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Expression of human β3-adrenergic receptor induces adipocyte-like features in CHO/K1 fibroblasts

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SUMMARY

It is reported here that CHO/K1 cells stably transfected with the human β3 AR gene (CHO/K1-β3), grown in the presence of differentiation-stimulating agents accumulate triglycerides. This lipid formation is mediated through the β3 AR, since non-transfected CHO/K1 cells, or cells expressing the human β2 AR, accumulate no significant amount of lipids when grown in supplemented medium. Moreover, lipid production can be inhibited significantly by the β AR antagonist bupranolol. CHO/K1 cells expressing the W64R polymorphism (Trp to Arg polymorphism at position 64 of the human β3 AR), which has been associated with morbid obesity, show increased lipid accumulation as compared to CHO/K1 cells expressing the wild-type β3 AR. Semi-quantitative RT-PCR experiments reveal that a major gene regulating adipocyte differentiation, peroxisome-proliferator-activated-receptor γ (PPARγ), is expressed in CHO/K1 cells. Concomitantly with the formation of lipid droplets, the expression of PPARγ mRNA is increased in CHO/K1-β3 cells, but not in non-transfected CHO/K1 cells. We furthermore detected constitutive expression of another adipocyte-associated protein: hormone sensitive lipase, while leptin or uncoupling protein-1 transcripts were not expressed. These data suggest that the frequently used CHO/K1 fibroblasts display several preadipocyte-like features, and that the sole expression of the β3 AR modifies the expression of PPARγ mRNA in these cells, and induces lipid formation under certain culture conditions.

Key words: Adrenergic receptor, β AR, Adipocyte, Adipogenesis, PPARγ, CHO

INTRODUCTION

The β_3 adrenergic receptor (β3 AR) is a G-protein-coupled receptor that is expressed mainly in adipose tissue (Strosberg and Pietri-Rouxel, 1996). It is not expressed in preadipocytes but appears in mature, white as well as brown adipocytes. In rodent adipocytes, catecholamine stimulation of the β3 AR leads to a G_S-mediated increase in intracellular cAMP concentration, subsequent activation of protein kinase A (PKA), and phosphorylation of several target proteins, e.g. hormone sensitive lipase (HSL) resulting in lipolysis. Stimulation of the human β3 AR leads to significant increases in intracellular cAMP concentrations in transfected cells, but its role in the regulation of energy balance in human adipocytes is still debated (e.g. Arch and Wilson, 1996). However, a naturally occurring polymorphism at position 64 (W64R) of the human β3 AR has been associated with morbid obesity and insulin resistance (reviewed by Strosberg, 1997), although it is still unknown how this mutation affects adipocyte biology (Li et al., 1996; Snitker et al., 1997). No differences in binding or adenylyl cyclase activation have been observed between CHO cells expressing the wild-type or the mutated β3 AR (Candelore et al., 1996; Piétri-Rouxel et al., 1997) whereas maximal accumulation of cAMP was shown to be lower in cells expressing the mutant receptor (Piétri-Rouxel et al., 1997).

In addition to the role of the β3 AR in lipolysis, this receptor has more recently been suggested to be involved in the regulation of adipocyte proliferation/differentiation, especially in brown adipose tissue (BAT). In rodents, treatment with β3 agonists results in a hypertrophy of brown adipocytes (Ghorbani et al., 1997) and in culture, activation of the β3 AR stimulates brown adipocyte differentiation (Bronnikov et al., 1992). In patients with pheochromocytoma, a catecholamine-secreting tumour, brown adipose tissue appears around the tumour (Himms-Hagen, 1990).

Adipogenesis requires the activation of signalling pathways including extracellular regulated kinase (ERK) 1/2 (Sale et al., 1995), phosphatidylinositol-3 kinase (PI3K) (Christoffersen et al., 1998) and protein kinase B (PKB) (Magun et al., 1996). The β3 AR may stimulate adipocyte differentiation by directly or indirectly interfering with such signal transduction pathways. Indeed, in rat adipocytes, stimulation of the β3 AR leads to activation of ERK 1/2 (Shimizu et al., 1997), and PKB (Moule et al., 1997). We have shown recently that the human β3 AR, upon heterologous expression in CHO/K1 cells, can

also stimulate ERK and PKB via a PI3K-dependent mechanism (Gerhardt et al., 1999).

Most studies on adipocyte cell biology have been performed on either primary cultures of fibroblast-like preadipocytes, or several immortalised preadipocyte cell lines, such as the murine white preadipocyte cell lines 3T3-L1 (Green and Kehinde, 1974) and 3T3-F442A (Green and Kehinde, 1976), and the human brown preadipocyte cell line PAZ-6 (Zilberfarb et al., 1997). Primary as well as immortalised preadipocytes can be differentiated in vitro into mature, lipid droplet producing adipocytes by adding mixtures of differentiation-stimulating agents (such as insulin, pioglitazone, dexamethasone) to confluent preadipocytes (Cornelius et al., 1994; Gregoire et al., 1998). During adipogenesis, intracellular fat droplets appear and transcription of several adipocyte-specific marker genes such as the γ form of peroxisome proliferator activated receptors (PPARγ) (Tontonoz et al., 1994a) or the adipocyte satiety hormone leptin (Maffei et al., 1995; Rentsch and Chiesi, 1996), is increased.

Here we report that when confluent CHO/K1 cells stably expressing the human β3 AR (CHO/K1-β3) are cultured in a medium supplemented with adipocyte differentiation-stimulating agents, intracellular lipid droplets appear after 5 days. This lipid accumulation seems to be due to the presence of the β3 AR, as non-transfected CHO/K1 cells, or CHO/K1 cells expressing β2 AR (CHO/K1-β2) do not produce any lipids. Moreover, lipid accumulation is increased in cells expressing the W64R mutated β3 AR (CHO/K1-β3W64R). Semi-quantitative RT-PCR experiments show that CHO/K1-β3 cells express some (e.g. PPARγ and HSL) but not all (e.g. no leptin or uncoupling protein 1) adipocyte-specific marker genes.

Our data thus demonstrate that CHO/K1 fibroblasts, abundantly used for transfection experiments, express some preadipocyte-like features. Moreover, mere expression of the human β3 AR in these cells suffices to modulate PPARγ mRNA expression and to induce lipid accumulation when cultured in the presence of adipogenesis-stimulating agents.

MATERIALS AND METHODS

Cell culture

CHO/K1 (Chinese hamster ovary/K1) cells stably expressing the human wild-type β3 AR (B_{max} = 0.47 pmol receptor/mg protein), here referred to as CHO/K1-β3, or the mutated W64R form (B_{max} = 0.32 pmol receptor/mg protein), here referred to as CHO/K1-β3W64R, were described before (Piétri-Rouxel et al., 1997). CHO/K1 cells stably expressing the human wild-type β2 adrenergic receptor (B_{max} = 0.30 pmol receptor/mg protein), here referred to as CHO/K1-β2, were generously provided by Dr S. Marullo (Paris, France). Cells were cultured in 1:1 mixture of Ham's F12/Dulbecco's modified Eagle's medium (Gibco-BRL) supplemented with 20 mM Hepes (Gibco-BRL), 5% decomplemented foetal calf serum (Gibco-BRL) and antibiotics (Gibco-BRL) until confluence. After reaching confluency, cells were changed to medium supplemented with biotine (33 μM, Sigma), panthothenate (18 μM, Sigma), triiodothyronine (1 nM, T3, Sigma), insulin (85 nM, Sigma), dexamethasone (1 μM, Sigma), IBMX (3-isobutyl-1-methyl xanthine; 250 nM, Sigma), naphthiazo (5-[2-(2-naphthylmethyl)-5-benzofuranylmethyl]-2,4-thiazolidinedione; 1 μM, generous gift from Dr J. Duhault, IRIS, France). Cells were maintained in this medium for 10-12 days changing the medium every three days. As indicated in the corresponding figure legend, 10 μM of bupranolol (1-(2-chloro-5-methylphenoxy)-3-[(1,1-dimethylethyl)amino]-2-propanol; Schwarz Pharma AG) was added to the supplemented medium.

Oil Red O staining and quantification of lipid accumulation

The Oil Red O solution (Sigma) was prepared as described before by Ramirez-Zacarias et al. (1992). Multiwell-6 plates were washed twice with phosphate buffered saline (PBS), fixed for 1 hour at room temperature with 10% para-formaldehyde in PBS, washed with water, stained for 2 hours by complete immersion in a solution of Oil Red O and rinsed twice with water. Excess of water was evaporated by placing the dishes at 37°C. The dye was extracted with 200 μl of isopropyl alcohol per well, and the absorbance of an aliquot was measured immediately at 492 nm. Lipids were quantified using a triolein (C18: 1, [cis]-9, Sigma) calibration curves as described by Ramirez-Zacarias et al. (1992).

RT-PCR

Total RNA was extracted from CHO/K1 cells using Trizol RNA isolation agent (Life Technology). Hamster RNA was extracted from the interscapular brown adipose tissue and the omental abdominal white adipose tissue.

Total RNA was treated for 60 minutes at 37°C with 0.3 U of RNase-free DNAse I (RQ1 DNAse, Promega) per μg nucleic acid in 40 mM Tris-HCl, pH 7.9, 10 mM NaCl, 6 mM $MgCl_2$, 10 mM $CaCl_2$ in the presence of 2 U/μl of RNase inhibitor (RNAsin, Promega). After phenol/chloroform extraction and precipitation of the RNA, 2 μg was used to synthesize cDNA. RNA was incubated for 90 minutes at 42°C with 75 U of Moloney Murin Leukaemia Virus reverse transcriptase (SuperScript™II, Gibco-BRL) in 10 μl of reverse transcriptase buffer (50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$ and 10 mM dithiothreitol) containing 0.4 mM each dNTP, 0.003 U random hexanucleotides (Pharmacia, France), and 2 U of RNase inhibitor.

Semi-quantitative PCR was performed in a final volume of 50 μl containing 0.25 mM primers, 2.5 U of Taq polymerase (Gibco-BRL), 0.25 mM of each dNTP, 1× reaction buffer (Gibco-BRL) and supplemented with 1.5 mM $MgCl_2$. Uncoupling protein 1 (UCP1) PCR was performed in the presence of 10% DMSO. 200 ng cDNA template was denatured for 4 minutes at 94°C and amplified as followed: UCP1 and Ob (94°C, 30 seconds; 52°C, 30 seconds; 72°C, 45 seconds, 40 cycles); PPARγ (94°C, 30 seconds; 51°C, 30 seconds; 72°C, 40 seconds, 35 cycles); HSL (94°C, 30 seconds; 55°C, 30 seconds; 72°C, 45 seconds, 35 cycles); β-actin (94°C, 30 seconds; 60°C, 30 seconds; 72°C, 30 seconds, 25 cycles). A final extension was performed for 3 minutes at 72°C. In order to compare the PCR products in a semi-quantitative way, we (i) determined the exponential phase of amplification by performing 25-30-35-40 cycles and (ii) amplified the gene for β-actin (15-20-25 cycles) as internal control for cDNA quantity and quality. Sequences of the primers were; UCP1: forward 5′-ATT AGG TAT AAA GGT GTC CT-3′ and reverse 5′-ATG TTT GTG TGC TTT GAA-3′; Ob: forward 5′-CAG GAT GAC ACC AAA ACC-3′ and reverse 5′-AAA GCC ACC ACC TCT GT-3′; PPARγ: forward 5′-AGA CAA CAG ACA AAT CAC CAT-3′ and reverse 5′-AAG TTT GAG TTT GCT GTG AAG-3′; HSL: forward 5′-GCT GGT GTC CTT CGG GG-3′ and reverse 5′-GCG GCT TAC CCT CAC GG-3′; β-actin: forward 5′-GAG ACC TTC AAC ACC CC-3′ and reverse 5′-GTG GTG GTG AAG CTG TAG CC-3′. Amplified PCR products were visualised on a 1% agarose gel: UCP1 715 bp; PPARγ 401 bp; Ob ±350 bp; HSL ±250 bp, β-actin ±250 bp).

Statistical analyses

The data were expressed as the mean ± s.e.m. of two to five independent experiments performed in duplicate. Significance between differences were assessed using the unpaired Student's *t*-test.

RESULTS

Quantification of lipid accumulation in CHO/K1-β3 and CHO/K1-β3 W64R cells

We have previously shown that stimulation of the β3 AR expressed in CHO/K1 cells leads to activation of PKB and extracellular signal regulated kinase 1/2 (ERK1/2) (Gerhardt et al., 1999). Since these signalling proteins play important roles in adipocyte differentiation, we wondered how CHO/K1-β3 cells would react to the presence of adipocyte differentiation-stimulating agents. CHO/K1-β3 cells were cultured in normal medium until they reached confluency, and were then transferred to a supplemented medium containing the following differentiation-stimulating agents: biotine, panthothenate, triiodothyronine, insulin, dexamethasone, IBMX and thiazolidinedione naphthiazo. After five days of culture in this medium, small lipid droplets appeared in the cells. After ten days, lipid accumulation reached a maximum (with lipids present in ±40% of the cells). As shown in Fig. 1, CHO/K1-β3 cells cultured in the presence of differentiating agents and stained with Oil Red O display intracellular lipid accumulation while CHO/K1-β3 cells cultured in normal medium did not accumulate fat droplets. Non-transfected CHO/K1 cells cultured in the presence of differentiation-stimulating agents did not accumulate fat droplets either (data not shown).

The accumulation of lipid droplets in the CHO/K1-β3 cells (that express 0.47 pmol receptor/mg protein) was not due to a clonal modification, for a cell line exhibiting a higher β3 AR density (B_{max} = 2.3 pmol/mg protein) similarly accumulated Oil Red O stainable lipid droplets (data not shown). We chose the CHO/K1-β3 cells for further characterisation, as the receptor level in these cells approximates that described in the human brown adipocyte cell line PAZ-6 (~0.4 pmol/mg protein; Zilberfarb et al., 1997).

In order to quantify the amount of lipids, cells were stained with Oil Red O solution and an aliquot of the extracted dye was monitored spectrophotometrically at 492 nm using a triolein concentration range as a standard calibration curve. When cultured in the supplemented medium, 901±117 μg triglyceride/ml (*n*=5) was extracted from CHO/K1-β3 cells/well. In non-transfected CHO/K1 cells, a basal level of 210±21 μg triglyceride/ml/well (*n*=5) was detected (Fig. 2A).

In order to determine whether the observed lipid accumulation is selective for CHO/K1-β3 cells, we cultured CHO/K1 cells stably expressing a similar amount of the human β2 AR (CHO/K1-β2 cells, 0.3 pmol/mg protein) in the presence of supplemented medium. Interestingly, CHO/K1-β2 cells did not accumulate significantly more lipids (212±40 μg triglyceride/ml/well; *n*=3) than the wild-type CHO/K1 cells (Fig. 2A).

Several studies have shown that the naturally W64R polymorphism is correlated with morbid obesity. To assess whether the W64R mutation affects the capacity of CHO/K1 cells to accumulate lipids, cells stably expressing the β3W64R mutant receptor (B_{max} = 0.32 pmol/mg protein) were cultured in the presence of adipocyte differentiating agents. Interestingly, CHO/K1-β3W64R cells accumulated 1.55±0.09 fold more triglycerides (1429±259 μg triglyceride/ml/well, *n*=5) than the CHO/K1-β3 cells (Fig. 2B).

When CHO/K1-β3 cells were cultured in the presence of the selective β AR antagonist bupranolol (10 μM), a drastic inhibition of lipid accumulation was observed (91%). This effect of bupranolol was similar in cells expressing the W64R mutant (92%) (Fig. 3).

Expression of adipocyte specific markers

To assess whether the lipid formation observed in β3 AR transfected CHO/K1 cells is correlated with adipogenesis, we performed semi-quantitative RT-PCR experiments on four transcripts expressed in adipocytes. These included PPARγ, the satiety hormone leptin (Ob), the brown adipose tissue specific uncoupling protein 1 (UCP1), and the HSL, a key enzyme in the catecholamine-mediated lipolysis. Among these genes, only the sequences encoding PPARγ (Aperlo et al., 1995) and UCP1 (Echtay et al., 1997) are known from hamster. As Ob and HSL have not yet been cloned from hamster tissues, we aligned the sequences for these genes known from other species (i.e. mouse, rat, chicken, dog, sheep, pig, monkey, human for Ob, and rat, pig, human for HSL) and generated oligonucleotides against the most conserved sequences (see Materials and Methods). RNA from hamster brown or white adipose tissue was extracted to serve as a positive control.

As shown in Fig. 4, preconfluent non-transfected CHO/K1 cells as well as preconfluent CHO/K1-β3 cells and CHO/K1-β3W64R cells all express equal levels of

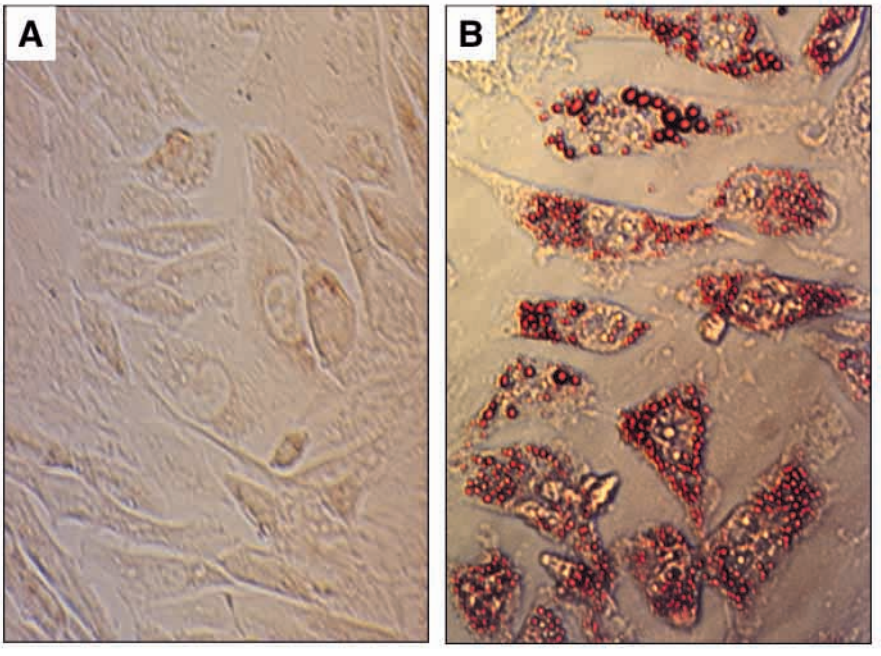

Fig. 1. Expression of human β3 AR stimulates lipid accumulation in CHO/K1 cells. CHO/K1-β3 cells were cultured until confluency and either maintained in normal culture medium (A) or cultured in a medium supplemented with differentiating-stimulating agents (B) for ten days. Cells were fixed and stained with Oil Red O (magnification ×40).

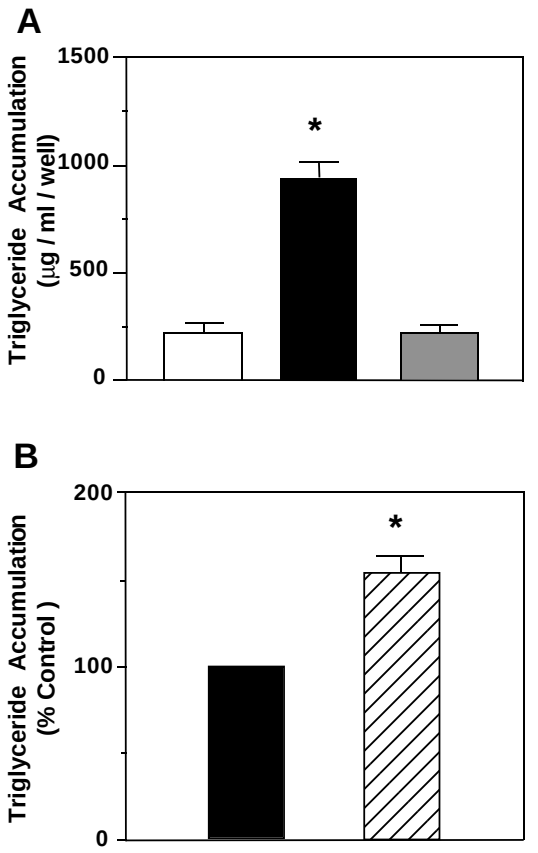

Fig. 2. Quantification of lipid accumulation in CHO/K1 cells. Cells were cultured in the presence of differentiating agents for 10 days and fixed. Lipids were stained with Oil Red O solution, extracted and quantified using a triolein calibration curve. (A) Absolute values of lipid accumulation in non-transfected CHO/K1 (white), CHO/K1-β3 (black), or CHO/K1-β2 cells (grey). (B) Relative difference between CHO/K1-β3 cells (black, control) and CHO/K1-β3W64R (hatched). Values correspond to 901±117 μg triglycerides/ml/well and 1429±259 μg triglycerides/ml/well, respectively. The data shown are expressed as the mean ± s.e.m. of three to five independent experiments performed in duplicate. **P*<0.001 (*t*-test).

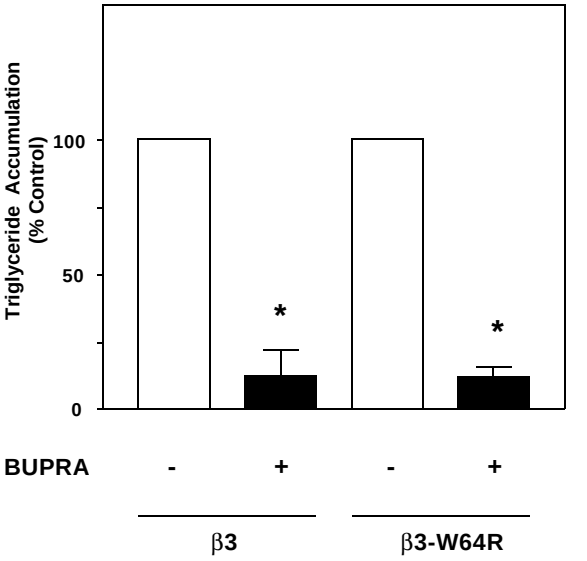

Fig. 3. Quantification of lipid accumulation in CHO/K1-β3 and CHO/K1-β3W64R cells in the presence of β AR antagonist. Relative difference between CHO/K1-β3 cells and CHO/K1-β3W64R cultured in the presence of differentiating agents without (white) or with (black) 10 μM β AR antagonist bupranolol. Lipids were quantified as described before. For CHO/K1-β3 cells, values correspond to 952±243 μg triglycerides/ml/well and 86±22 μg triglycerides/ml/well, respectively. For CHO/K1-β3W64R cells, values correspond to 1532±349 μg triglycerides/ml/well and 123±28 μg triglycerides/ml/well, respectively. The data shown are expressed as the mean ± s.e.m. of two independent experiments performed in duplicate. **P*<0.001 (*t*-test).

HSL. The expression of HSL was not modified in any of the three cell lines cultured in the absence or presence of differentiating agents.

Furthermore, we found that preconfluent CHO/K1 cells express PPARγ. The expression level of PPARγ was found to be lower in preconfluent CHO/K1-β3 cells and CHO/K1-β3W64R cells than in preconfluent non-transfected CHO/K1 cells (4 and 8 times, respectively). In addition, the expression level of PPARγ strongly increased when the β3-transfected cells, but not the non-transfected cells, were cultured in supplemented medium (Fig. 4).

No expression of Ob or UCP1 could be detected in preconfluent CHO/K1 cells, or in transfected cells. Also after culturing the cells in the presence of differentiating agents, no Ob or UCP1 transcripts were detected. In contrast, a single band of the expected size was efficiently amplified from cDNA prepared from control hamster WAT (Ob) or BAT (Ob and UCP1) (data not shown).

β-Actin transcripts served as internal standard. Equal amounts of β-actin were detected in CHO/K1 cells, CHO/K1-β3 cells, and CHO/K1-β3W64R cells, cultured with or without differentiating agents (Fig. 4).

DISCUSSION

We here report that stable expression of the human β3 AR in CHO/K1 cells is associated with the formation of intracellular lipid droplets when these fibroblasts are cultured in the presence of adipocyte differentiation-stimulating agents. The observed triglyceride accumulation in CHO/K1 cells was found to be due exclusively to the presence of the β3 AR. Wild-type CHO/K1 cells, cultured similarly in a supplemented medium, did not or hardly produce any triglycerides, nor did cells expressing the closely related human β2 AR. The effects of the β3 AR were not due to a clonal modification, since two independent CHO/K1-β3 cell lines, expressing 0.47 (Piétri-Rouxel et al., 1997) and 2.3 pmoles (Gros et al., 1998) receptors/mg protein, respectively, similarly accumulated intracellular triglycerides.

Apparently, the presence of the β3 AR by itself is sufficient to induce the formation of lipid droplets. Although the molecular basis of this observation remains to be elucidated, the fact that the β AR antagonist bupranolol completely blocks lipid accumulation suggests that this process requires the presence of a functional receptor protein. The induction of lipid production occurs without addition of β3 AR agonists, suggesting that the receptor in its 'basal' state might be

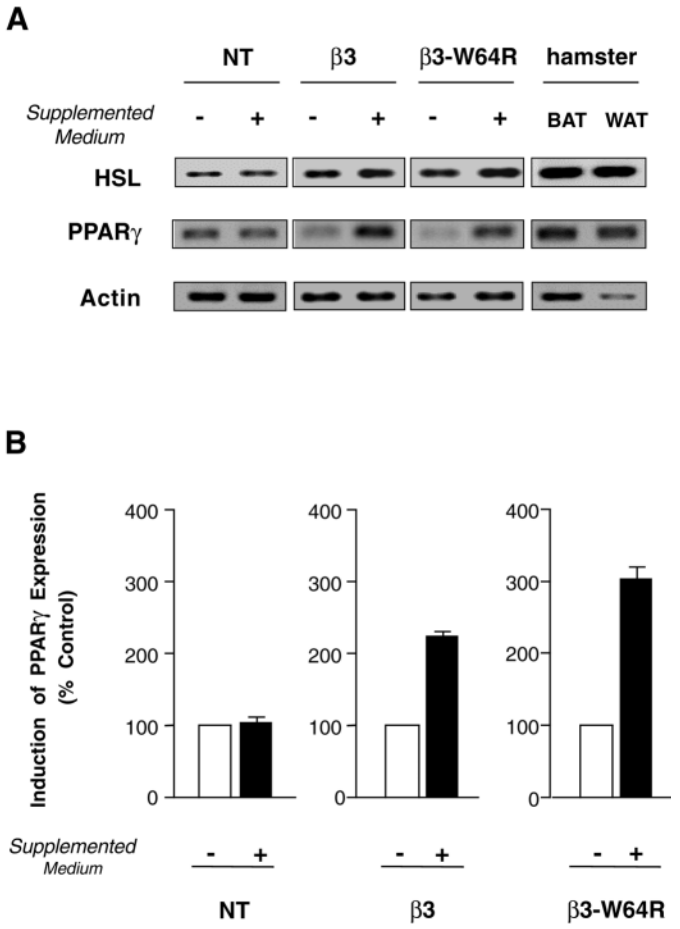

Fig. 4. Expression of adipocyte differentiation markers during lipid accumulation in CHO/K1 cells. Representative semi-quantitative RT-PCR experiment, performed as described in Materials and Methods. (A) Non-transfected CHO/K1 cells (NT), CHO/K1-β3 cells (β3), or CHO/K1-β3W64R (β3–W64R), were cultured in the absence (-) or presence (+) of differentiation-stimulating agents. Brown (BAT) or white (WAT) adipose tissue isolated from hamster served as positive control. (B) Relative increase in PPARγ expression in CHO/K1 cells cultured in the abscence (-) or presence (+) of differentiation-stimulating agents. Two independant PCR experiments were performed on cDNA derived from cells incubated with or without differentiation-stimulating agents, and the optic density of the DNA bands was determined. The values obtained from cells cultured in the absence of differentiation stimulating agents were normalised to 100%.

sufficiently activated to exert its effects. Indeed, bupranolol has been shown to act as inverse agonist in CHO/K1-β3 cells (F. Pietri-Rouxel and M. F. Drumare, personal communication). Basal cAMP levels (that varied from 20 to 100 pmol/10^6 cells) were completely abolished after incubation with bupranolol (n=3).

A W64R polymorphism in the β3 AR gene has been associated with morbid obesity and insulin resistance (for a recent review see Strosberg, 1997). Here we show that CHO/K1 cells expressing a β3 AR carrying this mutation accumulate more lipids upon cultivation in supplemented medium than cells expressing the wild-type β3 AR (6.5 times and 4.5 times more than in non-transfected CHO/K1 cells, respectively). Since the level of receptor expression in CHO/K1-β3W64R cells is similar to that of CHO/K1-β3 cells, a yet unknown signal may be transduced from the receptor in its 'basal' state, and this signal is stronger in the W64R form of the β3 AR. Previous work from our laboratory has indicated that the basal level of cAMP in CHO/K1-β3W64R cells is actually *lower* than that in the CHO/K1-β3 cells (Pietri-Rouxel et al., 1997). However, we have recently presented evidence for a double coupling of the β3 AR to $G\alpha_s$ and $G\alpha_{i/o}$ in CHO/K1 cells (Gerhardt et al., 1999). Therefore, the decreased precoupling of the W64R mutant receptor to $G\alpha_s$ may be accompanied by an increased precoupling to $G\alpha_{i/o}$. We are currently investigating this possibility.

How can the presence of the β3 AR, but not the β2 AR, induce lipogenesis? Both receptor subtypes are highly homologous and functionally related. Both receptors couple to a $G\alpha_s$ protein to activate adenylyl cyclase, and subsequent target proteins. The human β2 AR is however, expressed in a wide variety of tissues, including in preadipocytes and adipocytes, whereas the β3 AR is preferentially expressed in mature adipocytes, suggesting that the β3 AR may have a function which is specific for adipocytes. Such β AR-subtype-specific function has been suggested before in rodent adipocytes. The β1 AR is the major subtype expressed in rodent preadipocytes and is present as a minor component in adipocytes while the β3 AR is the major subtype expressed only in adipocytes. Whereas both β1 and β3 AR are coupled to adenylyl cyclase, it appears that stimulation of the β1 AR leads to preadipocyte proliferation, while activation of the β3 AR stimulates differentiation into adipocytes (Bronnikov et al., 1992).

PI3K (Christoffersen et al., 1998) as well as PKB (Magun et al., 1996) and ERK1/2 (Sale et al., 1995) have all been shown to play a role in adipocyte differentiation. Interestingly, we have recently shown that the human β3 AR, expressed in CHO/K1 cells can activate PI3K, PKB and ERK1/2 (Gerhardt et al., 1999). The role of the β3 AR to stimulate adipogenesis in rodent preadipocytes, together with the activation of PI3K, PKB and ERK by the human β3 AR expressed in CHO/K1 cells, suggests that the observed lipid formation in CHO/K1-β3 cells actually constitutes a process of adipogenesis. We have therefore examined the expression of adipocyte marker genes in preconfluent and confluent, lipid producing CHO/K1-β3 cells by semi-quantitative RT-PCR.

An important, so called 'master' gene, regulating adipocyte differentiation is PPARγ (Spiegelman and Flier, 1996; Fajas et al., 1998). Expression of PPARγ is induced before transcriptional activation of most other adipocyte marker genes (Braissant et al., 1996; Forman et al., 1996) and ectopic expression of PPARγ in NIH 3T3 cells induces adipogenesis (Tontonoz et al., 1994b). Here we show that CHO/K1 cells express significant amounts of PPARγ. The level of PPARγ expression is increased when CHO/K1-β3 cells, but not non-transfected CHO/K1 cells, are cultured in supplemented medium. As shown before in adipocytes, this increase could be correlated to an advanced state of adipogenesis (for reviews see Auwerx et al., 1996; Spiegelman and Flier, 1996). On the other hand, the increase in PPARγ expression may also be a consequence of the long-term treatment with thiazolidinedione (Gimble et al., 1996). However, here we show that prolonged treatment with the thiazolidinedione naphtazio does not

increase the expression of PPARγ mRNA in non-transfected CHO/K1 cells. This suggests that the increased PPARγ expression in CHO/K1-β3 cells is not simply a consequence of long-term thiazolidinedione treatment, and may indeed be related to the development of an adipocyte-like phenotype. While PPARγ plays a well-established role in adipogenesis (Tontonoz et al., 1994a), its expression has been detected in some preadipocytes as well (Chawla et al., 1994; Spiegelman and Flier, 1996; Gregoire et al., 1998). Moreover, PPARγ has been detected in ovary cells (Lambe and Tugwood, 1996) which could explain why we detected PPARγ in CHO/K1 cells. Currently we do not know why the level of PPARγ expression is reduced in preconfluent CHO/K1-β3 cells as compared to non-transfected cells. This observation does, however, indicate that the expression of the β3 AR per se can change the expression of a gene playing a major role in adipogenesis.

We furthermore detected the mRNA encoding HSL, of which the levels remained unchanged in non-transfected CHO/K1 cells and in transfected CHO/K1-β3 or CHO/K1-β3W64R cells.

Taken together, our RT-PCR results suggest that stable expression of the human β3 AR in CHO/K1 cells is able to modulate the expression of a master adipogenic gene PPARγ, first by decreasing its expression in preconfluent cells and secondly by increasing its expression during lipid accumulation in confluent cells. Cultivation with differentiation-stimulating agents does not modify the transcription of HSL nor induces expression of leptin, suggesting that CHO/K1-β3 cells display some, but not all of the molecular attributes of adipocytes.

In conclusion, this paper shows that the widely used CHO/K1 fibroblasts may have a previously unknown preadipocyte-like predisposition. Moreover, mere expression of the human β3 AR in these cells, when cultured in supplemented medium, induces a certain, although partial, adipogenesis with lipid formation and increased expression of PPARγ mRNA.

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