Volume 274, number 1,2, 185-188 FEBS 09098 November 1990

Induction of type II iodothyronine 5'-deiodinase and mitochondrial uncoupling protein in brown adipocytes differentiated in cell culture

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Received 20 September 1990

Brown adipocytes differentiated in primary cell culture were found to contain a type II iodothyronine 5'-deiodinase (5'D). Incubation of confluent cells with norepinephrine or dibutyryl-cAMP caused up to 17-fold increase in 5'D activity with a maximum after 8 h. Activation of 5'D required mRNA and protein synthesis and was accompanied by parallel, up to 5.8-fold increase in the amount of mitochondrial uncoupling protein with a maximum after 24 h. Analysis of adrenergic stimulation of 5'D suggested predominant involvement of the β-receptors and increased intracellular cAMP levels, while the contribution of α_1-receptors was small.

Iodothyronine 5'-deiodinase; Uncoupling protein; Brown adipose tissue; Cell culture; Catecholamine

1. INTRODUCTION

Brown adipose tissue (BAT), the major site of non-shivering thermogenesis in newborn and cold-acclimated animals (for review see [1]) contains, similarly to the brain, pituitary and pineal gland, type II iodothyronine 5'-deiodinase (5'D) [2]. In contrast to type I enzyme present in liver and kidney, type II 5'D is insensitive to propylthiouracil, exhibits different kinetics and has low K_m values for T_4 and reverse T_3 in the nanomolar range [3,4].

Activity of 5'D in BAT is largely stimulated during perinatal recruitment of thermogenic function [5-7], in hypothyroid and cold-exposed animals [8-11] and by sympathetic stimulation [12]. The local production of T_3 by 5'D in BAT in concurrence with norepinephrine [13,14] is essential for the synthesis of mitochondrial uncoupling protein (UCP), the key and rate-limiting component of BAT thermogenesis [1,15]. When fully activated, 5'D of BAT represents a substantial source of systemic T_3, in hypothyroid animals in particular [9-11].

Hormonal regulation of 5'D in BAT is still not very clear. Involvement of α_1-adrenergic receptors in activation of 5'D by norepinephrine was demonstrated in vivo [12] but a large increase in 5'D activity was also induced by isoproterenol, insulin and glucagon [16]. Activation by insulin and α_1- but not β-adrenergic agonists was further observed in some experiments with isolated adipocytes from rat BAT [17,18], while in others [19], a pronounced effect of β- and synergistic influence of α_1-adrenergic agents was found.

Experiments are reported here that were carried out to examine 5'D in brown adipocytes which differentiated in primary culture from precursor cells to a state allowing full expression of the UCP gene [20].

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Abbreviations: BAT, brown adipose tissue; dbcAMP, dibutyryl-cAMP; 5'D, iodothyronine 5'-deiodinase; UCP, uncoupling protein

2. MATERIALS AND METHODS

The stromal-vascular cells from BAT of 3-4-week-old mice (Balb/c, outbred strain) were isolated and cultivated as previously described [20] at a standard inoculation of 2×10^4 cells/cm^2 in 35-mm Petri dishes using modified [20,21] Eagle's minimal essential medium containing 10% fetal calf serum. Experiments were performed with cells cultured for 7 days. Incubation of cells with the agents indicated was terminated by two brief washes with PBS and cells were harvested by scraping into 150 μl PBS. They were homogenized (10 s sonication) and frozen in liquid nitrogen for later analysis.

5'D activity was assayed according to [3] with the following modifications: the incubation mixture (200 μl) contained 100 mM sodium phosphate (pH 7.0), 1 mM EDTA, 10 mM DTT, 0.15 nM [^{125}I]T_4 (~ 50000 cpm of L-[3',5'-^{125}I]T_4, > 44TBq/g; from IZINTA, Hungary), 2.25 nM unlabeled T_4 and 1 mM 6-*n*-propyl-2-thiouracil. After 1 h at 37°C, 50 μl of 2% BSA was added followed by 350 μl of ice-cold 10% TCA. Released $^{125}I^-$ was separated from iodothyronines on Dowex 50W-X2 columns and counted as previously described [3]. The tracer was purified by paper electrophoresis before each assay and blank values (no protein) were less than 1% of the radioactivity added. The results are expressed as fmol T_3 produced per h per mg protein.

The content of mitochondrial UCP was measured by immunoblotting as before [6,20]. The protein content was determined as described [22] using BSA as standard.

Norepinephrine, isoprenaline, phenylephrine, propranolol, forskolin, dibutyryl-cAMP and cycloheximide were from Sigma, prazosin from Pfizer and actinomycin D from Merck.

3. RESULTS AND DISCUSSION

Cultured BAT cells produced around confluence (6-7 days after inoculation) a measurable 5'D activity as

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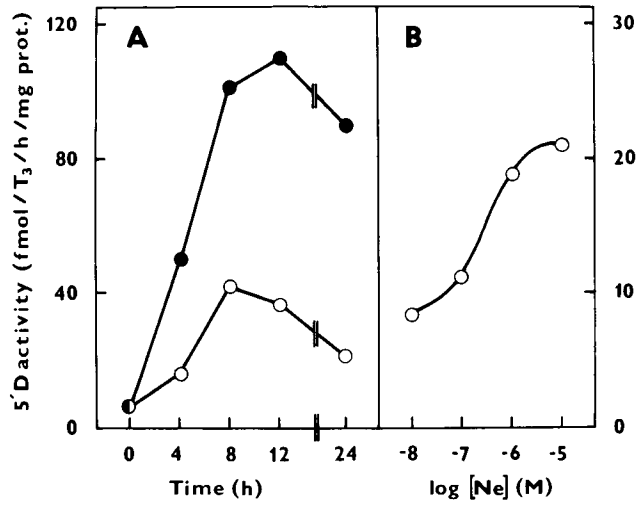

Fig. 1. Time course (A) and concentration dependence (B) of the increase in 5'D activity induced by 10 μM norepinephrine (○) and 1 mM dibutyryl-cAMP (•). In B, cultured cells were exposed for 24 h to the indicated concentration of norepinephrine. Values are the means of two independent experiments performed in duplicate.

well as a detectable amount of spontaneously synthesized UCP. When the 7-day cells were supplemented with 10 μM norepinephrine or 1 mM dibutyryl-cAMP (dbcAMP), 5'D became largely stimulated resulting in a maximally 7-fold and 17-fold increase in 5'D activity, respectively (Fig. 1A). The time course of both the norepinephrine and dbcAMP effect on 5'D is seen to be similar. While the enzyme activity quickly increased during the first 8–12 h there was no measurable increase in the 5'D activity in control cells for 24 h. The dose-response curve for norepinephrine stimulation of 5'D was sigmoid with a maximum at 10 μM norepinephrine (Fig. 1B). When exposed to norepinephrine or dbcAMP for more than 24 h cells started to detach from the dishes.

The K_m for T_4 of the 5'D induced in cultured adipocytes by norepinephrine and dbcAMP was around 10^{-9} M, similar to microsomes of cerebral cortex [3] or infranatants of BAT [3] from hypothyroid adult rats. The 5'D of cultured cells was also insensitive to 1 mM propylthiouracil (lower than 10% inhibition at 0.2 nM and 2 nM T_4 as substrate). Therefore, the 5'D induced in brown adipocytes differentiated in cell culture corresponds to the type II enzyme present in BAT of different rodent species [2,10] and not to the type I enzyme of ovine and bovine BAT [23,24].

Incubation of cultured adipocytes with dbcAMP (1 mM) together with cycloheximide (10 μg/ml) or actinomycin D (60 ng/ml) which were added 10 min before addition of dbcAMP completely prevented the stimulatory effect of dbcAMP (Table I). This suggests that activation of 5'D in cultured cells is fully based on de novo protein synthesis which requires active mRNA synthesis and is not due to increased translation of preexisting mRNA, similarly as in the case of norepinephrine- and cold-activated BAT 5'D in vivo [16,25]. Both inhibitors also induced large and rather fast decrease of 5'D when added 8 h after dbcAMP (Table I). This implies fast turnover of both the 5'D protein and its mRNA, in agreement with their short half-lives observed in vivo ($t_{0.5}$ of protein 0.4–0.7 h, $t_{0.5}$ of mRNA below 2 h [16,25]).

Table I

Effect of cycloheximide and actinomycin D on dibutyryl-cAMP-stimulated 5'D activity in cultured BAT cells

Cell treatment	5'D activity (fmol T_3/h/mg protein)
(A)	
dbcAMP (1 mM)	109.2
dbcAMP + cycloheximide (10 μg/ml)	0
dbcAMP + actinomycin D (60 ng/ml)	2.6
(B)	
dbcAMP (1 mM)	90.1
dbcAMP + cycloheximide (10 μg/ml)	20.5
dbcAMP + actinomycin D (60 ng/ml)	22.7

In (A), cells were exposed for 8 h to dbcAMP and inhibitors were added 10 min before dbcAMP. In (B), cells were exposed to dbcAMP for 24 h and inhibitors were present for the last 16 h (added 8 h after dbcAMP). The values are the means of 2 experiments performed in duplicate.

Addition of norepinephrine and dbcAMP to cultured adipocytes markedly elevated also the specific content of UCP (Fig. 2). In contrast to changes in 5'D, the UCP content continuously increased with time until the maximum was obtained after 24 h (4.9-fold and

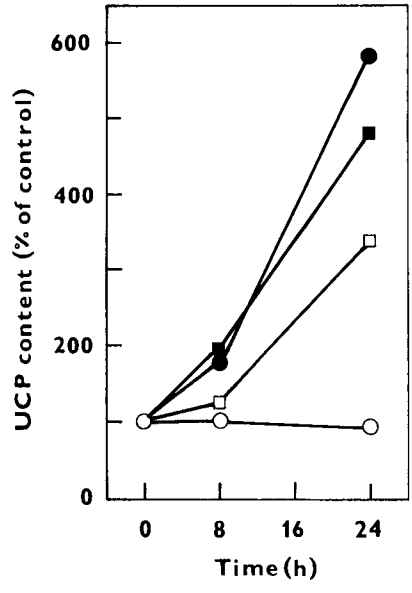

Fig. 2. Specific content of mitochondrial uncoupling protein (UCP) in cells exposed to norepinephrine and dibutyryl-cAMP. Cultured BAT cells were incubated for the indicated times with 10 μM (□) or 0.1 μM norepinephrine (■) or 1 mM dbcAMP (•). Untreated cells (○) served as controls. 10 μg protein aliquots of cells harvested by scraping were analysed by immunoblotting for UCP content. Data are the means of two experiments performed in duplicate.

Table II

Effects of adrenergic and cAMP-concentration-modulating agents on 5'D activity in cultured BAT cells

Agent	5'D activity (fmol T_3/h/mg protein)	(*n*)
Control	6.0 ± 3.7	23
Norepinephrine (10 μM)	42.4 ± 12.1	12
Isoprenaline (10 μM)	37.2 ± 9.5	7
Phenylephrine (10 μM)	8.8 ± 8.4	5
Forskolin (1 μM)	98.2 ± 38.3	5
dbcAMP (1 μM)	101.1 ± 36.7	7
Norepinephrine (1 μM)	27.1 ± 5.1	6
+ propranolol (10 μM)	9.8 ± 3.7	4
+ prazosin (10 μM)	21.3 ± 5.7	4

Cultured BAT cells were exposed for 8 h to the agents indicated. Values are the means ± SD from *n* experiments performed in duplicate.

5.8-fold, respectively) and the optimal concentration of norepinephrine was 0.1 μM. The lower induction of UCP at 10 μM norepinephrine is in accordance with previous data on synthesis of UCP and its mRNA in postconfluent cells [7,20,26]. The data indicate that the same adrenergic and cAMP-mediated stimulation activates the synthesis of both 5'D and UCP but the control of expression of the two genes appears to be different. This is not surprising in the light of evidence that active 5'D and binding of locally produced T_3 to nuclear receptors [27] is required for induction of UCP synthesis. Interestingly, the perinatal development of BAT in several species shows coincidental, but not exactly parallel changes in 5'D activity and UCP content [5,7,24,28].

The extent of stimulation of 5'D activity in cultured BAT cells exposed for 8 h to different adrenergic and cAMP modulating agents is shown in Table II. Isoproterenol (10 μM), the β-selective agonist, was found to be similarly effective as α_1, β-agonist, 10 μM norepinephrine (6.2–7-fold increase, respectively) while the effect of the α_1-agonist phenylephrine (10 μM) was negligible. An even more pronounced, 16-fold increase in 5'D activity was induced by 1 μM forskolin, an activator of adenylate cyclase, in agreement with activation of 5'D caused by membrane-permeant dbcAMP (1 mM). The effect of the subtype-selective adrenergic antagonists was tested by using propranolol and prazosin in a 10-fold excess over norepinephrine (Table II). The induction of 5'D activity in cultured brown adipocytes by 1 μM norepinephrine was almost completely abolished (82%) by 10 μM propranolol, the β-selective antagonist. The α_1-selective antagonist prazosin (10 μM) was significantly less inhibitory and prevented only 27% of norepinephrine-induced 5'D activity.

The results obtained with cultured BAT cells thus convincingly demonstrate that adrenergic stimulation of 5'D synthesis involves predominantly β-adrenergic receptors and changes in cellular cAMP, similarly as found in cultured astroglial cells [29], or pineal glands [30]. The large extent of 5'D stimulation and in vitro conditions rule out the possibility of an indirect effect of β-agonist [16]. At variance with in vivo experiments [12,16], and experiments with cells isolated from BAT of euthyroid and hypothyroid rats [19], little stimulation of 5'D by phenylephrine and low inhibitory effect of prazosin as well as significantly higher activation of 5'D by dbcAMP and forskolin than by norepinephrine suggest that α_1-receptors are only weakly involved in adrenergic stimulation of 5'D in cultured BAT cells.

Acknowledgements: Expert technical assistance of V. Fialová and H. Nováčková is gratefully acknowledged.

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