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Thyroid hormone-induced expression of the ADP/ATP carrier and its effect on fatty acid-induced uncoupling of oxidative phosphorylation

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Abstract Liver mitochondria from rats made hypothyroid by administration of 2-mercapto-1-methylimidazole were less sensitive to the uncoupling effect of myristic acid, as measured by the increase of resting state respiration, than mitochondria from euthyroid animals, whereas subsequent administration to the animals of triiodothyronine ('hyperthyroidism') resulted in an increased uncoupling action of myristate. 'Hyperthyroidism' also resulted in doubling of the carboxyatractyloside-sensitive portion of the myristate-stimulated respiration. Parallel to this was a twofold increase of the mitochondrial content of the ADP/ATP carrier protein and an over threefold increase of its activity. The uncoupling effect of phytanic acid was less sensitive to carboxyatractyloside and was increased in the hyperthyroid state to a smaller extent than in the case of myristate. These results provide further support to the thesis [Skulachev, V.P., FEBS Lett. 294 (1991) 158–162] that the ADP/ATP carrier is involved in the mechanism of the uncoupling effect of long-chain fatty acids.

Key words: ADP/ATP carrier; Fatty acid; Uncoupling; Thyroid hormone; Respiration; Mitochondria

1. Introduction

Administration of thyroid hormones to rats markedly increased oxygen consumption of liver, heart and kidney tissue (for review see [1,2]). This stimulation of the cellular respiratory capacity is attributed to a thyroid hormone-induced expression of several nuclear-encoded genes, mainly those of the mitochondrial energy coupling system. Thus, increased tissue levels of mRNA-encoding proteins, such as cytochrome c/c_1, glycerol 3-phosphate dehydrogenase, ADP/ATP carrier, have been found in rat after treatment with T3 [1,2]. These findings explain why the control pattern of certain proteins on mitochondrial respiration changes with alteration of the thyroid hormone status [3].

AAC exists in several isoforms in mammals [4,5]. In rat two isoforms are expressed in a tissue-specific manner, the muscle-specific AAC1 form and the ubiquitous AAC2 form [6,7]. In rat liver, AAC2 is the main isoform and only its mRNA tissue level responds to T3 [7,8]. The mRNA level of AAC2 increased manyfold in rat liver tissue in a time scale of days when hypothyroid rats were treated with T3 [7,8]. AAC is a protein of particular interest in the understanding of the mitochondrial energy coupling process because (i) it exerts high control on mitochondrial respiration [9,10], (ii) it is involved in the mitochondrial permeability transition [11] and (iii) it mediates uncoupling of oxidative phosphorylation by non-esterified long-chain fatty acids (for review see [12,13]). Participation of AAC in FFA-linked uncoupling is clearly seen from the observation that ligands of AAC (e.g. carboxyatractyloside, atractyloside and acyl-CoA derivatives) depress FFA-stimulated resting state respiration [14]. In addition, the AAC-mediated uncoupling effect of FFA increases with the mitochondrial AAC content [15] and, finally, photoaffinity immobilisation of FFA on AAC makes mitochondria less sensitive to the uncoupling by FFA [16]. It is now widely believed that FFAs act as protonophoric uncouplers and that AAC mediates the transmembrane movement of the fatty acid anion across the inner mitochondrial membrane ([12,13], but see also [17]). However, to date it is not known how the thyroid hormone status modulates the uncoupling activity of FFA. In the present report we show that, in rat liver mitochondria, T3 affects strongly the FFA-stimulated (uncoupled) respiration and that the T3-induced increase in the AAC protein content accounts quantitatively for the AAC-sensitive uncoupled respiration.

2. Materials and methods

2.1. Animals and isolation of mitochondria

The thyroid hormone status of adult female Wistar rats (150–180 g) was manipulated either by supply of 2-mercapto-1-methylimidazole (methimazole; an inhibitor of thyroid hormone synthesis) in drinking water (0.04%) for 15 days (hypothyroidism) or by intraperitoneal injection of T3 (20 μg/100 g body weight per day) administered over 3 days after methimazole treatment (hyperthyroidism). The thyroid hormone status was verified by measuring T3 and T4 levels by radio-immunoassay of sera collected at the time of killing the animals. Mitochondria from liver and heart were isolated essentially as described in [18] and [16], respectively. The prepared mitochondria had routinely a respiratory control ratio greater than 8 with glutamate plus malate as respiratory substrates. Protein content in mitochondrial stock suspensions was determined by a modified biuret method.

2.2. Measurement of respiration

The medium was composed of 110 mM mannitol, 60 mM KCl, 60 mM Tris-HCl, 10 mM potassium phosphate and 0.5 mM Na_2EDTA, pH 7.4. Oxygen uptake was measured polarographically using a Clark-type electrode in a thermostatted chamber maintained at 25°C using 3 mg mitochondrial protein suspended in 2.0 ml of the respiration medium. For calculation of respiration rates, oxygen concentration of 230 nmol O_2/ml was adopted. Partly uncoupled states were adjusted by addition of micromolar concentrations of 5 mM ethanolic solutions of myristate or phytanate.

2.3. Content and activity of the ADP/ATP carrier, (ATP+ADP) pool size

The content of AAC protein in samples of mitochondria was esti-

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Abbreviations: AAC, ADP/ATP carrier; ATR, atractyloside; CAT, carboxyatractyloside; FCCP, *p*-trifluoromethoxycarbonylcyanide phenylhydrazone; FFA, non-esterified fatty acid; T3, 3,3′,5-triiodo-L-thyronine; T4, L-thyroxine

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mated from the high affinity binding of [^{3}H]ATR to the AAC protein [18]. Liver mitochondria (1 mg protein) were incubated in 1.0 ml medium supplemented with 1 nmol [^{3}H]]ATR (specific activity, 220 dpm/pmol). Non-specific binding of [^{3}H]ATR to mitochondria was determined by incubation of samples with 100 μM unlabeled ATR prior to the addition of [^{3}H]ATR. The activity of AAC was measured at 4°C by the atractyloside-stop method of Pfaff and Klingenberg [19] as described in [16]. From the [^{3}H]ADP uptake determined during initial 10 s periods translocation activities were calculated and expressed as nmol ADP/min per mg protein. The size of the exchangeable pool of adenine nucleotides (ATP+ADP) was estimated from the uptake of [^{3}H]ADP after a 5 min incubation period.

2.4. Materials

Myristic acid (C14:0), phytanic (3,7,11,15-tetramethylhexadecanoic) acid, atractyloside, carboxyatractyloside, 2-mercapto-1-methylimidazole, 3,3′,5-triiodo-L-thyronine, FCCP, ADP, glutamate, malate and succinate were from Sigma (St. Louis, MO, USA). Trypsin (bovine pancreas) was from Serva (Heidelberg/New York). [^{3}H]ADP was obtained from NEN-DuPont (Boston, MA, USA). [^{3}H]Atractyloside was prepared and purified by Dr. S. Noll (Institute of Bioinorganic and Radiopharmaceutical Chemistry, Dresden-Rossendorf, Germany).

3. Results

3.1. Thyroid hormone status and mitochondrial respiration

The consequences of manipulation of the thyroid hormone status in rats can be clearly seen from serum levels of T3 and T4 and from mitochondrial respiration data (Table 1). In line with others (e.g. [3]), the respiration data show that switching from hypo- to hyperthyroidism increased twofold state 3 respiration and respiration of the fully FCCP-uncoupled state.

3.2. The effect of triiodothyronine on the activity and the content of the ADP/ATP carrier and the (ATP+ADP) pool size

The action of T3 on adenine nucleotide translocation of liver mitochondria from euthyroid, hypothyroid and hyperthyroid (hypothyroid+T3) rats can be seen from the data shown in Fig. 1. Our results provide confirmation of earlier observations [20] that mitochondria from hypo- and hyperthyroid rats differ markedly in their AAC activity. Thus the translocation activity of 'hyperthyroid' mitochondria (14.6 ± 2.4 nmol/mg protein/min) is almost fourfold that found in 'hypothyroid' mitochondria (4.10 ± 0.7 nmol/mg protein/min).

It has been reported that T3 can stimulate the activity of AAC in a way which cannot be attributed to an increased content of AAC [20]. Therefore, the observed increase in the AAC activity was compared with T3-induced expression of AAC in liver. For that, the content of AAC was measured

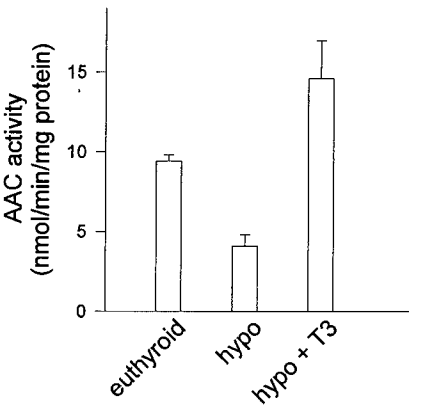

Fig. 1. Effect of the thyroid hormone status on the activity of the ADP/ATP carrier. The data represent mean values ± S.D. of five separate mitochondrial preparations obtained from each thyroid state.

using [^{3}H]ATR binding to the AAC protein. We found that T3 administration to hypothyroid rats increased the mitochondrial AAC content twofold, i.e. to the level measured in euthyroid animals (see Fig. 2). This increase was due to the enrichment of the mitochondrial AAC protein rather than to an increased content of mitochondria, since it was similar (on a protein basis) in the mitochondrial fraction and in the whole homogenate (Fig. 2). With heart mitochondria from hypo- and hyperthyroid rats, only a slight increase in the AAC protein content was found (from 232 ± 24 to 299 ± 65 pmol/mg protein, $n = 5$). This was parallelled by only a small increase of the myristate-induced respiration (not shown).

The content of exchangeable adenine nucleotides in liver mitochondria was also affected by the thyroid hormone status. This pool (ATP+ADP) decreased from 3.8 ± 0.6 nmol/mg protein ($n = 4$) in euthyroid animals to 2.6 ± 0.2 nmol/mg protein ($n = 5$) after methimazole treatment and was restored to the original level (4.1 nmol/mg protein ± 0.8 nmol/mg protein, $n = 4$) after administration of T3.

3.3. Protonophoric activity of fatty acids

The protonophoric effect of long-chain fatty acids on 'hypothyroid' and 'hyperthyroid' mitochondria was studied with myristic acid. Fig. 3 shows that myristate stimulates state 4 respiration in 'hyperthyroid' liver mitochondria much more than in 'hypothyroid' mitochondria. Moreover, the CAT-sensitive portion of this respiration was doubled, which fits well with the twofold increase of the mitochondrial AAC protein content.

Table 1
Effect of the thyroid hormone status on the respiration of rat liver mitochondria

		Thyroid status	Respiration (nmol O_2/min/mg protein)	
		Serum level (nmol/l)	State 3	Uncoupled
Euthyroid	T3	2.1 ± 0.6	47 ± 6	76 ± 10
	T4	189 ± 39	(100%)	(100%)
Hypothyroid	T3	1.4 ± 0.1	30 ± 4	52 ± 7
	T4	59 ± 9	(64%)	(68%)
Hypothyroid+T3	T3	14.1 ± 1.3	66 ± 5	111 ± 11
	T4	70 ± 6	(140%)	(146%)

Rat liver mitochondria (1.5 mg protein/ml) were incubated as described in Section 2 with medium. State 3 respiration was measured with 5 mM glutamate plus 5 mM malate as respiratory substrate. Uncoupled respiration was measured with 10 mM succinate plus 1 μM rotenone by titration of mitochondria with FCCP. The data are means ± S.D. of seven preparations.

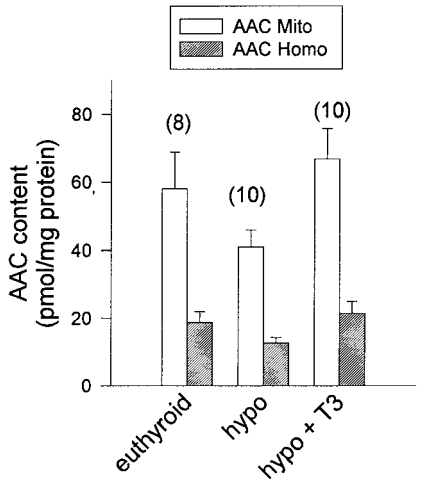

Fig. 2. Effect of the thyroid hormone status on the content of the ADP/ATP carrier. The data represent mean values ± S.D. obtained from each thyroid state. The numbers of separate preparations are given in parentheses.

The effect of T3 on myristate-uncoupled respiration was compared with that obtained with phytanic acid. This compound is easily formed in mammals from phytol, which is a natural constituent of milk products and vegetable fats. This fatty acid accumulates extensively in liver tissue with a defective peroxisomal α-oxidation for branched chain fatty acids (e.g. the Refsum syndrome) [21]. Fig. 4 shows that phytanic acid also exerted an uncoupling effect on liver mitochondria, though to a somewhat lower extent than myristic acid (compare Fig. 3). However, the CAT-sensitive portion of phytanic acid-stimulated respiration was considerably lower than that in the case of myristic acid (18 ± 5% as compared to 39 ± 4%). Moreover, the magnitude of CAT sensitivity was independent of the thyroid hormone status.

4. Discussion

Uncoupling of oxidative phosphorylation by fatty acids has been ascribed to their protonophoric action, comparable to that of 'classical' uncouplers (for review see [13]). However, because spontaneous transbilayer passage of the fatty acid anion is very slow [22], it has been proposed that mitochondrial carriers such as the ADP/ATP carrier [12,14], the glutamate carrier [23] and the dicarboxylate carrier [24] facilitate its movement from the inner to the outer leaflet of the inner mitochondrial membrane, whereas the movement of the protonated (undissociated) form moves in the opposite direction by unmediated flip-flop. The present results provide further strong support for this proposed role of AAC [12–17]. This is based on the following observations.

Firstly, T3 administration to hypothyroid rats resulted in a twofold increase in the CAT-sensitive myristate-induced mitochondrial respiration which was parallelled by a similar enrichment of the AAC protein. Increased expression of AAC by T3 was also reflected by the increase of the adenine nucleotide translocation activity. The discrepancy between the increase in the translocation activity (fourfold) and the enrichment of the AAC protein in mitochondria (twofold) can be partly attributed to a difference in the exchangeable (ATP+ADP) pool size of 'hypothyroid' and 'hyperthyroid' mitochondria and in addition might also result from a different cardiolipin content in the inner mitochondrial membrane [25,26], as it is known from experiments on AAC reconstitution into liposomes that cardiolipin is essential for restoration of AAC activity [27].

Secondly, FFA-stimulated respiration of heart mitochondria only slightly depended on the thyroid hormone status of the animals. This was parallelled by small differences in the content of AAC in these mitochondria. This, in turn, could be explained by the fact that in heart tissue the content of the AAC1 mRNA isoform is 2–5 times higher than that of the AAC2 mRNA isoform and that only the AAC2 gene responds to thyroid hormones [4,7,8].

Finally, uncoupling of liver mitochondria by phytanic acid was less stimulated by treatment of hypothyroid rats with T3 than the uncoupling by myristic acid, which is in line with the low sensitivity of the former respiration to CAT.

The results of the present study also show that the increase of myristate-induced respiration is only partly sensitive to CAT. This indicates that it is not entirely accounted for by an increased expression of the AAC protein. It can therefore be concluded that the thyroid hormone status also affects other constituents of the inner mitochondrial membrane which may be involved in the protonophoric action of fatty acids.

It has been reported [28,29] that hyperthyroidism-associated thermogenesis can result from a decrease in the H^+/O ratio ('slip') or, rather, an increased H^+ conductance of the inner mitochondrial membrane ('leak'). Recently, Skulachev and coworkers [30] have reported on seasonal variations in coupling efficiency of rat liver mitochondria and have related this phenomenon to the level of thyroid hormones. Moreover, it is known that the plasma concentration of FFA is elevated in hyperthyroid humans and animals and is diminished in hypothyroid subjects (for review see [31]). Our results indicate that

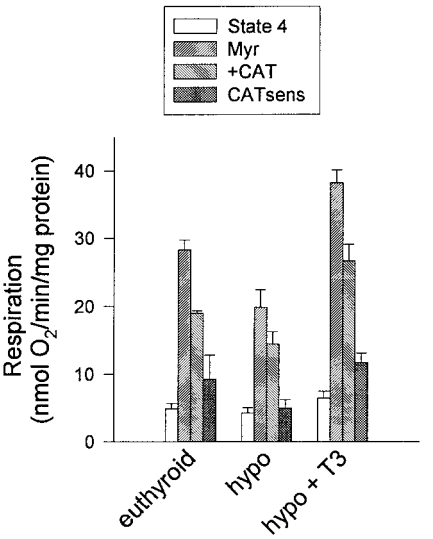

Fig. 3. Effect of the thyroid hormone status on myristate-stimulated resting state respiration in the presence and absence of carboxyatractyloside. The medium was supplemented with 5 mM glutamate plus 5 mM malate as substrates. Respiration was stimulated by the addition of 40 nmol myristate/mg protein. At stationary respiration carboxyatractyloside was added to a final concentration of 2 μM. The data are means ± S.D. of 7–9 preparations obtained from each thyroid state.

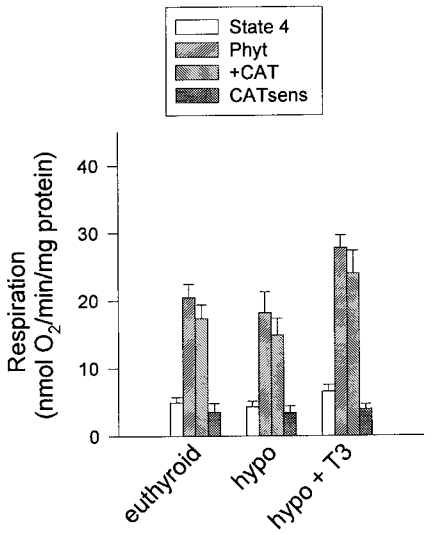

Fig. 4. Effect of the thyroid hormone status on phytanate-stimulated resting state respiration in the presence and absence of carboxyatractyloside. Experimental conditions were as described in the legend to Fig. 3. The data are means ± S.D. of 7–9 preparations obtained from each thyroid state.

elevated tissue concentration of non-esterified fatty acids may be responsible for increased thermogenesis in thyroid hormone-responsive tissues.

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