Adrenergic control of induction of type II iodothyronine 5'-deiodinase activity in cultured mouse brown adipocytes

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Iodothyronine 5'-deiodinase (5'D) of mouse brown adipocytes differentiated in cell culture was characterized in detail with respect to the adrenergic control of its biosynthesis. The stimulation of 5'D required mRNA and protein synthesis and was dependent on the stage of differentiation of the cells. The maximum induction was observed around confluence (7-day-old cells), in pre- and post-confluent cells the 5'D activity was significantly less induced. The transient responsiveness of brown fat-cells to the stimulatory effect of adrenergic agents was reflected also in the time course of the induction of 5'D by different concentrations of agonists. The maximum response occurred regularly after an 8 h incubation and implicated a rather fast turnover of the induced enzyme. On the basis of the inhibitory effects of cycloheximide and actinomycin D, the half-life of the

induced 5'D and its mRNA were estimated to be 1.5 and 3.3 h respectively. The noradrenaline-induced 5'D activity was shown to be that of the type II enzyme, insensitive to propylthiouracil (PTU). The estimated values of its apparent K_m for thyroxine, K_m for the co-substrate dithiothreitol, and V_{max} in the presence of 1 mM PTU were 2 nM, 2.6 mM, and 0.1 pmol of I⁻/h per mg of protein respectively. The 5'D activity was effectively induced by forskolin and dibutyl cyclic AMP, as well as by isoprenaline, noradrenaline and CGP-12177, but not by phenylephrine, cirazoline or oxymetazoline. This indicates that, contrary to previous observations *in vivo*, stimulation of 5'D in cultured brown fat-cells involves elevated cyclic AMP levels and is mediated predominantly via β -receptors, particularly via the so-called β_3 -adrenoceptors.

INTRODUCTION

Brown adipose tissue (BAT) is the most important site of non-shivering thermogenesis in mammals (for a review, see [1]). A unique 32 kDa uncoupling protein (UCP) known as thermogenin is present in the inner membrane of BAT mitochondria, where it dissipates the proton gradient created by the respiratory-chain complexes [2,3]. The synthesis of UCP *in vivo* is controlled by the sympathetic nerve fibres, releasing noradrenaline (NA; norepinephrine) after stimulation either by decreased environmental temperature or by nutritional status [1]. Thyroid hormones have also been reported to exert control on BAT function [4–6]. In addition, as shown first in the rat, BAT of some species contains a type II iodothyronine 5'-deiodinase (5'D) which catalyses the conversion of thyroxine (T_4) to biologically active 3,5,3'-triiodothyronine (T_3) [7]. From a kinetic standpoint, BAT type II 5'D is indistinguishable from the enzyme present in the central nervous system, the anterior pituitary and pineal gland [7–9]. At variance with the enzyme in these tissues, however, rodent BAT 5'D can be activated *in vivo* by the sympathetic nervous system [10,11]. However, conclusions drawn from experiments aimed at a pharmacological characterization of the response [10–13] are contradictory. Involvement of α_1 -adrenergic receptors in the stimulation of BAT 5'D by NA was demonstrated *in vivo* by the inhibitory effect of the α_1 -antagonist prazosin [10]. Activation of 5'D by α_1 - but not β -adrenergic agonists was also observed in some experiments with dispersed rat brown adipocytes [12], while in other studies [13] a pronounced effect of β -adrenergic agents and a synergistic effect of α_1 - and β -adrenergic agonists

was found. The recent development of cell-culture techniques for *in vitro* studies of brown fat-cells [14,15] made it possible to ascertain the effects of individual hormones. We used this approach previously in studies of synthesis of mitochondrial UCP and induction of deiodinase activity in mouse brown adipocytes differentiated in cell culture [16,17] and showed that, at around confluence, i.e. 6–7 days after inoculation, these cells produced a measurable thyroxine 5'-deiodinating activity. This activity could be markedly increased by NA or dibutyl cyclic AMP [(Bu)₂-cAMP] treatment of the cells [17].

The aim of the present studies was to characterize in greater detail the adrenergic control of the induction of 5'D activity in cultured brown fat-cells. From the results obtained we conclude that the ability of these cells to optimally express the 5'D gene is a transitory phenomenon. The stimulation of 5'D involves β_3 -adrenoceptors and elevated cyclic AMP levels.

MATERIALS AND METHODS

Cell isolation and culture

Brown fat precursor cells were isolated from interscapular, cervical, and axillary BAT depots of 3–4-week-old mice (Balb/c, outbred strain) as described previously [16]. The cells were inoculated (2×10^4 viable cells/cm²) either into 35 mm plastic dishes (Nunc) containing 1.5 ml of culture medium or into 25 cm² culture flasks (Sterilin) containing 5 ml of medium and were grown at 37 °C in an atmosphere of CO₂/air (1:19) with 80% humidity. The medium was exchanged on days 1 (completely) and 3 (partly) after plating [16]. Unless otherwise

Abbreviations used: 5'D, iodothyronine 5'-deiodinase; BAT, brown adipose tissue; (Bu)₂-cAMP, dibutyl cyclic AMP; DTT, dithiothreitol; NA, noradrenaline (norepinephrine); PTU, 6-n-propyl-2-thiouracil; rT_3 , 3,3',5'-tri-iodothyronine; T_3 , 3,5,3'-tri-iodothyronine; T_4 , thyroxine; UCP, uncoupling protein.

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specified, the cultures were analysed on day 7 after plating, i.e. at around confluence. When required, the following agents or their combinations were added to the cultivation medium for the indicated times before harvesting: NA, isoprenaline, phenylephrine, CGP-12177 and propranolol, all dissolved in 0.15 mM sodium ascorbate; (Bu)₂-cAMP, cirazoline, oxymetazoline, and cycloheximide all dissolved in water; forskolin and actinomycin D both dissolved in ethanol; and prazosin dissolved in dimethyl sulphoxide. Stock solutions of the agents used were stored at -20°C . The final concentration of the solvent in culture was lower than 0.2 %.

Cell harvesting and analysis

The medium was discarded, the cell monolayer was washed twice with ice-cold PBS and cells were detached by scraping (with a rubber policeman) into 500 μl of PBS. The cells were sedimented by 1 min centrifugation at 14000 g (Eppendorf centrifuge) and pellets were suspended in 100–200 μl of 0.1 M sodium phosphate/1 mM EDTA (pH 7.0) by sonication for 10 s (MSE N146 sonifier equipped with a microtip) on ice. The homogenates were rapidly frozen and stored at -70°C for analysis. The thyroxine 5'-deiodinase activity was assayed as described in [18] with the modifications specified in [17]. The protein content was determined by the Lowry method [19], with BSA as standard.

Isolation of BAT microsomes

Interscapular BAT of cold-acclimated (3–4 weeks at 4°C) Syrian hamsters was dissected and homogenized in a glass/Teflon homogenizer in the isolation medium (0.25 M sucrose/10 mM Tris/HCl/0.2 mM EDTA, pH 7.4; 10 %, w/v, homogenate). The microsomal fraction was isolated from the homogenate by differential centrifugation (the postmitochondrial supernatant from the 10000 g centrifugation was further centrifuged at 140000 g for 60 min). The final pellet was suspended in a minimum volume of 0.1 M sodium phosphate/1 mM EDTA (pH 7.0). Aliquots of this suspension were rapidly frozen (in liquid N₂) and kept at -70°C until use.

Materials

The source of the materials used for cell isolation and culture was the same as previously described [16]. (–)-Arterenol bitartrate [noradrenaline (NA); norepinephrine], (–)-isoproterenol hydrochloride (isoprenaline), L-phenylephrine hydrochloride, DL-propranolol hydrochloride, *N*⁶,2'-*O*-dibutyryl adenosine 3':5'-cyclic monophosphate [(Bu)₂-cAMP], oxymetazoline, cycloheximide, and forskolin were all obtained from Sigma. CGP-12177, cirazoline, actinomycin D, and prazosin were from Ciba-Geigy, Synthelabo Recherches, Merck, and Pfizer respectively. All other chemicals were of analytical grade.

RESULTS

Differentiation-related stimulation of 5'D by adrenergic agents

Initial experiments were performed with the aim to characterize the dependence of the stimulation of 5'-deiodinase by adrenergic agents on the stage of differentiation of the cultured brown fat-cells (Figure 1). The cells grown in culture for 5, 6, 7, 8 or 9 days were supplemented on the respective day with a membrane-

permeant cyclic AMP analogue [1 mM (Bu)₂-cAMP], a dual α_1 , β -agonist (10 μM NA), a β -agonist (1 μM isoprenaline) or an α_1 -agonist (10 μM phenylephrine) for 8 h before harvesting. The most pronounced effects of all adrenergic agents tested occurred around confluence, while in the preconfluent (day 5) and postconfluent cells (day 9) the stimulatory effects were much weaker. In contrast, the basal 5'D activity in control cells was very low, regardless of the differentiation stage of the cells. It is also seen (Figure 1) that the stimulation of 5'D was brought about mostly by a cyclic AMP-dependent mechanism.

Characterization of the deiodinase activity induced by NA

Here we investigated in detail the time course of the stimulation of 5'D in cultured confluent brown adipocytes by different concentrations of NA (Table 1). The maximum response, more than 8-fold increase, occurred regularly after 8 h incubation of the cells with the highest, 10 μM , concentration of NA. Both at 4 h and 12 h time points only about 50 % response was observed, indicating a very narrow time window of the 5'D activation. Importantly, using the same cultured cells, a 100 times lower concentration of NA was necessary for optimal expression of the UCP gene, as measured either by the amount of UCP mRNA (0.1 μM NA, 4 h) [20] or by the amount of synthesized UCP (0.1 μM NA, 24 h) [16].

In order to characterize kinetically the induced 5'D activity, we measured the K_m for T₄ as substrate and the K_m for the co-substrate dithiothreitol (DTT) in the whole homogenate of stimulated cultured BAT cells and in isolated microsomal fraction [21] from BAT of cold-acclimated Syrian hamsters respectively. The activity was measured in the presence and absence of 1 mM propylthiouracil (PTU), a specific inhibitor of type I deiodinase [18]. Examples of double-reciprocal plots of the rate of I[−] generation as a function of T₄ concentration and DTT concentration are shown in Figures 2(a) and 2(b) and in the insets. The standard preparation of hamster BAT microsomes (Figure 2a) contains type II 5'-deiodinase [21]. It resembles the enzyme found in fetal, neonatal and cold-exposed adult rat BAT (7,11,21,22) and is characterized by low K_m for T₄ and relative

Figure 1 Differentiation-dependent stimulation of 5'-deiodinase by (Bu)₂-cAMP and different adrenergic agents

Brown adipocytes grown in culture for 5, 6, 7, 8 or 9 days had been exposed to 1 mM (Bu)₂-cAMP (cAMP), 1 μM isoprenaline (ISO; β -agonist), 10 μM NA (α_1 , β -agonist) or 10 μM phenylephrine (PHE; α_1 -agonist) for 8 h before the cells were harvested for 5'D assay. Control cells (addition of solvent, 0.15 mM sodium ascorbate) were also assayed for 5'D every day. Results are means $\pm$ S.E.M. from two representative experiments performed in triplicate dishes.

Table 1 Time course of 5'D activity as induced by different concentrations of NA

Brown fat precursor cells were isolated and grown in culture for 7 days. On day 7, NA was added to the indicated final concentration and cells were exposed to NA for 2, 4, 8, 12 and 24 h. The cells were then harvested for 5'D assay as described in the Materials and methods section. Values are means $\pm$ S.E.M. from *n* experimental series performed in duplicate flasks.

Incubation time with NA (h)	[NA] (μ M)	5'D activity (fmol of I ⁻ /h per mg of protein)	<i>n</i>
0	0 (Control)	5.7 $\pm$ 0.6	12
2	0.1	9.3 $\pm$ 1.4	2
	1	11.2 $\pm$ 0.9	3
4	0.1	15.5 $\pm$ 2.9	3
	1	16.7 $\pm$ 1.8	4
	10	20.1 $\pm$ 3.5	3
8	0.1	21.9 $\pm$ 6.7	4
	1	35.8 $\pm$ 6.2	6
	10	48.8 $\pm$ 7.0	12
12	0.1	16.3 $\pm$ 4.7	4
	1	14.5 $\pm$ 1.7	8
	10	23.9 $\pm$ 5.5	4
24	0 (Control)	6.1 $\pm$ 0.9	6
	0.1	9.0 $\pm$ 1.2	6
	1	11.8 $\pm$ 1.9	6
	10	14.6 $\pm$ 1.6	6

insensitivity to PTU. Estimated values from Figure 2(a) of the apparent K_m for T_4 , K_m for DTT and maximum velocity (V_{max}) in the absence and presence of 1 mM PTU were 2.2 nM, 1.8 mM, and 8.0 and 7.1 pmol of I⁻/h per mg of protein respectively, in good agreement with the previous studies [7,21]. PTU reduced the 5'D activity by less than 10% and did not influence the apparent K_m for T_4 . In comparison with these data (Figure 2a), it is evident from Figure 2b that the 5'D activity induced by NA in brown adipocytes differentiated in cell culture displayed typical features of the type II enzyme. The estimated apparent K_m for T_4 and V_{max} values in the presence of 10 mM DTT and in the absence or presence of 1 mM PTU were 2.0 nM, and 0.103 and 0.092 pmol of I⁻/h per mg of protein respectively. The apparent K_m for DTT was about 2.6 mM. As expected, the specific T_4 5'-deiodinating activity of crude cell homogenates was about 1.3% of the 5'D activity of the standard preparation of hamster BAT microsomes, and was relatively insensitive to PTU inhibition (Figure 2b). However, addition of excess T_4 (1 μ M) or 3,3',5'-triiodothyronine (rT_3 ; 1 μ M) decreased the radioiodide release from [¹²⁵I] T_4 by more than 95% in both enzyme preparations, while 1 μ M T_3 had a negligible inhibitory effect on T_4 5'-deiodination (results not shown).

Effect of inhibition of protein and mRNA synthesis

To investigate the involvement of *de novo* protein synthesis in the stimulation of 5'D in cultured brown fat cells, the cells were preincubated for 20 min with cycloheximide (60 μ M) and then 1 μ M NA, 1 μ M forskolin or 1 mM (Bu)₂-cAMP were added. In all cases the cycloheximide pretreatment fully abolished the induction of the deiodinase. There was even a tendency to a decrease in 5'D activity in the control cells after a short (4 h) incubation with cycloheximide (results not shown), indicating a rather short half-life of the enzyme (see below). These results

Figure 2 Double-reciprocal plots of the rate of T_4 5'-deiodination catalysed by BAT microsomes (a) and by cultured brown fat-cells (b) as a function of T_4 concentration and DTT concentration (in the insets)

A microsomal fraction (a) was isolated as described in the Materials and methods section. Cells (b) were stimulated by incubation for 8 h with 10 μ M NA. The T_4 concentration was varied between 0.5 and 20 nM in the presence of 10 mM DTT and 0 or 1 mM PTU. DTT concentration was varied between 1 and 20 mM in the presence of 2.4 nM T_4 and absence of PTU. Individual points represent the means $\pm$ S.E.M. from two independent determinations using $\sim$ 30 μ g of microsomal protein and $\sim$ 100 μ g of total cellular protein/assay tube.

indicate that the adrenergically induced increase in the specific activity of the deiodinase requires rapid *de novo* protein synthesis and cannot be due to a modification of pre-existing enzyme.

Up to now, the type II 5'D has not been isolated, and cDNA clones corresponding to the mRNA for 5'D have not been identified. However, by the use of the transcription inhibitor actinomycin D it is possible to investigate the control of the deiodinase gene expression and the mRNA half-life indirectly. The cultured cells were preincubated for 20 min with actinomycin D (0.4 μ M) and then adrenergic agents were added. Similarly to the incubation with cycloheximide, the increase in 5'D activity was totally abolished by actinomycin, indicating that the increase was due to mRNA synthesis and not to increased translation of pre-existing mRNA (results not shown).

To assess the apparently short half-life of 5'D and its mRNA, the confluent cells were stimulated by incubation for 4–13 h with 1 μ M NA or 1 mM (Bu)₂-cAMP and then cycloheximide (60 μ M)

Figure 3 5'D activity in (Bu)₂-cAMP-stimulated cultured brown fat-cells after actinomycin D or cycloheximide treatment

Cells were stimulated by a 4 h (a) or a 13 h (b) incubation with 1 mM (Bu)₂-cAMP. Thereafter, 0.4 μ M actinomycin D (Act D) or 60 μ M cycloheximide (Cyclo) or corresponding volume of ethanol (Control) were added and cells were further incubated with (Bu)₂-cAMP together with the respective inhibitor. At the indicated time points, the cells from two dishes per treatment were harvested for 5'D assay. The lines concerning actinomycin D and cycloheximide treatments are drawn after linear regression analysis of time points 0–8 h (a) and 0–7 h (b) after the addition of the inhibitors; note the logarithmic y-axes. Values in (a) and (b) each represent the means from two independent experimental series.

Table 2 Effects of adrenergic agonists and cyclic AMP-concentration-modulating agents on 5'D activity in cultured BAT cells

Confluent (7 days) cells were exposed for 8 h to the agents and concentrations indicated. Values are means $\pm$ S.E.M. for *n* experiments performed in duplicate or triplicate.

Agent	Concentration (μ M)	5'D activity (fmol of I ⁻ /h per mg of protein)	<i>n</i>
Control		5.9 $\pm$ 0.5	25
(Bu) ₂ -cAMP	1000	75.7 $\pm$ 10.7	9
Forskolin	1	70.6 $\pm$ 22.1	5
NA	0.1	21.9 $\pm$ 6.7	4
	1	31.6 $\pm$ 4.9	7
	10	48.1 $\pm$ 3.2	12
Phenylephrine	10	9.3 $\pm$ 3.4	6
Cirazoline	1	6.0 $\pm$ 0.4	2
	10	6.5 $\pm$ 0.2	2
Oxymetazoline	1	6.7 $\pm$ 1.9	4
	10	8.1 $\pm$ 1.8	3
Isoprenaline	0.1	31.5 $\pm$ 2.5	4
	1	37.0 $\pm$ 4.1	7
	10	34.6 $\pm$ 2.6	8
CGP-12177	0.1	22.4 $\pm$ 2.7	4
	1	29.0 $\pm$ 1.7	6
	10	32.6 $\pm$ 2.6	4

or actinomycin D (0.4 μ M) were added. The cells were exposed to the agents together with the respective inhibitor for different periods of time up to 12 h and the 5'D activities were measured in 1 h intervals. As may be seen in Figures 3a and 3b, cycloheximide completely blocked further stimulation of 5'D, re-

gardless of the time of addition, and caused an immediate and fast decrease in the enzyme activity with a half-life of approx. 1.5 h. Actinomycin D also completely blocked the stimulation of 5'D caused by NA or (Bu)₂-cAMP. However, in contrast with cycloheximide, actinomycin was effective only about 1 h after addition, and the rate of decrease in 5'D activity caused by the inhibition of 5'D mRNA synthesis was lower, namely about 40% of the rate with cycloheximide.

Pharmacological characterization of the adrenergic stimulation of 5'D activity

There is evidence for the presence of all the described types of adrenergic receptors (α_1 , α_2 , β_1 , β_2 and β_3) on brown fat-cells. Table 2 summarizes the results of a detailed analysis of the participation of these cellular receptors in the induction of 5'D activity in cultured brown adipocytes. Two alternative means of increasing the cyclic AMP level in the cell were used. We tested the effect of (Bu)₂-cAMP, a membrane-permeant derivative of cyclic AMP, which had been found to induce type II 5'D activity in cultured rat astroglial cells [23]. We also tested the effect of forskolin, an adenylate cyclase activator, which had been earlier shown to rapidly stimulate thermogenesis in mature BAT cells [24] and which is therefore able to increase cyclic AMP in these cells in an adequate manner. In agreement with our previous results [17], forskolin (1 μ M) caused a 12-fold increase in 5'D activity in confluent cells in the course of an 8 h incubation. Similarly, (Bu)₂-cAMP (1 mM) caused a 13-fold increase (Table 2). From these observations it is clear that the β -adrenergic stimulation, mediated via an increase in cytosolic cyclic AMP, is effective in the induction of 5'D activity.

The interpretation of the effects of the dual adrenergic agonist NA *in vitro* is more complicated [13,20]. In view of the limited receptor-subtype-selectivity of the available adrenergic agents, both the ability of the antagonists to inhibit the NA effect on the induction of the deiodinase activity, and the ability of different agonists to mimic this effect has to be tested. Previously, we described the effects of adding the subtype-selective antagonists propranolol (β) or prazosin (α_1) in a 10-fold excess over NA, together with NA, to brown-fat-cell cultures [17]. The NA-induced increase in 5'D was inhibited by about 80% by 10 μ M propranolol, while prazosin at the same concentration decreased the NA effect by less than 30%, indicating the activation of both pathways, with prevailing mediation via β -receptors [17]. This was further supported by the observed modest effect of phenylephrine (10 μ M), an agent with a higher ratio of α_1 - to β -agonist activity than NA (Table 2). The pure α_1 -agonist cirazoline was essentially without any effect at the concentrations tested (1 and 10 μ M). The same was true for oxymetazoline (1 and 10 μ M). On the other hand, isoprenaline, a relatively pure β -agonist, was as effective as NA at a wide range of concentrations (10⁻⁷–10⁻⁵ M) (Table 2).

There is overwhelming evidence from studies using both antagonists and agonists that the functional β -adrenoceptor in brown adipocytes can be differentiated from classical β_1 - and β_2 -adrenoceptors. The existence of atypical β -adrenoceptors in brown adipocytes has been confirmed by the discovery of agonists, such as the BRL series, that selectively stimulate these receptors [25]. This group of receptors are described as β_3 -adrenoceptors [26]. The adrenergic agent CGP-12177, generally accepted as a β_1 -antagonist, has recently been shown to be an agonist for thermogenesis in isolated brown fat-cells, as well as in BAT *in vivo* [27] and for induction of UCP synthesis in cultured confluent brown fat-cells [20,28]. CGP-12177 can be considered as a qualitatively selective β_3 -adrenergic agonist,

Table 3 Time course of 5'D activity as induced by different concentrations of the β_3 -agonist CGP-12177

Brown fat-cells grown in culture for 7 days were treated with CGP-12177 in the same way as described for NA in Table 1. Values given are means $\pm$ S.E.M. from n cultures grown in duplicate flasks.

Incubation time with CGP-12177 (h)	[CGP-12177] (μ M)	5'D activity (fmol of I ⁻ /h per mg of protein)	n
0	0 (Control)	5.5 $\pm$ 1.2	10
2	1	6.9 $\pm$ 0.2	3
4	1	11.3 $\pm$ 3.4	4
8	0.1	22.4 $\pm$ 2.6	4
	1	29.0 $\pm$ 1.7	8
	10	32.6 $\pm$ 2.7	4
12	0 (Control)	5.7 $\pm$ 1.1	10
	0.1	12.7 $\pm$ 3.6	4
	1	11.2 $\pm$ 1.5	10
	10	15.9 $\pm$ 2.8	8

having a stimulatory effect only on β_3 -receptors in BAT [29]. We have therefore also investigated in detail the effect of CGP-12177 on the stimulation of 5'-deiodinase (Tables 2 and 3). As seen in Tables 2 and 3, CGP-12177 was able to induce the 5'D activity in cultured brown adipocytes, and the time course and magnitude of the increase in 5'D were comparable with the effect of NA at the same concentrations (0.1, 1 and 10 μ M).

DISCUSSION

In the present paper we demonstrate, in accordance with our previous experiments [17], that induction of 5'D activity in cultured mouse brown fat-cells is mediated via β -adrenergic receptors and elevated cyclic AMP levels, while α -receptors are only weakly involved. This contrasts with the original view of Silva and Larsen [10,30], based on *in vivo* studies, that NA- and cold-induced increase in 5'D in BAT is due to α_1 -adrenergic stimulation. Recently, however, these authors concluded that both α_1 - and β -agonists are involved in 5'D stimulation [31].

The β -adrenergic induction of 5'D activity is shown both by the equal efficiency of NA and a relatively pure β -agonist isoprenaline, and by the effect of an increase in intracellular cAMP, the expected second messenger. For the first time we also demonstrate that the β_3 -agonist CGP-12177 [29] was able to induce 5'D in cultured brown fat-cells to an extent and with a time course comparable with those with NA (Tables 1–3). Since CGP-12177 is the β_1 -antagonist and virtually free of any β_2 -activity (in contrast with the β_3 -agonists of BRL series) [27,29] it appears that an involvement of β_3 -adrenoceptors is crucial for induction of 5'D in BAT, similarly as it seems to be for induction of UCP [20,28].

Primary cultures of mouse BAT cells, as used here, exhibit a rather fast adipose differentiation [16]. In non-stimulated cells an equal basal 5'-deiodinating activity was detected at pre-confluence, confluence and postconfluence. Although at all these stages (Bu)₂-cAMP, NA or isoprenaline induced an increase in 5'D activity, the intensity of these effects was the highest at around confluence and followed closely the changes in differentiation and morphological pattern of the cells. There is a clear analogy between differentiation-dependent inducibility of 5'D and the extent of NA-induced UCP mRNA expression [20].

Close correlation between the 5'D activity and UCP content or UCP mRNA levels were also observed in rat [22], hamster [32,33], cow [34] and man [32] during perinatal development. The common regulatory mechanism operating through β_3 -adrenoceptors and cyclic AMP levels is thus likely to control both the UCP and 5'D genes.

There are at least two outer-ring iodothyronine 5'-mono-deiodinases, a high- K_m ($\approx 1 \mu$ M for T₄) PTU-sensitive ($K_i \approx 1 \mu$ M) type I enzyme, which is typical for liver and kidney and decreases in hypothyroidism [35], and a PTU-insensitive (at higher than 5 mM DTT), low- K_m (1–2 nM for T₄) type II enzyme, which occurs mainly in the pituitary, brain and BAT and increases in hypothyroidism [35]. Although rodent BAT 5'D is a typical type II enzyme [7,8,10,21], the bovine BAT 5'D is a PTU-sensitive type I enzyme [34], and ovine BAT contains in fact two distinct 5'-deiodinating activities, one with a high K_m and another with a low K_m [36]. The present study proves quite unambiguously that only a type II enzyme is induced in mouse brown adipocytes differentiated in cell culture.

In accordance with experiments in rat BAT *in vivo* [11,30] and in dispersed rat brown adipocytes [31], the adrenergic induction of 5'D activity in cultured brown adipocytes is fully due to *de novo* synthesis of the 5'D itself and requires both the active gene expression and subsequent protein synthesis. Ongoing 5'D synthesis is also necessary for the maintenance of 5'D basal activity. Both the 5'D protein and mRNA apparently exert rapid turnover. Their half-lives, as deduced from inhibitor studies, are rather short (about 1.5 h and 3 h respectively) and agree well with the values observed in BAT of the cold-stressed or NA-treated rats [11,30]. There is an apparent distinction between 5'D and UCP in this respect. The UCP half-life of 7–9 days *in vivo* [37] and 20–70 h in cultured BAT cells [37] as well as the UCP mRNA half-life values of 10–45 h [38] respectively clearly indicate much slower turnover rates. Fast turnover of 5'D may be advantageous for regulation and control of the intracellular T₃ level and saturation of nuclear T₃ receptors [4–6] which is necessary for UCP induction by catecholamines.

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