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Regulation of UCP gene expression in brown adipocytes differentiated in primary culture. Effects of a new β-adrenoceptor agonist

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Summary

Primary cultures of precursor cells from mouse and rat brown adipose tissue (BAT) were used to study the effect of a new β-agonist (ICI D7114) on the uncoupling protein (UCP) gene expression. ICI 215001 (the active metabolite of D7114) increased the expression of UCP and its mRNA in brown adipocytes differentiating in vitro in a dose-dependent manner. This stimulating effect was not inhibited by propranolol, a non-specific β-antagonist, but was partially reduced by bupranolol, a β_3-antagonist. No expression of UCP mRNA was ever induced by ICI 215001 in white adipocytes differentiated in vitro. It was concluded that the drug could affect the brown adipose cells through a β_3-pathway. It could clearly modulate the expression of UCP in brown adipocytes differentiated in vitro, but was not able by itself to turn on the gene.

Introduction

Brown adipose tissue (BAT) is characterized by its thermogenic capacity, due to the presence of a mitochondrial protein, unique to this tissue, the uncoupling protein (UCP) (Ricquier and Bouillaud, 1986). BAT is responsible for the increase in heat production induced by cold exposure or by excess of food intake (diet-induced thermogenesis, DIT) in young mammals. A defect in BAT-mediated DIT may have a role in the development of obesity, since it has been shown that genetically obese mice (*ob/ob*) and Zucker rats (*fa/fa*) have defective brown fat thermogenesis (Rothwell and Stock, 1986; Trayhurn, 1986; Himms-Hagen, 1989). For these reasons interest of obesity research has been focused on this tissue in recent years. Even though in large mammals (ovine, bovine, man) BAT is only recognizable in neonates and is undetectable, or present only in small quantities, in adults (Lean and James, 1986; Casteilla et al., 1989), UCP has been found in perirenal adipose tissue of human patients with some pathologies as pheochromocytoma or aldosteronism (Ricquier and Mory, 1984; Lean et al., 1986; Garruti and Ricquier, 1992). UCP gene expression can therefore be induced in

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adult adipose tissues in certain conditions. BAT is under control of the sympathetic nervous system and is stimulated by catecholamines via a mechanism implying atypical β-adrenoceptors (Arch et al., 1984; Arch, 1989; Scarpace and Matheny, 1991). Several β-agonists have therefore been developed in recent years and tested on animals for their ability to stimulate heat production and decrease food efficiency (Arch et al., 1987). A novel β-adrenoceptor agonist, ICI D7114 (ICI Pharmaceuticals) (Holloway et al., 1991) was recently shown to induce the appearance of UCP mRNA in different adipose depots in adult dogs, whilst no UCP could be detected in untreated animals (Champigny et al., 1991). In parallel to these in vivo experiments on whole animal, cell culture was used to confirm that this drug was able to act directly on the adipose cells differentiating in vitro.

Cell culture is indeed a useful tool to study the regulation of gene expression by different effectors, the mechanisms involved and the timing for maximum efficacy. Many preadipose cell lines have been established, but the attempt to obtain a brown cell line able to express UCP has proven unsuccessful (Forest et al., 1987a, b). Therefore interest has been focused in the last 10 years on primary culture. Only recently conditions have been defined for some species including mouse (Rehnmark et al., 1989), Djungarian hamster (Klaus et al., 1991b) and ovine (Casteilla et al., 1991), but no expression of UCP has ever been obtained in rat BAT cells differentiating in primary culture (Néchad et al., 1983, 1987; Néchad, 1983; Cigolini et al., 1986; Forest et al., 1987b; Herron et al., 1989).

In the present work we have determined the culture conditions necessary for the achievement of full differentiation of precursor cells from rat BAT. Primary cultures of preadipocytes from mouse and rat BAT were then used to study the effect of a new β-agonist developed by ICI Pharmaceuticals (ICI D7114) on UCP gene expression and provide more evidence on the β-adrenergic regulation of this gene. Since ICI D7114 is metabolized in vivo to yield an active acid metabolite, ICI 215001 (B. Law, unpublished observation), we have used this compound for in vitro studies.

Materials and methods

Materials

Culture media and fetal calf serum were purchased from Gibco. Collagenase and crystalline bovine insulin were obtained from Boehringer. Triiodothyronine, human transferrin, isoproterenol, propranolol, anti-sheep immunoglobulins linked to peroxidase and all other compounds were purchased from Sigma Chemical Co. Bupranolol was obtained from Laboratoires Logeais. Nylon membranes (Hybond-N) for Northern blotting, [^{32}P]dATP and Multiprime DNA labelling system were obtained from Amersham. Nitrocellulose membranes for Western blotting were purchased from Sartorius.

Cell isolation and culture

Three-week-old male mice (BALB/c outbred strain) or rats (Wistar strain) were obtained from Iffa-Credo and kept for several days at 22°C. They were killed by cervical dislocation and interscapular brown fat was immediately removed. Stromal vascular cells were isolated according to Rehnmark et al. (1989).

Mouse cells were inoculated into 100 mm plastic dishes (Nunclon; 1500 cells/cm^2) and cultivated in a mixture of Dulbecco modified Eagle's medium (DMEM)/Ham's F-12 nutritive medium (1 : 1; v/v) supplemented with biotin 0.016 mM, pantothenic acid 0.018 mM, glutamine 5 mM, glucose 16 mM, Hepes 15 mM, penicillin 100 U/ml, streptomycin 50 μg/ml and ascorbate 100 μM. Insulin 20 nM and triiodothyronine 2 nM were added to this 'complete' medium, which was supplemented with 10% fetal calf serum (FCS). The medium was changed on day 1 and partially (5 ml out of 10 replaced by 10 ml fresh medium) on day 3. When cells were to be harvested on day 10, the medium was changed again on day 9. ICI 215001 was added in some dishes either from day 1 or 3 (chronic treatment), or 4 h before harvesting (acute treatment). The β-antagonists were added together with ICI 215001 in the case of chronic treatment, and 1 h before the drug in the case of acute treatment.

Precursor cells isolated from rat BAT were suspended in the 'complete' medium described above supplemented with 10% FCS and plated

into 100 mm plastic dishes at a density of 3500 cells/cm^2. On day 1 the dishes were washed with phosphate buffered saline (PBS) and filled with 10 ml of 'complete' medium supplemented with insulin 510 nM, triiodothyronine 0.2 nM and transferrin 10 μg/ml. This serum-free chemically defined medium has been referred to as ITT medium according to Deslex et al. (1987). ICI 215001 was added in some dishes either from day 1 or 3 (chronic treatment) or 4 h before harvesting (acute treatment) and cells were harvested on days 5 and 7.

Cell harvesting, RNA extraction and mitochondrial isolation

For RNA extraction cells were washed twice with PBS and scraped directly in specific lysis buffer (10 mM sodium acetate, 50 mM NaCl, 0.5% sodium dodecyl sulfate, 50 mM aurintricarboxylic acid). Total RNA was extracted by the hot phenol method according to Brawerman (1983).

For mitochondria isolation, cells were washed twice with PBS, scraped in 10 mM Tris, 250 mM sucrose, 1 mM EDTA and homogenized in a glass/Teflon Potter-Elvehjem homogenizer. Mitochondria were isolated by differential centrifugation.

Northern and Western blotting

Samples (15–20 μg) of total RNA were electrophoresed either in 1.5% agarose gels in 20 mM NaH_2PO_4 pH 7.0, 2.2 M formaldehyde or in 1.2% agarose gels in 20 mM Mops buffer, 0.37 M formaldehyde. They were transferred to nylon membranes in 20 × SSC (saline sodium citrate buffer: 0.3 M citrate acid trisodium, 3 M NaCl) according to the Northern procedure. A cDNA probe coding for rat UCP (Bouillaud et al., 1985) was ^{32}P-labelled and was used to hybridize RNA at 42°C in the presence of 50% formamide (v/v) as previously described (Champigny and Ricquier, 1990). The same blots were also hybridized with a cDNA probe coding for lipoprotein lipase (LPL) (Kirchgessner et al., 1987) and with mouse mitochondrial genomic DNA (Bibb et al., 1981).

Samples (50 μg) of mitochondrial proteins were electrophoresed in 12% polyacrylamide gels in 25 mM Tris, 200 mM glycine, 0.1% sodium dodecyl sulfate (SDS) and electrotransferred onto nitrocellulose membranes. UCP was revealed by immunodetection, using an anti-rat UCP raised in sheep (Ricquier et al., 1983). Anti-sheep immunoglobulins (Sigma) linked to peroxidase were used as second antibody.

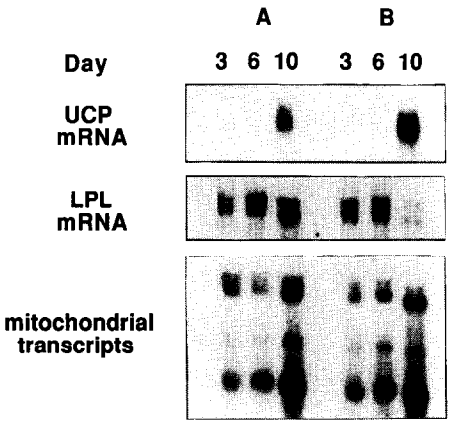

Fig. 1. Chronology of appearance of UCP mRNA in mouse brown adipocytes differentiating in vitro. Comparison with LPL mRNA and mitochondrial transcripts. Each lane corresponds to 20 μg of total RNA, isolated from control cells (*A*) or cells treated with ICI 215001 10^{-6} M (*B*). Cells were cultured in DMEM/F-12 medium supplemented with nutrients, antibiotics and hormones, as described in Materials and methods, and with 10% fetal calf serum. The medium was changed on day 1, partially on day 3 and renewed on day 9. ICI 215001 was added to half the dishes on day 1 and renewed on days 3, 6 and 9. Cells were harvested on days 3, 6 and 10.

Results

Chronology of appearance of UCP mRNA in mouse brown fat cells differentiating in vitro

Precursor cells from mouse BAT were cultivated in serum-supplemented medium as described in Materials and methods. Cells were harvested on days 3 (preadipocytes), 6 (about confluence) and 10 (differentiated cells filled with lipid droplets). Total RNA was extracted and analyzed on Northern blots, as described in Materials and methods.

UCP mRNA could not be detected in cells harvested on days 3 and 6 (Fig. 1); this confirmed that there was no contamination of the precursor cells by mature brown adipocytes at the start of the culture. On day 10 the proportion of differentiated cells was high: 80–90%. A signal corre-

sponding to UCP mRNA was then clearly visible on the Northern blot. The hybridization of the same blot with mitochondrial genomic DNA revealed a clear increase in the mitochondrial transcripts at day 10, when UCP mRNA appeared. LPL mRNA was already present on day 3 of culture.

The presence in the medium, from day 1, of ICI 215001 did not induce a premature appearance of UCP mRNA, but stimulated its expression in mature brown adipocytes. It had no effect on mitochondrial transcripts, but induced a decrease in LPL mRNA on day 10.

Evolution of the cell sensitivity to β-stimulation with the time of culture

The effect of chronic or acute addition of ICI 215001 to the culture medium on UCP gene expression was compared at different times of culture.

In a preliminary experiment (results not shown) cells were harvested 6, 9, 12, 15 and 18 days after plating. Acute treatment with the drug (4 h before harvesting) on day 6 (confluence) was able to trigger the expression of UCP mRNA, while chronic treatment had no effect at this time. From day 15 on, cells accumulated larger droplets

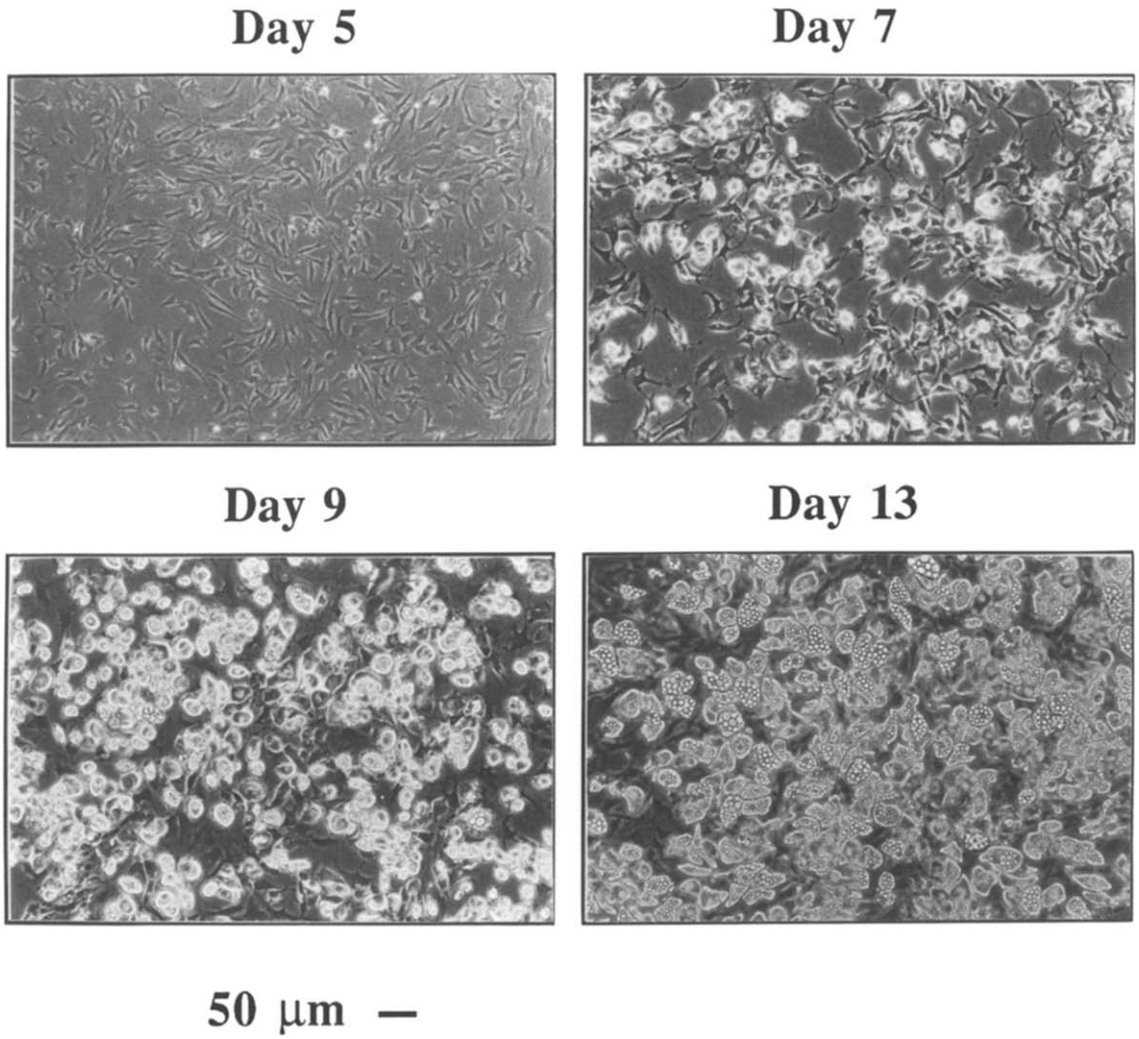

Fig. 2. Growth, differentiation and aging of mouse BAT cells in primary culture. Conditions of culture are the same as in Fig. 1. Magnification of the microphotographs: 50×.

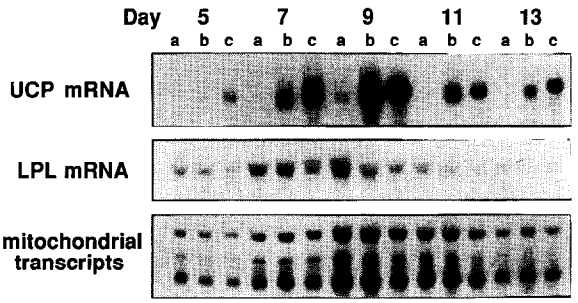

Fig. 3. Variations in UCP gene expression according to the duration of cell culture; effect of chronic or acute treatment with ICI 215001. Mouse BAT cells were cultured as previously described. ICI 215001 10^{-7} M was added to some dishes either on day 3 of culture and renewed every 3 days (chronic treatment; lanes b), or 4 h before harvesting (acute treatment; lanes c). Lanes (a) correspond to control cells. Cells were harvested on days 5, 7, 9, 11 and 13. Each lane corresponds to 15 μg of total RNA.

of lipids and β-stimulation of UCP mRNA was no longer observable. Another experiment was carried out to study more precisely the period of sensitivity of the cells to the drug. Cells were harvested on days 5, 7, 9, 11 and 13 (Fig. 2). They expressed UCP mRNA as soon as on day 5, when they were acutely stimulated by ICI 215001 (Fig. 3), though their aspect was still fibroblastic (Fig. 2). Surprisingly there was a difference between chronic and acute treatment, though the pattern of stimulation was the same; the maximal response occurred on day 7 for acute treatment and on day 9 for chronic treatment. With increasing time of culture, cells seemed to become less sensitive to the drug and the level of UCP mRNA decreased. The expression of LPL mRNA followed a similar pattern of evolution with the duration of culture, being maximal on days 7–9 and decreasing afterwards. There was again a decrease in LPL mRNA in the cells treated with the β-agonist, especially after acute treatment. Maximum expression of mitochondrial transcripts was observed on day 9. It was on the whole not affected by the presence of the drug; only the acute treatment led apparently on day 9 to an unexplained decrease in mitochondrial transcripts.

A new model: the rat BAT cell culture. Efficacy of ICI 215001 on UCP mRNA expression

When cultured in the same conditions as described for mouse cells, rat BAT preadipocytes grew to confluence, acquired the morphology of mature adipocytes accumulating lipids, but never expressed UCP mRNA, whatever the treatment tested: catecholamines, glucocorticoids, β-agonists (results not shown). We therefore decided to try a culture in serum-free medium conditions, as described for rat white fat cells by Deslex et al. (1987).

Rat BAT precursor cells were plated at a density of 3500 cells/cm^2 in 'complete' medium supplemented with 10% FCS. On day 1 the cells were washed with PBS and refed with ITT medium. ICI 215001 10^{-7} M was added in some dishes either on day 1 or 3 (chronic treatment), or 4 h before harvesting (acute treatment). The removal of FCS induced a growth arrest. Cell morphology was rapidly modified and lipid accumulation began. From day 5 on there was a good proportion of differentiated cells: 60–70%. Cells were harvested on days 5 and 7. Northern blot analysis revealed that UCP mRNA was clearly expressed. The β-stimulation by chronic or acute addition of ICI 215001 increased this expression (Fig. 4).

We have therefore succeeded in obtaining a new model of primary culture for brown fat cells:

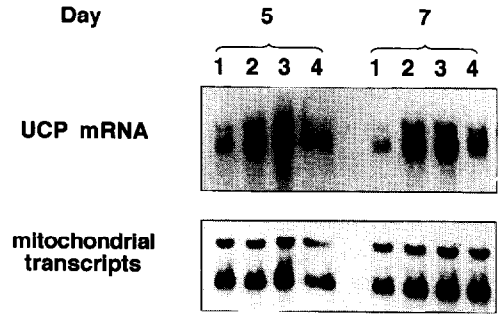

Fig. 4. Expression of UCP mRNA in rat brown adipocytes differentiated in vitro; effect of β-stimulation. Cells were cultured in ITT serum-free medium from day 1 of culture, as described in Materials and methods. Each lane corresponds to 15 μg of total RNA isolated from control cells (lanes 1), or cells treated with ICI 215001 10^{-7} M from day 1 (lanes 2), from day 3 (lanes 3), or 4 h before harvesting (lanes 4).

the rat model. Culture in the absence of serum is interesting because the medium is chemically defined. The effect of different substrates or hormones can therefore be studied precisely without interference of undefined serum or variable components. The only disadvantage is the smaller yield of cells, because of the growth arrest induced by the deprivation of serum growth factors. This implies the killing of a greater number of animals to obtain enough cells to test different treatments. Thus, for a pharmacological study the utilization of cells differentiating in serum-supplemented medium was more convenient.

Efficacy of the drug at different doses

Different doses of ICI 215001 were tested on mouse cells: 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} M. The drug was present in the medium from day 1; cells were harvested on day 10. At 10^{-7} M the drug was as effective as at 10^{-6} M, as revealed by Northern and Western blot analysis (Fig. 5). Even at the lowest dose tested this β-agonist elicited an increase in UCP mRNA. Therefore in the following experiments the dose utilized was usually 10^{-7} M.

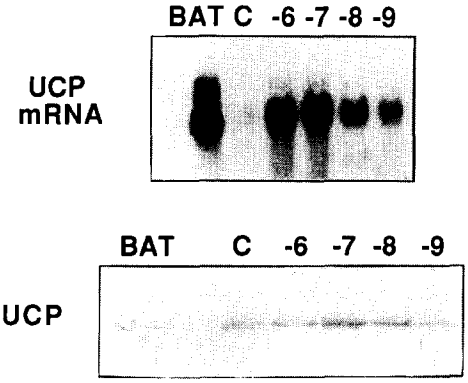

Fig. 5. Effect of different doses of ICI 215001 on UCP expression in mouse brown adipocytes differentiated in vitro. Northern blot: the first lane (BAT) corresponds to 5 μg of total mRNA isolated from rat BAT; the other lanes correspond to 20 μg of total mRNA isolated from differentiated cells. Western blot: the first lane corresponds to 5 μg of mitochondrial proteins from BAT; the other lanes correspond to 25 μg of mitochondrial proteins from differentiated cells. Cells were cultured as previously described and harvested on day 9. ICI 215001 was added from day 3 at different concentrations: 10^{-6} M (−6), 10^{-7} M (−7), 10^{-8} M (−8), 10^{-9} M (−9). Lane C corresponds to control untreated cells.

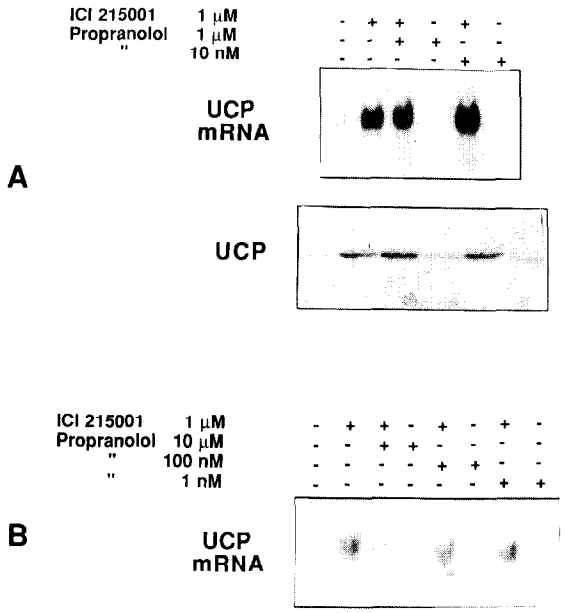

Fig. 6. Effect of propranolol on the stimulation of UCP expression induced by ICI 215001. Chronic experiments. Mouse BAT cells were cultured as previously. *A*: ICI 215001 was added on day 3 at 10^{-6} M, either alone or with propranolol at 10^{-6} or 10^{-8} M; some dishes received propranolol alone. The drugs were renewed on day 6. Cells were harvested on day 9. Northern blot: 20 μg of total RNA isolated from differentiated control cells or cells treated as indicated on the figure. Western blot: 25 μg of mitochondrial proteins isolated from the same cells as for the Northern blot. *B*: ICI 215001 was added on day 3 at 10^{-7} M, either alone or with propranolol at 10^{-5}, 10^{-7} or 10^{-9} M; some dishes received propranolol alone. The drugs were renewed on day 6. Cells were harvested on day 9. Each lane corresponds to 20 μg of total RNA isolated from differentiated control cells or cells treated as indicated on the figure.

Efficacy of the drug in the presence of a β-antagonist

Chronic experiments. ICI 215001 10^{-6} M was added to some dishes either alone, or with propranolol at two different concentrations: 10^{-6} and 10^{-8} M, from day 3 of culture; some dishes received the β-blocker alone. Cells were harvested on day 9. According to Northern and Western blot analysis the presence of propranolol did not prevent the stimulation of UCP gene expression by the β-agonist (Fig. 6*A*). The presence of propranolol alone did not affect UCP expression.

Similar experiments were carried out with ICI 215001 10^{-7} M and propranolol 10^{-5}, 10^{-7} and 10^{-9} M. Only when the β-blocker was 10^{-5} M was the drug-induced stimulation of UCP mRNA expression reduced (Fig. 6*B*). Otherwise there was apparently no effect of propranolol on UCP induction by ICI 215001.

Acute experiments. At 09.00 h on day 9 propranolol (10^{-6}, 10^{-7} or 10^{-8} M) was added to some dishes; at 10.00 h half the propranolol-treated dishes received ICI 215001 10^{-7} M. Cells were harvested at 14.00 h. The stimulation of UCP mRNA expression by the β-agonist was apparently unaffected by the presence of the β-blocker. In a similar experiment cells were treated with norepinephrine 10^{-6} M, or isoproterenol 10^{-7} M, or ICI 215001 10^{-7} M, either alone or in the presence of propranolol (10^{-6} and 10^{-7} M). There was no observable decrease of UCP mRNA induction by any of the agonists in the presence of the β-blocker (results not shown).

Propranolol is a non-specific β_1/β_2-antagonist. A third type of β-receptor has been described recently in fat cells, for which there is no selective antagonist. To better assess the specificity of ICI 215001 for β-adrenoceptors we used bupranolol, which was reported to be a probe for the presence of an atypical β-adrenoceptor in the heart (Kaumann, 1989). Bupranolol 10^{-6} or 10^{-7} M was added 1 h before ICI 215001 10^{-7} M, as described above. Propranolol at the same concentrations was used in comparison. Bupranolol was tested also on cells treated with isoproterenol 10^{-7} M. In contrast to propranolol, bupranolol partially inhibited the stimulating effect of ICI 215001 and isoproterenol on UCP mRNA expression when its concentration was 10 times that of the β-agonists (Fig. 7).

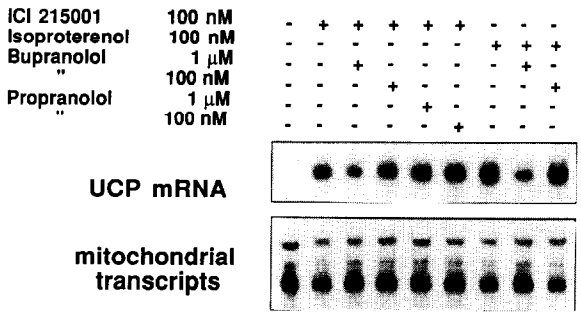

Fig. 7. Effects of different β-antagonists on the stimulation of UCP expression induced by ICI 215001. Acute experiments. Mouse BAT cells were cultured as previously described. On day 8 some dishes received at 09.00 h either bupranolol or propranolol at doses of 10^{-6} or 10^{-7} M. One hour later some control dishes as well as the antagonist-treated ones received either ICI 215001 10^{-7} M, or isoproterenol 10^{-7} M. Cells were harvested at 14.00 h. Each lane corresponds to 20 μg of total RNA isolated from control cells or cells treated as indicated on the figure.

Would a β-agonist stimulation trigger the expression of UCP in white adipose cells differentiating in culture?

The most important difference between white and brown adipocytes is the presence of UCP in the latter. Treatment of whole animals in vivo with ICI D7114 has proven efficient in inducing the appearance of UCP mRNA in several depots in which no such expression was detectable in control animals (Holloway et al., 1991). Was it due to the transformation of white adipocytes into brown ones, or to the recruitment of 'dormant' brown fat cells present in the white fat deposit? Could precursor cells from white fat pads express UCP if treated with a β-agonist at a given time of their development? To answer this question some experiments were made in parallel with precursor cells isolated from mouse epididymal white adipose tissue as well as from BAT. The same protocol of isolation and culture was followed for the two types of cells. ICI 215001 10^{-6} M was added in some dishes either chronically (from day 1 or day 3 on) or acutely (24 h or 4 h before harvesting). Cells from white adipose tissue grew well, reached confluence at the same time as brown fat cells, but their differentiation in the dishes was not as homogenous: clusters of differentiated adipocytes were surrounded by undifferentiated fibroblastic cells. This was not affected by the presence of the drug in the medium. Cells were harvested on day 9. No expression of UCP mRNA was observed in the cells originating from epididymal adipose tissue, whatever the duration and timing of the drug treatment.

Discussion

Primary cultures are a useful tool to study the regulation of gene expression by different effec-

tors and to determine the mechanisms involved in this regulation. In the present work we have used two models of BAT cell culture, mouse and, for the first time, rat, to demonstrate the direct effect on brown adipocytes of a novel β-agonist and bring further evidence of the presence in brown adipocytes of an atypical β-adrenoceptor.

UCP mRNA was clearly expressed in mouse BAT cells differentiated in vitro. Its appearance was concomitant with an increase in mitochondrial transcripts, confirming the link between UCP expression and mitochondriogenesis during cell development.

ICI 215001 had no effect on mouse cell growth and on the proportion of differentiated cells, contrary to what happens with BAT cells from *Phodopus sungorus* (Klaus et al., 1991a, b). However, it must be noted that the percentage of differentiation was already high for control mouse cells. The presence of the drug from day 1 in the culture medium did not induce a premature appearance of UCP mRNA, but stimulated its expression in mature brown adipocytes. LPL mRNA, an early marker of differentiation, was already expressed on day 3 of culture, that is to say before confluence. Its expression was clearly decreased by ICI 215001 in mature cells. This is in contradiction to the report that lipoprotein lipase gene transcription is positively regulated in brown adipose tissue by the cellular level of cAMP (Carneheim et al., 1988; Giralt et al., 1990). However, it agrees with reports on decrease of LPL or its mRNA in adipocytes treated in vitro by dibutyryl cyclic AMP (Ben Sadoun and Marita, 1986) or isoproterenol (Raynolds et al., 1990).

An acute treatment with ICI 215001 was able to induce UCP mRNA expression as early as on day 5 of culture, before cells were at confluence. Surprisingly the same cells, when cultured in the presence of ICI 215001 from day 1 on, did not seem yet able to express UCP mRNA on day 5, though the presence of LPL mRNA indicated that they were engaged in the differentiation program. The sensitivity of the cells to β-stimulation would differ according to the precise time of application. This sensitivity decreased with the time of culture. This could be explained by a desensitization of the cells in the case of chronic treatment, but not of acute treatment. It must be noticed that the expression of the different mRNAs studied (UCP, LPL, mitochondrial mRNA) followed a similar pattern of evolution with the time of culture: increase until day 7 or 9, then decrease afterwards. Aging of the cells could lead to such a decrease, together with the development of a new growing cell population appearing in the dishes from day 13 on.

Rehnmark et al. (1989), as well as Houstek et al. (1990), have also reported an induction of UCP gene expression in cells at confluence, on day 6, after stimulation by norepinephrine or β-agonists. However, their conditions differ somewhat from ours, in that the density of inoculation was higher and they describe the cells on day 6 as already polyhedric or round in shape, or even as typical adipocytes.

ICI 215001 has been tested on a new model of BAT cell primary culture: the rat model. For the first time the expression of UCP mRNA was obtained in rat brown adipocytes differentiated in vitro. Several attempts had been made before, using medium supplemented with serum, which were unsuccessful (Néchad et al., 1983, 1987; Néchad, 1983; Cigolini et al., 1986; Forest et al., 1987b; Herron et al., 1989).The high mitogenic potency of serum seems to lead to a loss of the potentiality for full differentiation of rat brown fat preadipocytes. Indeed, when left in the presence of serum during 3 days after plating, rat precursor cells grew, but were then unable in serum-free medium to differentiate into brown adipocytes expressing UCP mRNA (results not shown). Amri et al. (1986) had already noticed that there is an inverse relationship in culture between the potentiality to overproliferate and the potentiality to convert into adipose cells. Rat BAT cells seem to be more sensitive than mouse cells to this opposition between growth and differentiation. These results underline once more the interspecies differences as for the needs of brown fat cells to achieve terminal differentiation. There are differences not only between large (ovine) and small (rodents) mammals, but also between different species of rodents: mouse or Djungarian hamster, and rat.

As observed for mouse BAT cells, the presence of ICI 215001 in the culture medium of rat brown fat cells did not increase the proportion of

differentiated adipocytes, but stimulated their expression of UCP mRNA.

The effect of the drug on UCP mRNA expression in mouse brown adipocytes differentiating in primary culture was dose-dependent and already visible at a concentration of 10^{-9} M. This drug has been claimed as an atypical β-agonist (Holloway et al., 1991), acting via a newly identified β-receptor called β_3 (Emorine et al., 1989; Muzzin et al., 1991; Nahmias et al., 1991). Indeed the stimulation of UCP mRNA by ICI 215001 10^{-7} M was not affected by propranolol at concentrations of 10^{-7} or 10^{-6} M, either in short-term (4 h) or in long-term (several days) studies. Only propranolol 10^{-5} M had an inhibitory effect on this stimulation of UCP mRNA. However, at high concentrations the effects of β-antagonists are generally considered to be non-specific and often related to effects on membrane stabilization. Bupranolol, which is the only β-blocker known to act on β_3- as well as on β_1- and β_2-adrenoceptors (Kaumann, 1989), was tested too. Although it blocked stimulation of UCP gene expression by ICI 215001 when dosed at 10^{-6} M (10 times the agonist concentration), this concentration is still much greater than those required to block β_1- or β_2-adrenoceptors. Therefore the effects of the acid metabolite of ICI D7114 on UCP gene expression appear to be resistant to blockade by propranolol or bupranolol, suggesting that this effect may also be mediated by an atypical (β_3)-adrenoceptor.

Would this new β-adrenoceptor agonist be able to trigger UCP gene in adipose cells which do not usually express it? We have observed in vivo the appearance of UCP mRNA in several adipose deposits of adult dogs treated with ICI D7114, whilst no UCP could be detected in untreated animals (Champigny et al., 1991). Several experiments were therefore carried out in vitro with precursor cells from mouse or rat white (epididymal) adipose tissue differentiating in primary cultures. No expression of UCP was ever obtained.

In conclusion a new model of BAT cell culture has been developed: the rat model. It has been used together with the mouse model to study the effect of a new β-agonist on UCP gene expression. The ICI D7114 acid metabolite clearly modulates the expression of UCP in mature brown adipocytes differentiated in vitro, through β_3-adrenoceptor activation, but it does not seem able to have a triggering action on the repressed gene.

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