RNA probe. Each experimental point

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was determined in triplicate. At saturation, the RNase-resistant radioactivity was about 5% of the input in the samples and 1% of the input in the controls. The statistical significance of differences in RNA concentration between treated and untreated animals was determined by Student's t-test. Northern blot hybridization was carried out as reported elsewhere [1].

3. RESULTS

The measurement of the steady-state level of rat liver CoI mRNA was carried out by a quantitative solution hybridization assay in which total mtRNA was hybridized to a CoI riboprobe. The mass of RNA hybridized at saturation/mass of total mtRNA was normalized with respect to the mtDNA co-extracted with 1 μg of total mtRNA. Table I reports the amount of RNA hybridized, expressed as the number of CoI mRNA molecules per mtDNA molecule in the animals after various treatments. It can be seen that hypothyroidism causes a decrease of about 50% in the level of CoI mRNA. A 3 h pre-treatment of hypothyroid rat with acetyl-L-carnitine is able to raise the level of CoI mRNA almost to the level of euthyroid rat. As already reported [8], a 48 h pre-treatment of hypothyroid rats with T_3 is able to bring back the concentration of CoI mRNA to that of control rat. When acetyl-L-carnitine was administered to T_3 treated rat it caused almost a doubling of CoI mRNA content. These observations were confirmed also at cellular level: the same amount (about 15 μg) of total rat liver RNA was run on a 1.4% methylmercuric agarose gel, transferred to a nitrocellulose filter and blot-hybridized to a CoI riboprobe. Fig. 1 shows that hypothyroid rats treated with acetyl-L-carnitine have a CoI mRNA content similar to that of T_3 treated rats and that the effect of the two substances is additive.

Table I

Effect of various treatments on the level of CoI mRNA in the liver of hypothyroid rats.

Animals	Number of molecules of CoI mRNA/mtDNA molecule
Control	3.40 ± 0.31
$-T_3$	1.94 ± 0.20
$+T_3$	3.49 ± 0.44
+ A	2.88 ± 0.20
$+T_3$ + A	6.12 ± 0.35

The number of molecules of mtRNA/mtDNA was calculated according to the formula $N=A$ (K/L) where A is the mass (pg) of RNA hybridized at saturation with the probe/mass (μg) of total mtRNA. K is constant for each mtRNA preparation and comes from the ratio of 29.4/mass (ng) of mtDNA coextracted with 1 μg of total mtRNA (the factor 29.4 contains constant factors such as the molecular weight of the nucleotides and the Avogadro number). L is the length of the probe in nucleotides. The amount of the mtDNA co-extracted with total mt nucleic acids was determined as described in section 2 by at least 3 independent measurements for each individual. The values reported in the Table are the mean ± SD of at least 5 hybridization experiments repeated each time on 3 mtRNA preparations. One individual was used for each RNA preparation. No appreciable statistical difference in the hybridization data on the single individuals of either group tested was observed. P was always less than 2×10^{5}, except for the comparison between T_3 and acetyl-L-carnitine treated rats where it was less than 10^{-2}. A, acetyl-L-carnitine.

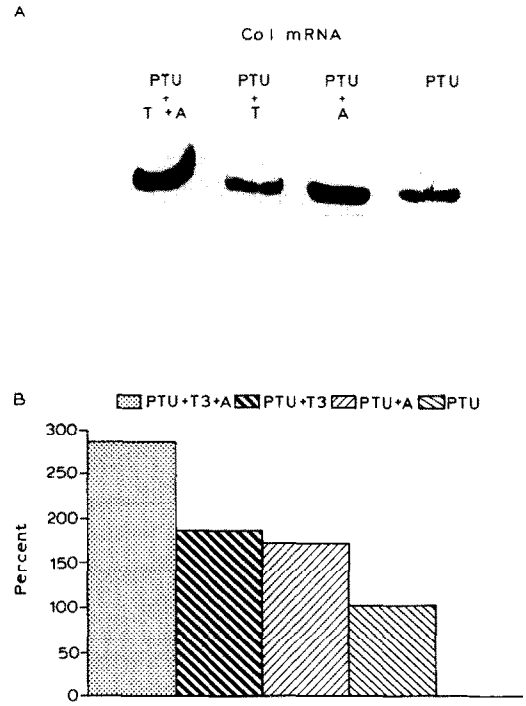

Fig. 1. Content of CoI mRNA in the total RNA population from treated and untreated hypothyroid rat. (A) Northern blot hybridization of total RNA isolated from treated and untreated hypothyroid rat. The same amount (15 μg) of total RNA from treated and untreated hypothyroid rat was run on a 1.4% methylmercuric agarose gel, transferred to a nitrocellulose filter and hybridized to a labelled CoI riboprobe. RNA transfer and hybridization were carried out as reported by Gadaleta et al. [1]. Total cellular RNA was extracted from the post-nuclear supernatant by repeated phenol/chloroform extractions. (B) Densitometric estimate of the hybridization data. The intensity of the bands, measured by densitometry (LKB-Ultroscan XL), was referred to the same number of liver cells. Cell number was inferred by measuring the total DNA concentration of homogenate. Peak areas were then multiplied for the following normalization factors, 1.53, 1.66, 1.05, 1.00 for PTU + T_3 + A, PTU + T_3, PTU + A and PTU samples, respectively. The results are expressed as percentage of increase over hypothyroid rat, whose value was set at 100.

4. DISCUSSION

Acetyl-L-carnitine acts by stimulating directly or indirectly the energetic metabolism [11,12]. Recently we found that acetyl-L-carnitine affects also mtRNA metabolism [1]: pre-treatment of senescent rats with acetyl-L-carnitine raised the level of CoI mRNA in the brain and heart up to that of adult individual. The data reported in this paper show that a 3 h pre-treatment with acetyl-L-carnitine is able to increase the steady-state level of CoI mRNA of hypothyroid rat liver to that of euthyroid and that the effect of acetyl-L-

carnitine on T_3-treated hypothyroid individuals is additive to that of T_3. These results could be extended to all the mtDNA coded RNAs, since it has been shown that in rat liver the concentrations of most of the mt mRNAs are similar [8] and that they are equally affected by T_3 treatment [9]. The results here reported are relevant firstly because they increase the range of altered metabolic conditions in which acetyl-L-carnitine is effective on mt transcription and secondly because they extend the evidence about the relationship between the steady-state level of mtRNAs and the cell metabolism [8–10, 13–22]. The real mechanism of the acetyl-L-carnitine action on the mtDNA transcription both in senescence and in hypothyroidism remains to be understood. However, it could be hypothesized that in both cases acetyl-L-carnitine might be able to re-establish the correct structure and function of mitochondrial membranes which appear to be altered in these two conditions [23–29]. Some recent findings in our laboratory confirm this hypothesis: acetyl-L-carnitine treatment normalizes age-induced alterations in the lipid composition of rat plasma [30] and of brain and heart rat mitochondria (Ruggiero and Gadaleta, in preparation). Mitochondrial membrane permeability could be relevant for mtDNA transcription influencing aspects such as optimal mitochondrial concentration of ATP and/or monovalent and divalent cations [31], import into mitochondria of RNA polymerase and of other nuclear DNA encoded proteins required for mtRNA synthesis [32] and processing [33].

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