Induction of UCP_2 mRNA by thyroid hormones in rat heart

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Abstract The possible regulation of the expression of uncoupling protein-2 (UCP_2) mRNA by thyroid hormones in different tissues was examined in rats. Triiodothyronine (T_3) was found to produce an organ-specific enhancement of UCP_2 expression in rat tissues. The effect of T_3 was markedly observed in heart, whereas a moderate effect was seen in skeletal muscle and no effect in kidney or liver. These results suggest that UCP_2 is a protein that may be involved in the nuclear-mediated effect of T_3 on resting metabolic rate in the rat.

Key words: Uncoupling protein; Thyroid hormone; Heart; Skeletal muscle; Hypothyroidism

1. Introduction

The thyroid hormones exert a wide variety of biological actions. It is well known that they induce metamorphosis in amphibia and play a fundamental role in regulating mammalian development and energy metabolism. Administration of T_3 to hypothyroid rats leads to a significant increase in the metabolic rate of the whole animal after 36–72 h. At the mitochondrial level a stimulation of respiration can be observed as a result of an increased activity in the respiratory chain and an increase in oxidative phosphorylation [1].

The effects of thyroid hormones on mitochondrial activity may be divided into 'short-term' and 'long-term' effects. The 'short-term' effects are probably mediated by a direct interaction of diiodothyronines with mitochondria and possibly with non-catalytic subunits of the cytochrome oxidase complex as previously hypothesized by us [2,3]. The 'long-term' effects are mediated by T_3 and involve the nuclear pathway (for review see [4]). The early studies of Tata and Widnell [5] resulted in the first proposal that thyroid hormones exert their actions primarily by a stimulation of the transcription of genetic information at the nuclear level. The exact molecular mechanisms underlying these effects are poorly understood, although it seems that some of them can be attributed, at least in part, to the induction of nuclear encoded mitochondrial proteins such as cytochrome *c* [6], cytochrome c_1 [7], adenine nucleotide translocase and glycerol 3-phosphate dehydrogenase [8]. Recently, however, the discovery that, besides the uncoupling protein present in brown adipose tissue (termed UCP_1), two other uncoupling proteins (termed UCP_2 and UCP_3) are expressed in several tissues in humans and rats [9–12] has opened new, interesting perspectives in research on the regulation of energy metabolism. Uncoupling proteins produce heat by generating a pathway that allows dissipation of the proton electrochemical gradient across the inner mitochondrial membrane without a coupling to any other energy-consuming process. In mice, UCP_2 is present in metabolically very active tissues such as heart, kidney, skeletal muscle and liver (the last two at a lower level) and it has been suggested that UCP_2 plays a role in energy balance [9].

It has been shown that mitochondria from numerous tissues are 'imperfectly coupled' as a consequence of a proton leak that could account for as much as 15–33% of cellular energy expenditure; thyroid hormone seems to influence this mitochondrial proton leak (for review see [13]). It is conceivable that thyroid hormones might do this by regulating the expression of uncoupling proteins. Consequently, we thought it might be interesting to investigate in the rat the role played by thyroid hormones (T_4 and T_3) in the regulation of the expression of UCP_2 in metabolically active tissues such as liver, heart, skeletal muscle and kidney. In order to discriminate between the effects of T_4 and T_3, these hormones were injected separately into both normal euthyroid rats and rats in which hypothyroidism was induced by administration of propylthiouracil (PTU) and iopanoic acid (IOP). This combined treatment produces animals with severe hypothyroidism and with a strong inhibition of all three known types of deiodinase enzymes.

2. Materials and methods

2.1. Rat treatments and tissue samples

Male Wistar rats (220–230 g) living in a temperature-controlled room at 28°C were kept, one per cage, under an artificial lighting regime of 12 h light:12 h darkness. A commercial mash was available ad libitum and the animals had free access to water. Hypothyroidism was produced in rats by a daily i.p. administration of PTU (1 mg/100 g body weight) for 3 weeks together with a weekly i.p. administration of IOP (6 mg/100 g body weight). Euthyroid controls were sham injected with saline.

Chronic hyperthyroidism was induced in euthyroid and hypothyroid rats by seven daily i.p. injections of either 30 μg T_4/100 g body weight or 15 μg T_3/100 g body weight; control rats (euthyroid and hypothyroid) received saline injections. The iodothyronine doses and the treatment duration were chosen to obtain a change in the levels of the thyroid hormones without significantly changing the body weight of the animals. At the end of the treatments (24 h after the last dose of

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Abbreviations: UCP1, uncoupling protein 1; UCP2, uncoupling protein 2; UCP 3, uncoupling protein 3; T_3, triiodothyronine; T_4, thyronine; BAT, brown adipose tissue; PTU, propylthiouracil; IOP, iopanoic acid; N, euthyroid control rats; P+I, rats made hypothyroid by a combined treatment with PTU and IOP for 3 weeks; ID-I, type I deiodinase activity; ID-II, type II deiodinase activity; ID-III, type III deiodinase activity

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T_4 or T_3), rats were anesthetized by i.p. administration of chloral hydrate (40 mg/100 g body weight) and killed by decapitation. Trunk blood was collected and serum was isolated and stored at −20°C until hormone levels were to be analyzed.

The liver, heart, skeletal muscles (hind leg muscles), kidneys, brown adipose tissue and brain were isolated from each rat, immediately frozen in liquid nitrogen, then stored at −80°C until further processing. All experiments were performed in accordance with local and national guidelines covering animal experiments.

2.2. Deiodinase assays and determination of hormone concentrations

Type I deiodinase activity (ID-I) was determined in the liver microsomal fraction by analysis of the production of radioiodide from outer-ring-labeled rT_3. To this end, 0.1 μM rT_3 and ≈100 000 cpm [3′,5′-^{125}I]rT_3 were incubated for 30 min at 37°C with 2 μg microsomal protein in 200 μl of 0.2 M phosphate buffer (pH 7.2), 4 mM EDTA and 5 mM dithiothreitol (DTT), using the method described by others [14].

Type II deiodinase activity (ID-II) was determined in BAT according to the method of Leonard et al. [15]. This involved measuring the release of radioiodide from [3′,5′-^{125}I]rT_3 following the incubation of 20 μg BAT infranatant proteins for 60 min at 37°C with 2 nM rT_3 and ≈100 000 cpm [3′,5′-^{125}I]rT_3 in 200 μl of 0.1 M phosphate buffer (pH 7.2), 2 mM EDTA and 20 mM DTT. In both the type I and type II deiodinase assays, the reactions were stopped by the addition of 100 μl 5% bovine serum albumin at 0°C. Protein-bound iodothyronines were precipitated by the addition of 500 μl of 10% (w/v) trichloroacetic acid. After incubation of the mixtures at 0°C for 10 min, they were centrifuged and the radioactivity in the supernatant subsequently determined. Enzymatic deiodination was corrected for non-enzymatic ^{125}I production (as determined in blank incubations without enzymes) and multiplied by 2 to account for random labeling and the deiodination of the 3′ and 5′ positions in [3′,5′-^{125}I]rT_3.

Type III deiodinase activity (ID-III) was determined in brain microsomes by measuring the formation of 3[3′-^{125}I]T_2 from [3′-^{125}I]T_3 by HPLC analysis, as reported by Schoenmakers et al. [16]. A 100 μg sample of brain microsomal protein was incubated for 60 min at 37°C with 1 nM T_3 and ≈100 000 cpm [3′-^{125}I]T_3 in phosphate buffer (pH 7.2) with 4 mM EDTA and 10 mM DTT. The reactions were stopped by the addition of 300 μl methanol on ice. After centrifugation of precipitated proteins, the supernatants were analyzed for 3[3′-^{125}I]T_2 formation by HPLC analysis following elution with a 45:50 (v/v) mixture of methanol and 20 mM ammonium-acetate (pH 4.0) at a flow rate of 0.8 ml/min.

The protein concentration was determined by the method of Hartree [17] using bovine serum albumin as standard.

Serum levels of T_3 and T_4 (in unextracted sera) were estimated by means of specific RIAs using reagents and protocols supplied by Becton-Dickinson (Orangeburg, NJ, USA).

2.3. RNA isolation and Northern blotting

Total RNAs from skeletal muscle, liver, heart and kidney were purified by the method of Chomczynski and Sacchi [18]. For Northern blots, 20 μg of total RNA was separated on a 1% (w/v) agarose/formaldehyde gel and transferred onto nylon membranes with 20×SSC (1×SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0).

To detect specific mRNA, we used a 900 bp probe derived from murine UCP_2 cDNA (GenBank accession number U69135 [9]) which was labeled with ^{32}P using a random priming system.

Hybridization and washing were carried out as described by Church and Gilbert [19]. A 28S-derived oligonucleotide was used to normalize the amount of RNA for each line.

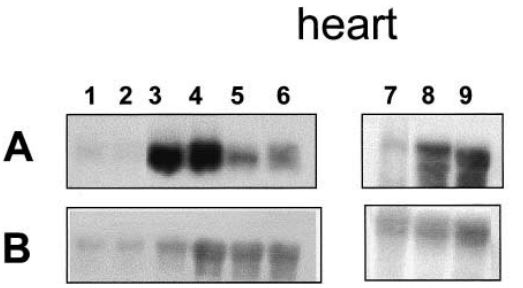

Fig. 1. A: Expression levels of UCP_2 mRNA in the heart of hypothyroid rats (left panel) and normal euthyroid rats (right panel) before and after T_4 and T_3 administration. B: Hybridization with a 28S-derived oligonucleotide. The Northern blotting procedures are described in Section 2. Lines: 1, 2 = hypothyroid rats; 3, 4 = hypothyroid rats treated with T_3; 5, 6 = hypothyroid rats treated with T_4; 7 = normal euthyroid rats; 8 = normal euthyroid rats treated with T_3; 9 = normal euthyroid rats treated with T_4.

3. Results

Table 1 shows the effects of 3 weeks' treatment with PTU and IOP on total serum T_4 and T_3 levels, as well as on deiodinase activities. The combined administration of PTU and IOP produces rats with severe hypothyroidism: the total T_3 and T_4 levels are, in fact, significantly lower in such hypothyroid rats than in euthyroid ones. Another effect of the PTU+IOP treatment is to inhibit the activities of all three deiodinase enzymes: they were strongly inhibited (>80% for type III and >91% for type I) or, in the case of type II deiodinase, greatly reduced (by 64%).

In our conditions, T_3 greatly influenced the expression of UCP_2 mRNA in heart, had a weaker effect in skeletal muscle, but was almost ineffective in kidney and liver.

In hypothyroid rats, UCP_2 mRNA was weakly expressed in heart and, while the administration of T_3 strongly enhanced its expression, administration of T_4 was much less effective (see Fig. 1, left panel). When T_4 and T_3 were injected into normal euthyroid rats, the differences between T_4- and T_3-treated animals were not so evident (see Fig. 1, right panel).

In skeletal muscle, the situation was quite different. In hypothyroid rats, UCP_2 mRNA expression was not evident until after the administration of T_3 and T_4 (see Fig. 2, left panel). In normal euthyroid animals, a slight expression of UCP_2 mRNA was already evident, and administration of T_3 induced a stimulation of its expression. The administration of T_4 to normal euthyroid animals also enhanced the expression of

Table 1
T_3 and T_4 serum levels, liver ID-I activity, BAT ID-II activity and brain ID-III activity in hypothyroid (P+I) and euthyroid (N) rats

Parameter	Group	
	P+I	N
T_3 (nmol/l)	0.25 ± 0.02*	0.95 ± 0.08
T_4 (nmol/l)	8.8 ± 0.98*	45 ± 3
ID-I activity (pmol I/min/mg prot)	22 ± 2*	240 ± 14
ID-II activity (fmol I/h/mg prot)	16 ± 1*	44 ± 3
ID-III activity (fmol 3,3′-T_2/min/mg prot)	4 ± 1*	28 ± 1

Results are presented as mean ± S.E. of values from four rats in each group. Deiodinase activity is the mean of values from four experiments, each performed in triplicate. *$P < 0.05$ vs. N rats.
P+I = rats made hypothyroid by a combined treatment with PTU and IOP for 3 weeks. N = euthyroid control rats.

UCP_2 mRNA, but to a lesser extent (see Fig. 2, right panel).

In the kidney from hypothyroid rats, UCP_2 mRNA was moderately expressed, but thyroid hormones seemed not to have a significant influence on its expression (see Fig. 3). Similar results were obtained in normal euthyroid rats (data not shown).

In the whole liver, UCP_2 mRNA was poorly expressed in both normal and hypothyroid rats (in accordance with the reports of Fleury et al. [9] and Larrouy et al. [20]). Neither T_3 nor T_4 had any apparent effect on its expression under our conditions (data from normal euthyroid rats are not shown).

4. Discussion

Energy balance is a delicate equilibrium between energy intake and energy expenditure. Significant advances have been made in the last few years in the understanding of the highly complex processes underlying the control of energy intake. This control is now known to involve multiple neural circuits with specific neuropeptides and neurotransmitters all influenced by peripheral signals (such as leptin) and inputs from higher cortical centers. In contrast, much less is known about the molecular mechanisms involved in the regulation of energy expenditure. However, the discovery of UCP_2 and the possibility of the regulation of its expression by thyroid hormones opens a new line of enquiry in this field. In this report, we identify T_3 (and to a lesser extent, T_4) as the first hormonal factor that has been found to regulate the expression of UCP_2. To judge from the present study in the rat, the control exerted by T_3 is organ-specific, the greatest effect being seen in the heart, a clear effect in skeletal muscle (especially in normal euthyroid animals), and no effect at all in kidney and liver.

From the data presented here, it is evident that the heart preferentially utilizes T_3 for the control of UCP_2 expression and that it is normally derived from T_4. In fact, in hypothyroid rats the effect exerted in the heart by T_3 is stronger than that of T_4, while in normal euthyroid rats the effects due to T_4 and T_3 are almost equal. This difference may be explained by the greater conversion of injected T_4 to T_3 by deiodinase enzymes in euthyroid rats. In our hypothyroid rats, the deiodinase enzymes are strongly inhibited, and so the above conversion would be correspondingly reduced. The data from skeletal muscle suggest that this tissue utilizes T_3 collected from the serum, rather than relying on that produced in the tissue by the conversion of T_4 to T_3.

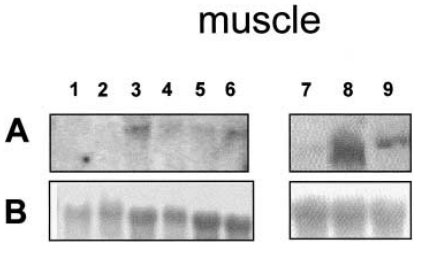

Fig. 2. A: Expression levels of UCP_2 mRNA in the skeletal muscle of hypothyroid rats (left panel) and normal euthyroid rats (right panel) before and after T_4 and T_3 administration. B: Hybridization with a 28S-derived oligonucleotide. The Northern blotting procedures are described in Section 2. Lines: 1, 2 = hypothyroid rats; 3, 4 = hypothyroid rats treated with T_3; 5, 6 = hypothyroid rats treated with T_4; 7 = normal euthyroid rats; 8 = normal euthyroid rats treated with T_3; 9 = normal euthyroid rats treated with T_4.

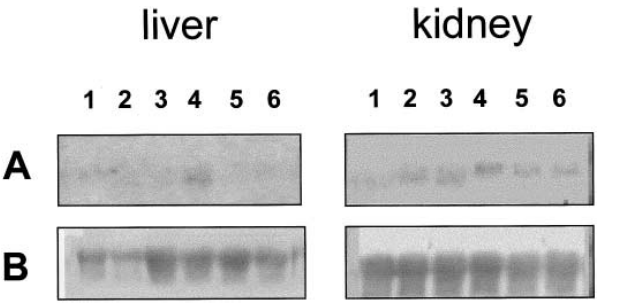

Fig. 3. A: Expression levels of UCP_2 mRNA in the kidney (right panel) and liver (left panel) of hypothyroid rats before and after T_4 and T_3 administration. B: Hybridization with a 28S-derived oligonucleotide. The Northern blotting procedures are described in Section 2. Lines: 1, 2 = hypothyroid rats; 3, 4 = hypothyroid rats treated with T_3; 5, 6 = hypothyroid rats treated with T_4.

The discovery of the role played by T_3 in controlling the expression of UCP_2 casts a new light on the problem of the nuclear-mediated regulation of energy expenditure by thyroid hormones. Such effects may be especially important in some specific circumstances such as fasting or cold exposure, in which variations in the circulating levels of thyroid hormones have been observed [21,22]. This idea is consistent with a recent finding by Boss et al. [23] of a tissue-dependent upregulation of UCP_2 expression in the rat in response to fasting and cold.

Previous reports have shown that thyroid hormones stimulate the mitochondrial proton leak (for review see [13]) and our results may suggest a molecular basis for this effect in skeletal muscle and heart.

In conclusion, the upregulation of UCP_2 mRNA expression is in agreement with the known role of thyroid hormones in resting metabolic rate and the genetic linkage of the UCP_2 locus to resting metabolic rate value in man [24].

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