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Characterization of the novel brown adipocyte cell line HIB 1B

Adrenergic pathways involved in regulation of uncoupling protein gene expression

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SUMMARY

The HIB 1B cell line, derived from a brown fat tumor of a transgenic mouse, is the first established brown adipocyte cell line capable of expressing the brown fat-specific mitochondrial uncoupling protein (UCP). UCP gene expression, which was virtually undetectable under basic conditions, was stimulated by acute catecholamine or cyclic AMP treatment to levels comparable to primary cultures of brown adipocytes. Elevation of UCP mRNA levels following stimulation was very rapid but transient, decreasing after about 4 hours with a half-life between 9 and 13 hours. Immunoblotting showed the presence of UCP in HIB 1B mitochondria, but expression was much lower than observed in BAT or primary cultures of brown adipocytes. Upon transfection of HIB 1B cells with a reporter gene containing the UCP promoter, the activity of the transgene was regulatable by cAMP and norepinephrine. Investigation of the possible adrenergic receptors involved in UCP stimulation showed that specific β3-adrenergic agonists were much less effective than nonspecific β-adrenergic agonists and that mRNA levels of the atypical, fat-specific β3-adrenoceptor were lower than those observed in brown adipocytes differentiated in primary culture. From pharmacological evidence we conclude that β3-adrenergic receptors account for approximately 30-40% of catecholamine induced UCP gene stimulation, whereas about 60-70% is stimulated via the classical β1/2 adrenergic pathway. We conclude that HIB 1B cells represent a functional system for the study of mechanisms related to brown adipose thermogenesis.

Key words: beta3 adrenergic receptor, brown fat thermogenesis, hormonal regulation of gene expression

INTRODUCTION

In addition to the energy-storing white adipose tissue (WAT), mammals possess a specialized type of fat, the brown adipose tissue (BAT). BAT functions as an energy-dissipating tissue, responsible for non-shivering thermogenesis in small and newborn mammals. BAT may also play an antiobesity function in overfed animals (Rothwell and Stock, 1986). Heat production in brown adipocytes requires the presence of a unique uncoupling protein (UCP) in the inner mitochondrial membrane, which acts as a proton channel and thus uncouples the respiratory chain from ATP production (reviewed by Nicholls et al., 1986; Ricquier et al., 1991; Klaus et al., 1991a). Histologically, adipocytes from white and brown adipose tissue can be distinguished quite easily. White adipocytes possess unilocular lipid depositions and scarce, poorly developed mitochondria. Brown adipocytes are typically smaller, characterized by numerous lipid depositions in multilocular lipid droplets, and a high number of well-developed mitochondria, which are bigger than those in white adipocytes and have a higher cristae surface area (for review see Néchad, 1986). Biochemically there are numerous quantitative differences between WAT and BAT, but so far the only qualitative difference that has been found is the unique presence of UCP in brown adipocytes (Cannon et al., 1982; Ricquier et al., 1992). White and brown adipose tissue are usually found in distinct depots, but there exists an apparent conversion of BAT into WAT in larger mammals (Casteilla et al., 1989). This and the fact that UCP-containing brown adipocytes have recently been detected in typical WAT depots (Loncar, 1991; Cousin et al., 1992) make it unclear as to whether white and brown adipocytes are truly distinct or if there is interconversion between the two cell types.

In vivo studies have shown that UCP expression in BAT is stimulated by catecholamines, originating from sympathetic innervation of BAT (Ricquier and Mory, 1984). Over the past years evidence has accumulated indicating the involvement of an atypical β-adrenoreceptor (β-AR) in this signalling pathway (Arch, 1989). Indeed, so called β3-ARs from human, mouse and rat species have recently been cloned and found to be expressed in both BAT and WAT (Emorine et al., 1989; Nahmias et al., 1991; Muzzin et al., 1991; Granneman et al., 1991).

In vitro studies on brown adipocyte development and the regulation of UCP gene expression have been hindered by the lack of an immortalized brown preadipocyte cell model that could be induced to express UCP upon differentiation in culture. Several groups have successfully established primary cell culture systems allowing the growth and differentiation (including UCP expression) of isolated brown preadipocytes in primary cell culture, first from mouse BAT (Rehnmark et al., 1989, 1990; Kopecky et al., 1990; Champigny et al., 1992) and then from other species like newborn lamb (Casteilla et al., 1991), Siberian hamster (Klaus et al., 1991b) and rat (Champigny et al., 1992). Kozak et al. (1992) obtained immortalized cells from hibernomas of transgenic mice. UCP expression, however, was transient in all these models and completely lost after passaging of cells. Only recently, the first immortalized cell line expressing UCP was obtained from the hibernoma of a transgenic mouse (Ross et al., 1992). These mice contained a transgene with the adipocyte-specific regulatory region from adipocyte P2 (aP2) gene linked to the simian virus 40 transforming genes. Most of these mice developed hibernomas, which we used to generate cell lines. One of these cells lines, HIB 1B, was found to be able to express the UCP gene in a differentiation-dependent manner when stimulated with catecholamines or cyclic AMP (Ross et al., 1992). In this study, we investigated more extensively the characteristics of UCP expression in HIB 1B cells with regard to kinetics, hormonal requirements, and possible receptor subtypes involved in adrenergic signal transduction.

MATERIALS AND METHODS

Cell cultures

Primary cultures of preadipocytes isolated from mouse interscapular BAT were performed as described by Champigny et al. (1992). The culture medium for HIB 1B cells and primary cultures was a mixture of Dulbecco's modified Eagle's medium (DMEM)/Ham's F-12 nutritive medium (1:1, v/v) supplemented with 0.016 mM biotin, 0.018 mM pantothenic acid, 5 mM glutamine, 15 mM glucose, 15 mM Hepes (pH 7.4), 100 i.u./ml penicillin, 50 μg/ml streptomycin. For primary cultures 20 nM insulin and 2 nM triiodothyronine (T3) were added and medium was supplemented with 10% fetal calf serum (FCS). HIB 1B cells were cultured in the standard medium supplemented with 10% FCS unless otherwise indicated in the text. At confluence (around 4-5 days after plating) cells were refed and insulin (17 nM) and T3 (1 nM) were added. Seven days later cells were usually differentiated and experiments performed. For experiments in serum-free medium plates were rinsed twice with PBS before addition of the medium. For repeated passaging cells were split 1:15 at confluence. Products for cell culture were obtained from Gibco-BRL, other chemicals from Sigma except CGP 12177 (Ciba Geigy) and the active metabolite of D7114, which was a gift from Dr B. Holloway (ICI Pharmaceuticals).

Transfection experiments

Differentiated HIB 1B cells were transfected using the calcium phosphate precipitation method. Cells were transiently cotransfected with a chloramphenicolacetyl transferase (CAT) reporter plasmid containing a 4.5 kb rat UCP gene promoter (Cassard-Doulcier et al., 1993) together with a plasmid encoding β-galactosidase. Detailed procedure and enzymatic assays were as described by Cassard-Doulcier et al. (1993).

RNA analysis

RNA was extracted by the single-step method using guanidinium thiocyanate extraction (Chomczynski and Sacchi, 1987). RNA electrophoresis and northern blotting were performed as described before (Ricquier et al., 1986). Equal loading of gels was checked visually after staining of membranes with bromophenol blue. The following probes were used for hybridization: rat UCP (Bouillaud et al., 1985), mouse aP2, mouse glycerol-3-phosphate dehydrogenase (GPD), mouse adipsin (Spiegelman et al., 1983), mouse lipoprotein lipase (LPL; Kirchgessner et al., 1987), total mouse mitochondrial genome (Bibb et al., 1981), human ribosome-associated protein (Laborda, 1991), and mouse β3-adrenergic receptor (a 1168 bp fragment, obtained by PCR amplification of a reverse transcription reaction on mouse brown adipose tissue poly(A)$^{+}$ RNA, with sense primer GCTCCGTGGCCTCACAGAAA matching positions 4-23 and antisense primer TCCCCTACCTGTTGAGCGGT matching positions 1167-1148 of the mouse β3-AR (EMBL data bank: MMB3AR)). All experiments were performed at least twice with cells of different passage number.

Western analysis of UCP

Mitochondria were isolated by differential centrifugation, and immuno-blotting was performed as described before (Klaus et al., 1991b), using an anti-rat UCP antibody raised in sheep.

RESULTS

Growth and differentiation

When grown in standard adipocyte culture medium supplemented with 10% FCS, insulin and T3, HIB 1B cells reached confluency after about 4 to 5 days of culture and showed a distinct differentiated phenotype about 1 week later. Neutral lipid accumulation could be confirmed by Oil Red O staining (not shown). Preadipocytes during exponential growth resembled preadipocytes grown in primary cell culture. After differentiation, however, HIB 1B cells could quite easily be distinguished from adipocytes in primary cell culture by their smaller size and their tendency to form multiple layers. FCS content could be reduced to 5% without significant changes in growth rate and differentiation of HIB 1B cells. Fig. 1 shows a time course of the gene expression of some adipocyte differentiation markers. Isoproterenol-induced UCP expression was undetectable in preconfluent cells and increased rapidly from day 4 after confluence. This coincided with the appearance of GPD (glycerol-3-phosphate dehydrogenase) mRNA, which is considered a late differentiation marker. A similar expression pattern was found for the mRNA of adipsin, a serine protease synthesized and secreted by adipocytes (Cook et al., 1985). The early differentiation marker lipoprotein lipase (LPL) appeared around confluence and aP2 expression was always detectable.

Detection of UCP and UCP mRNA expression

Fig. 2A shows the time course of UCP mRNA expression in differentiated HIB 1B cells after stimulation with isoproterenol or dibutyryl cAMP (db-cAMP). After addition of the respective compounds, UCP mRNA increased rapidly until 4 hours after addition, when a maximum was reached. After that, UCP mRNA levels decreased very rapidly, with a half-life of 8.6 hours in the case of isoproterenol stimulation. Part of this decrease could be due to a desensitization of the β adrenoreceptor, because in the presence of db-cAMP the decrease was

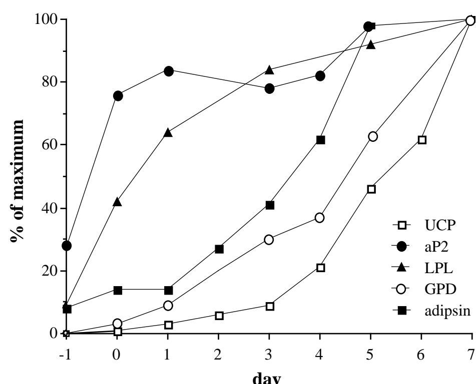
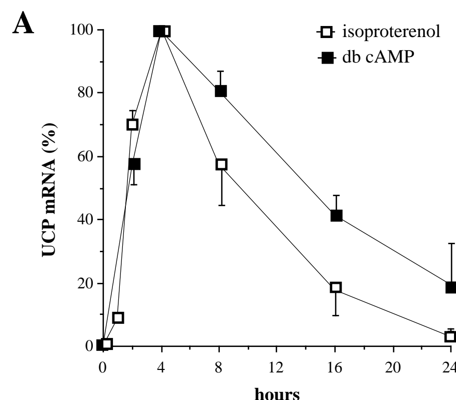


Fig. 1. Time course of expression of adipocyte genes during differentiation of HIB 1B cells. Cells were harvested for RNA extraction on the day indicated, day 0 being the day of confluence. For UCP, cells were treated with 1 μM isoproterenol 2 hours prior to harvest. Northern blotting was performed using 10 μg total RNA, and the membranes were probed sequentially with UCP, LPL, aP2, GPD and adipsin. Signal intensity was quantitated via PhosphorImager analysis (Molecular Dynamics) using ribosomal-associated protein probe as a control. These values were converted into percentage of the maximum level of expression for each marker. The average values from two independent experiments are represented.



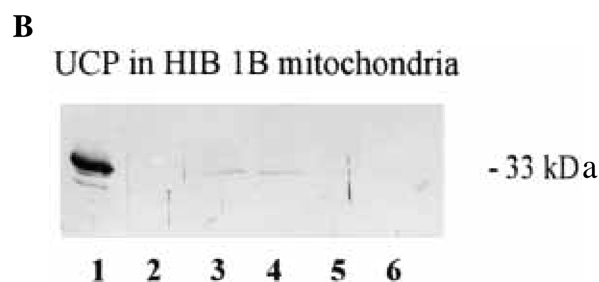


Fig. 2. Expression of UCP mRNA (A) and UCP (B) in differentiated HIB 1B cells that were kept overnight in a serum-free medium supplemented with insulin (17 nM) and T3 (1 nM). (A) Isoproterenol (1 μM) or db-cAMP (1 mM) were added into the medium and cells harvested at different times for RNA extraction. Northern blotting was performed with 20 μg of total RNA using a UCP cDNA probe. Densitometrical scanning of the autoradiographs was performed for quantification of the signal. Each curve represents the means ± s.e.m. of 3 to 4 independently performed experiments. (B) Mitochondria were isolated by differential centrifugation and 10 μg mitochondrial protein separated by SDS-PAGE (lanes 2 to 6). After transfer on a nitrocellulose membrane, western analysis was performed with an anti-rat UCP antibody. Cells were kept for 24 hours in a serum-free medium containing insulin (17 nM) and T3 (1 nM). Before harvest they were treated for 8 hours with 1 μM isoproterenol (lane 3), 1 mM db-cAMP (lane 4), 0.1 μM isoproterenol (lane 5) and for 24 hours with 0.1 μM isoproterenol (lane 6). Lane 1 contains 4 μg mitochondrial protein from rat brown fat.

slower (half-life 13 hours). Maximum levels of UCP mRNA expression were quite comparable to those observed in primary cultures of mouse brown adipocytes (data not shown), but in primary cultures no such rapid decrease of UCP mRNA levels was observed (Klaus et al., 1991b; Champigny et al., 1992).

To find out if UCP mRNA was translated into protein, we isolated mitochondria from differentiated HIB 1B cells and performed western analysis with an anti-rat-UCP antibody (Fig. 2B). Not surprisingly, no UCP could be found in control cells, but after 8 hours of treatment with isoproterenol or db-cAMP some UCP could be detected. The amount of UCP was decreased after 24 hours treatment and, contrary to UCP mRNA levels, UCP levels were much lower than those observed in tissue or primary culture of brown adipocytes.

We tranfected differentiated HIB 1B cells with a CAT reporter gene made of 4.5 kb upstream of the transcription start site of rat UCP gene. This region was shown to contain the *cis*-elements necessary for tissue specific expression as well as hormone-sensitive sites (Cassard-Doulcier et al., 1993). The transfection efficiency was lower than in primary cultures of brown adipocytes, CAT expression however was measurable (Fig. 3). In control cells CAT expression was very low, but a considerable increase (approximatively 4-fold) could be observed after treatment of transfected cells with db-cAMP or norepinephrine, indicating an increased activity of the transfected DNA.

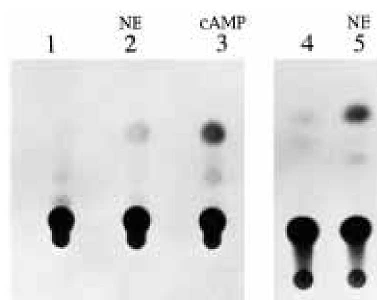


Fig. 3. CAT activity in cells transfected with UCP gene promoter. Differentiated HIB 1B cells were transiently transfected with 30 μg CAT plasmid containing 4.5 kb UCP promoter. CAT activity was normalized to β-galactosidase activity. Two independently performed experiments are shown (1-3, 4-5). Cells were untreated (lanes 1 and 4) or treated for 20 hours with 1 μM norepinephrine (lanes 2 and 5) or 1 mM db-cAMP (lane 3).

β-AR involvement in stimulation of UCP mRNA expression

Fully differentiated HIB 1B cells were treated for 4 hours with different β-adrenergic agonists in a serum-free medium (Fig. 4). Isoproterenol, an unselective β-agonist, proved to be most efficient, considerably increasing UCP mRNA levels at as little as 20 nM. Norepinephrine was somewhat less efficient, requiring a concentration of 200 nM for half-maximal UCP mRNA levels. The selective β3 agonist D7114 (Holloway et al., 1991) proved to be much less potent in stimulating UCP

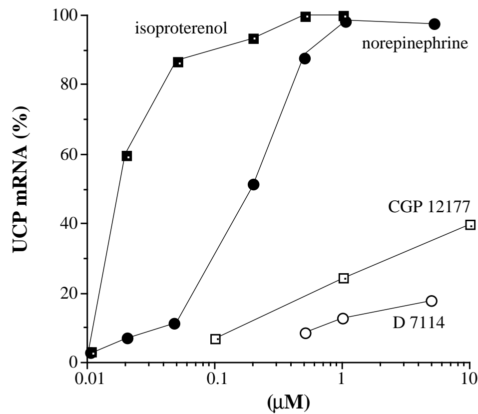


Fig. 4. Dose-response curve of β adrenergic UCP stimulation. Differentiated HIB 1B cells were kept overnight in a serum-free medium (supplemented with 17 nM insulin and 1 nM T3). Cells were harvested for RNA extraction 4 hours after indicated additions. Northern blotting was performed with 20 μg of total RNA using a UCP cDNA probe. Densitometrical scanning of the autoradiographs was performed for quantification of the signals. Values represent means of at least two independently performed experiments.

Table 1. Effect of β-adrenergic antagonists on norepinephrine- and isoproterenol-induced UCP expression

Agonist (M)	Antagonist (M)	UCP mRNA (% of maximum)
Isoproterenol (10^{-7})	–	100
Isoproterenol (10^{-7})	Propranolol (10^{-7})	33
Isoproterenol (10^{-7})	Propranolol (10^{-6})	11
Isoproterenol (10^{-7})	Propranolol (10^{-5})	1.4
Isoproterenol (10^{-7})	CGP 12177 (10^{-7})	31
Isoproterenol (10^{-7})	CGP 12177 (10^{-6})	34
Norepinephrine (10^{-6})	–	100
Norepinephrine (10^{-6})	CGP 12177 (10^{-7})	42
Norepinephrine (10^{-6})	CGP 12177 (10^{-6})	48

Differentiated HIB 1B cells were kept for 20 hours in a serum-free medium containing 17 nM insulin. Cells were harvested for RNA extraction 4 hours after indicated additions. Densitometrical scanning of the autoradiographs was performed for quantification of the signal.

expression; even at 5 μM, only about 25% of the maximal stimulation was observed. We also treated HIB 1B cells with CGP-12177, which is known to be a potent β1/β2-AR antagonist, but also acts as an agonist to the atypical β3-AR (Mohell and Dicker, 1989). This compound induced 33% of maximal UCP expression at a concentration of 10 μM. The stimulatory action of isoproterenol on UCP gene expression in HIB 1B cells could be completely inhibited by a 100-fold excess concentration of propranolol, a β1/2 AR antagonist. At equal concentrations propranolol blocked about 70% of the isoproterenol stimulation (Table 1). The selective β1/2 AR antagonist CGP 12177 inhibited 69% and 58% of the UCP stimulation by isoproterenol and norepinephrine, respectively (Table 1). CGP 12177 has a low affinity for β3-AR (Féve et al., 1992) and at 0.1 μM it acts predominantly as a β1/2 AR antagonist with very little β3-AR agonist action (Fig. 4). It can be concluded that between 30% and 40% of UCP mRNA induction was due to an involvement of β3-AR, whereas 60% to 70% involved the β1/2 adrenergic pathway. This is somewhat different from brown adipocytes differentiated in primary culture, where D7114 and other β3 agonists were shown to be as potent as norepinephrine or isoproterenol in stimulating UCP gene expression (Rehnmark et al., 1990; Klaus et al., 1991b; Champigny et al., 1992). We therefore decided to compare the expression of β3-AR mRNA in HIB 1B and primary mouse brown adipocytes in cultures.

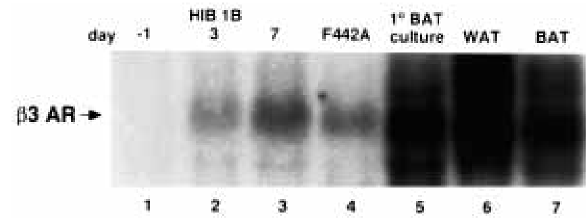


Fig. 5. Expression of β-3AR mRNA in HIB 1B cells and comparison with other adipocytes sources. Total RNA was isolated from HIB 1B adipocytes grown in 10% FCS, 17 nM insulin and 1 nM T3 on the day indicated (before or after confluence). Total RNA was also isolated from fully differentiated 3T3-F442A adipocytes and mouse brown adipocyte primary cultures, and from white and brown adipose tissue of C57 Bl/6 mice. Northern analysis was then performed using a 1168 bp murine β3-AR probe. Lanes 1-3, 30 μg total RNA from HIB 1B cells, day -1, 3 and 7, respectively. Lane 4, 20 μg total RNA from F442A adipocytes. Lanes 5-7, 10 μg total RNA from primary culture, WAT and BAT, respectively. Quantitation of the β3-AR mRNA signal was by PhosphorImager analysis.

β3-AR mRNA expression was detectable in northern blots using 30 μg of total RNA upon overnight exposure (Fig. 5). On the same northern blots we also loaded 20 μg of total RNA from F442A adipocytes, and 10 μg each of total RNA from mouse brown adipocytes differentiated in primary culture and white and brown mouse adipose tissue. The expression of the β3-AR mRNA was differentiation-dependent in HIB 1B cells. In all lanes except lane 1 containing HIB 1B preadipocyte RNA, a major band was observed at 2.4 kb, corresponding in size to previous results on rat BAT and WAT (Muzzin et al., 1991). The amount of β3-AR mRNA was much lower in HIB 1B cells than in primary cultures of mouse brown adipocytes or in adipose tissue. Correcting for the amount of RNA loaded in each lane, there is approximately 1.5-2-fold more β3-AR mRNA observed in F442A adipocytes, and 10- to 13-fold more in primary culture and adipose tissues, than in HIB 1B adipocytes. The relatively low level of β3-AR mRNA in HIB 1B cells compared to primary cultures is in agreement with the pharmacological data regarding the relatively low level of stimulation of UCP expression by β3 agonists in HIB 1B. Together, these results point to a predominantly β1/2-AR-mediated stimulation of UCP gene expression in HIB 1B cells, contrary to the predominant β3-AR effect in primary cell cultures.

Hormonal requirements in a serum-free medium

Differentiated HIB 1B cells could be maintained in a serum-free medium for several days. After 24 hours in a serum-free medium, cells in the presence of insulin had a much higher protein and total RNA content than cells without any hormonal addition or only T3 (Table 2). We also found insulin to be

Table 2. Hormonal requirements of differentiated HIB 1B cells in a serum-free medium

	Total protein (µg/3 cm dish)	Total RNA (µg/10 cm dish)	Isoproterenol-induced UCP mRNA (%)
24 h serum-free	94±11 (*n* =4)	116±9 (*n*=3)	83
24 h serum-free +insulin	234±5 (*n*=4)	309±31 (*n*=3)	100
24 h serum-free +T3	79±9 (*n*=4)	113±19 (*n*=3)	92
24 h serum-free +insulin+T3	237±18 (*n*=4)	364±23 (*n*=3)	98

Differentiated HIB 1B cells were placed for 24 hours in a serum-free medium with additions as indicated. For UCP mRNA measurements, 1 µM isoproterenol was added 4 hours before harvest. Values are means ± s.d.

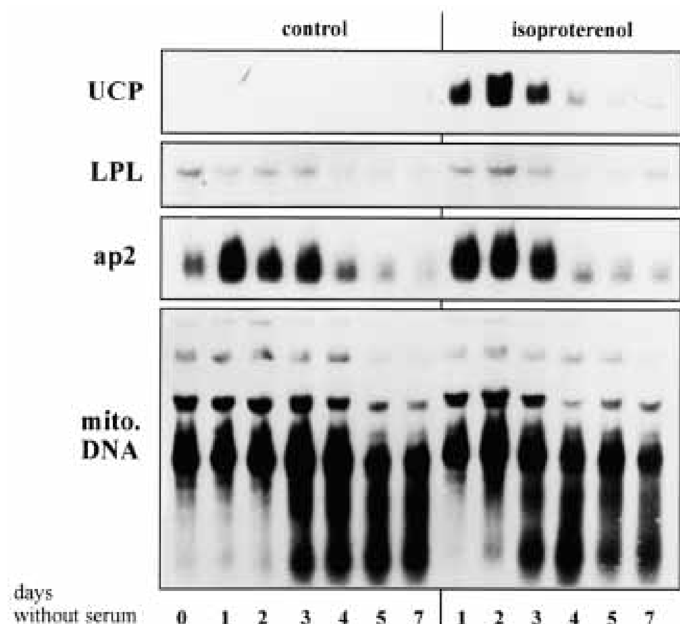


Fig. 6. Northern blot analysis of long-term culture of differentiated HIB 1B cells kept in serum-free medium (containing 17 nM insulin). UCP expression was induced by addition of 1 µM isoproterenol 4 hours before harvest where indicated. Each lane contains 20 µg total RNA. The blot was successively hybridized with a UCP, LPL, aP2 and mitochondrial DNA probe.

essential for growth and differentiation of HIB 1B cells in a serum-free medium (not shown). When we investigated whether UCP induction was influenced by insulin and/or T3 in a serum-free medium, we found that neither insulin nor T3 was necessary for isoproterenol-induced UCP stimulation (Table 2). Slightly higher levels could be observed in the presence of insulin; however, this seems to reflect the general effect of insulin on the differentiation status, as the mRNA levels of other differentiation markers like lipoprotein lipase (LPL), aP2, and mitochondrial transcripts were also slightly elevated in the presence of insulin (not shown). T3, conversely, had no effect on the investigated mRNAs. Maximum UCP mRNA levels were always found to be higher in a serum-free medium than in the presence of FCS (not shown).

We further investigated the effect of long-term culture on differentiated HIB1B cells by maintaining them for 7 days in a serum-free medium, supplemented with insulin (Fig. 6). aP2 mRNA expression considerably increased after 24 hours in serum-free medium and then decreased from day 2 on. LPL mRNA levels slightly decreased in serum-free medium, indicating that the effect of serum withdrawal on aP2 was specific and not an effect on the general differentiation status. When UCP mRNA expression was induced by adding isoproterenol 4 hours prior to harvest, a high level of induction was observed in cells cultured for up to 2 days. After 3 days there was a net decline in UCP mRNA levels. The overall quantity of mitochondrial transcripts did not change considerably during 7 days in serum-free medium but an increase in low molecular mass transcripts could be observed after 2 days, which indicates a degradation of mitochondrial transcripts. Total RNA content increased initially, but gradually decreased after 2 days (data not shown). This may indicate a general cell death after prolonged culture of differentiated cells in serum-free medium.

DISCUSSION

Previous attempts to create a brown adipocyte cell line using classical methods of immortalization had been unsuccessful because repeated passaging of brown preadipocytes resulted in a loss of UCP expression (Forest et al., 1987; S. Klaus, unpublished data), the only definitive marker of brown adipocytes. A much more elegant and less laborious method was used to establish a brown adipocyte cell line using the hibernoma of a transgenic mouse (Ross et al., 1992). The resulting cell line, HIB 1B, has so far been passaged for almost 30 generations without any loss of inducibility of UCP gene expression. HIB 1B cells represent a suitable model for the study of regulation of UCP expression because UCP expression is differentiation-dependent in these cells: essentially undetectable under normal culture conditions, and highly stimulated by catecholamine treatment (Ross et al., 1992; see Figs 1, 2). This is in agreement with transfection experiments (Fig. 3) where the UCP promoter, previously characterized in transfected brown adipocytes in primary cultures and transgenic mice (Cassard-Doulcier et al., 1993) exhibited a low activity in basal conditions and a considerably higher activity in cells treated with cAMP or norepinephrine. It should be noted that the dose-response curve of UCP mRNA induction by norepinephrine is shifted to the right as compared to isoproterenol (Fig. 4). This might indicate the presence of α2-adrenoceptors, which inhibit adenylate cyclase. However, this remains to be established.

HIB 1B cells could be maintained in a serum-free medium, which made it possible to study the possible role of different hormones in regulation of UCP gene expression. Although in vivo studies showed that the main stimulator of UCP expression are catecholamines, permissive and enhancing functions of T3 and insulin have also been demonstrated (Bianco et al., 1988; Reiter et al., 1990; Geloën and Trayhurn, 1990). Insulin has been reported to augment the catecholamine-induced UCP mRNA increase in primary brown fat cultures of mice, and T3 was shown to be essential for this increase (Rehnmark et al., 1990). In cultured brown adipocytes from another species, the Siberian hamster, insulin and T3 were found to synergistically stimulate UCP mRNA expression in serum-free medium to the same extent as cate-

cholamines (Klaus et al., 1991b). Contrary to this, HIB 1B cells obviously need neither T3 nor insulin for UCP gene activation (Table 2). Insulin had a slightly enhancing effect, due to an overall increase in differentiation. Interestingly, Kozak and coworkers (1992) obtained similar results with brown fat tumor cells. They used a hibernoma from a transgenic mouse containing the SV40 T-antigen under control of a mouse urinary protein promoter to establish a transplantable tumor line in nude mice. Brown fat precursor cells isolated from these tumors and grown in tissue culture also showed catecholamine-induced UCP gene expression that did not require the presence of insulin or T3.

Catecholamine action on BAT is thought to be mainly mediated by β-ARs coupled to adenylyl cyclase (Bronnikov et al., 1992, and references therein). The stimulatory action of catecholamines on UCP expression in HIB 1B cells could indeed be mimicked by db-cAMP (Fig. 2), which also stimulated expression of the transfected DNA in cells transfected with the UCP promoter (Fig. 3). It is well established in vivo as well as in tissue culture that the main β-AR involved in thermogenic function of brown fat is the atypical, β3-AR (Arch, 1989; Rehnmark et al., 1990; Klaus et al., 1991b; Bronnikov et al., 1992; Champigny et al., 1992). Investigation of the possible adrenergic receptors involved in induction of UCP expression revealed a somewhat different picture in HIB 1B cells. The dose-response and inhibition experiments (Fig. 4, Table 1) indicate a preferential utilization of classical β1/2-ARs in catecholamine-induced UCP gene stimulation, with β3-AR accounting for 30 to 40% of maximum induction. This correlates with low levels of β3-AR mRNA in HIB 1B cells compared to mouse brown adipocytes in primary culture (Fig. 5). Kozak et al. (1992) found no evidence of utilization of the β3 receptor in brown fat tumor cells and proposed activation of the β1-AR. However, they did not investigate the expression of the β3-AR mRNA in their cell system. Obviously, stimulation of UCP gene expression can result from both β1/2-ARs and β3-AR activation, which is not surprising as both β1 and β3-AR are able to stimulate the same adenylyl cyclase (Granneman, 1992). One intriguing observation, however, is the very rapid decrease of UCP mRNA levels in HIB 1B cells after initial stimulation in the presence of either isoproterenol or db cAMP. Not surprisingly, this results in much lower levels of UCP in the mitochondrial fraction as compared to primary cultures, where UCP mRNA levels stay elevated for a long time after initial stimulation (Klaus et al., 1991b; Champigny et al., 1992). UCP transcription is either very rapidly turned off or there is a rapid degradation of UCP mRNA (or both) in HIB 1B cells. It remains to be established if this is in any way connected to the preferential utilization of β1-AR in HIB 1B cells as opposed to β3-AR in primary cell cultures.

HIB 1B cells qualify as 'true' brown fat cells according to the definition that UCP expression is restricted to brown adipocytes. They also show expression of some adipocyte markers (i.e. GPD, adipsin, see Fig. 1) in a differentiation-dependent manner. However, HIB 1B cells also show some characteristics distinguishing them from brown fat cells in primary cultures as well as established lines of white preadipocytes. For example, they express the adipocyte markers aP2 and LPL even in the undifferentiated state (Ross et al., 1992; Fig. 1). This could be due to the use of a differentiation-linked regulatory element from the ap2 gene to drive T-antigen expression, thus possibly linking transformation to differentiation. HIB 1B cells show some similarities to the brown fat tumor cells described by Kozak and coworkers, indicating that there might be an effect of the transforming SV40 T-antigen on characteristics of these cells. However, some of the differences between HIB 1B cells and primary culture systems could be due to the fact that primary culture systems contain heterogeneous cell populations. For example, it is known that BAT contains many mast cells (Mory et al., 1983), and it is conceivable that these or other cell types, if present in the culture, might release paracrine or autocrine factors influencing gene expression.

HIB 1B cells should provide a very useful model for the study of regulatory factors involved in UCP gene transcription, which is facilitated by the fact that these cells do not need any hormones other than catecholamines for UCP gene stimulation. These cells are suitable for transfection studies and could be used for identification of hormone control elements in the UCP promoter. We are also currently investigating different subclones of HIB 1B, which seem to be more responsive to β3-AR agonists and show higher expression of β3-AR mRNA. These different subclones might be useful for the differential screening of potential atypical β3-adrenergic agonists.

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