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Transcriptional down-regulation by insulin of the β_3-adrenergic receptor expression in 3T3-F442A adipocytes: A mechanism for repressing the cAMP signaling pathway

(gene transcription/3T3 adipocyte)

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ABSTRACT **Modulation of the three β-adrenergic receptor subtypes (β-ARs) by insulin was investigated in mouse 3T3-F442A adipocytes. Saturation and competition experiments measuring binding of ^{125}I-labeled (−)-cyanopindolol to adipocyte membranes demonstrated that cell exposure to insulin for 4 days caused a 3.5-fold decrease in the density of the major β-AR component of the adipocyte, the β_3-AR, while β_1-AR sites remained unchanged and β_2-ARs were undetectable. This correlated with a lower potency of the β_3-AR-selective agonists CGP12177, ICI201651, and BRL37344 in stimulating adenylate cyclase. Northern blotting analysis indicated that insulin induced a rapid and sharp decrease in β_3-AR mRNA levels. This effect was detectable at low insulin concentrations (EC_{50} = 3 nM) and was not observed in the presence of insulin-like growth factor I, suggesting an insulin receptor-mediated phenomenon. Reverse transcriptase–PCR analysis showed that, in contrast to its dramatic down-regulatory effect on β_3-AR mRNA, insulin did not modify the levels of β_1- and β_2-AR transcripts. As assessed by nuclear run-on assays, insulin inhibited the β_3-AR gene transcription rate by 90% within 30 min. mRNA turnover experiments showed that the half-life of β_3-AR mRNA was short (90 min) and remained unaffected by insulin. These findings demonstrate the genetic control of a β-AR subtype expression by insulin and reveal a mechanism for the regulation by this hormone of cAMP-dependent biological processes in adipocytes.**

Because of the potential implication of hyperinsulinemia or insulin resistance in the pathogenesis of obesity and non-insulin-dependent diabetes mellitus, the long-term effects of insulin *in vivo* or *in vitro* have been studied extensively. In adipocytes, insulin inhibits catecholamine-stimulated lipolysis by a multistep control of the lipolytic cascade (1, 2). Part of this antilipolytic action of insulin is exerted by phosphorylation and activation of a cGMP-inhibited cyclic nucleotide phosphodiesterase (3), but few data are available concerning the heterologous regulation by insulin of the β-adrenergic receptor (β-AR)-sensitive adenylate cyclase complex. Engfeldt *et al.* (4) demonstrated that, in human adipocytes, short-term exposure to insulin caused a moderate and transient decrease in cell surface β-ARs. This phenomenon is related to a redistribution of β-ARs from plasma membrane to a microsomal fraction. However, due to the limited viability of adipose explants or isolated adipocytes in culture, chronic effects of insulin on adipocytes *in vitro* have been difficult to study. Mouse 3T3 preadipose cell lines (5), which mimic the morphological, metabolic, and hormonal features of adipose tissue development, offer a unique opportunity to evaluate the long-term effects of insulin on several cellular processes. During their adipose conversion *in vitro*, 3T3-L1 or 3T3-F442A cells acquire an increased insulin and catecholamine responsiveness, concomitant with a large increase in new insulin receptors and β-ARs (6, 7). A profound transformation of the β-AR subtype phenotype occurs during 3T3-F442A adipocytic differentiation (8, 9). Such rodent adipocytes exhibit a minor β_1-AR (<10%) population and overall a major adipose-specific β_3-AR component (90%) (9, 10). The β_3-AR gene has been cloned from genomic libraries (11, 12), and molecular and pharmacological properties of the resulting products have been studied in detail in adipocytes (9). While β_1- and β_2-AR gene expression is ubiquitous, that of the β_3-AR appears limited to the digestive tract and adipose tissue, in particular in humans (13).

In the present study, we have investigated the heterologous regulation by insulin of the three β-AR subtypes in 3T3-F442A adipocytes. By pharmacological and molecular approaches, we demonstrate that insulin rapidly induces a selective down-regulation of the β_3-AR mRNA and protein. At the same time, expression of β_1- and β_2-ARs remains unchanged. This phenomenon is detectable at physiological insulin concentrations, is insulin receptor mediated, and involves a transcriptional mechanism. This heterologous regulation of the β_3-AR by insulin could play a key role in the body adaptations to antilipolysis, lipogenesis, and thermogenesis that occur during hyperinsulinemic states.

MATERIALS AND METHODS

Cell Culture and Differentiation. 3T3-F442A cells (5) were grown and induced to differentiate in Dulbecco's modified Eagle's medium containing 4.5 g of glucose per liter and supplemented with 10% fetal calf serum. At confluence (day 0), insulin (100 nM) was added to the cell cultures. At day 7 more than 95% of the cells had the morphology of mature adipocytes. After two washes, cells were kept for 48 h in a defined medium consisting of Dulbecco's modified Eagle's medium/Ham's F12 medium (2:1, vol/vol) and 0.1% bovine serum albumin (BSA). From there on the cells were maintained in either the presence or the absence of insulin.

Pharmacological Experiments. For binding experiments with ^{125}I-labeled (−)-cyanopindolol (^{125}I-CYP), cells were harvested and homogenized at 4°C in 1 mM EDTA/25 mM Tris·HCl, pH 7.5. Homogenates were centrifuged for 10 min at 500 × *g* at 4°C. The supernatant was then further centrifuged for 30 min at 4°C at 40,000 × *g*. The pellet was resuspended in the homogenization buffer and stored at −80°C until it was used. Binding assays were performed as de-

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Abbreviations: β-AR(s), β-adrenergic receptor(s); BSA, bovine serum albumin; ^{125}I-CYP, ^{125}I-labeled (−)-cyanopindolol; IGF-I, insulin-like growth factor I; M-MLV, Moloney murine leukemia virus; RT, reverse transcriptase.

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scribed (10). Saturation experiments were performed with ^{125}I-CYP at 5 to 4000 pM. Competition experiments were carried out at 300 pM ^{125}I-CYP. Nonspecific binding was determined in the presence of 100 μM (±)-propranolol. Data from saturation and competition binding experiments were analyzed with the EBDA/LIGAND program (Biosoft–Elsevier, Cambridge, England). CGP12177 and CGP20712A were generous gifts from CIBA–Geigy. ICI118551 and ICI201651 were provided by ICI Pharma and BRL37344, by SmithKline Beecham. The Chemical Abstracts names of these β-AR ligands are mentioned in ref. 14. For adenylate cyclase experiments, cell extracts and assays were as previously described (9). Data are presented as mean ± SEM of at least four experiments. Statistical significance was assessed by the paired Student's *t* test.

RNA Analysis. RNA extraction, electrophoresis, and transfer were performed as described (9). Prehybridization and hybridization (in the presence of 2–3 × 10^6 cpm/ml of the probe) were carried out by the method of Church and Gilbert (16). Membranes were washed twice for 30 min at 60°C in 2× SSC (1× SSC = 150 mM NaCl/15 mM sodium citrate, pH 7.0)/0.1% SDS, then once in 0.2× SSC/0.1% SDS for 20 min at 60°C. For quantitation, autoradiograms were analyzed by videodensitometric scanning and β_3-AR signals were normalized relative to those of β-actin. Probes were labeled by random priming with [α-^{32}P]dCTP. The β_3-AR probe is a 305-bp amplification product of the cloned murine β_3-AR gene (12) between a sense 5′-GCATGCTCCGTGGCCTCACGAGAA-3′ and an antisense primer 5′-CCCAACGGCCAGTGGCCAGTCAGCG-3′.

For reverse transcriptase (RT)-PCR analysis of β-AR gene expression, RNA was digested for 15 min at 37°C with 0.1 unit of RNase-free DNase I (RQ1 DNase; Promega) per μg of 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 placental RNase inhibitor (RNAguard; Pharmacia) at 2 units/μl. RNA (1–4 μg) was reverse transcribed for 1 h at 42°C with Moloney murine leukemia virus (M-MLV) RT (400 units/μg; GIBCO/BRL) in the presence of 10 μM random hexanucleotides, placental RNase inhibitor at 2 units/μl, and each dNTP at 400 μM in a final volume of 40 μl consisting of 50 mM Tris·HCl at pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, and 10 mM dithiothreitol. A control without M-MLV RT was included for each RNA sample. cDNAs were denatured for 5 min at 94°C and then submitted to 30 cycles of amplification (94°C, 1 min; 50°C, 2 min; 72°C, 3 min) followed by a final extension of 7 min at 72°C. PCR was performed in 50 μl containing 2.5 units of *Taq* DNA polymerase (Bioprobe International, Richmond, CA), each dNTP at 125 μM, 10% (vol/vol) dimethyl sulfoxide, and 125 nM both sense and antisense oligonucleotides. The buffer consisted of 20 mM Tris·HCl, pH 8.55/16 mM $(NH_4)_2SO_4$/2.5 mM $MgCl_2$ and BSA at 150 μg/ml. Sequences of the sense and antisense oligonucleotides were 5′-GGATCCAAGCTTTCGTGTGCACCGTGTGGGCC-3′ and 5′-GGATCCAAGCTTAGGAAACGGCGCTCGCAGCTGTCG-3′ for the β_1-AR; 5′-GCCTGCTGACCAAGAATAAGGCC-3′ and 5′-CCCATCCTGCTCCACCT-3′ for the β_2-AR; 5′-ATGGCTCCGTGGCCTCAC-3′ and 5′-CTGGCTCATGATGGGCGC-3′ for the β_3-AR; and 5′-GAGACCTTCAACACCCC-3′ and 5′-GTGGTGGTGAAGCTGTAGCC-3′ for β-actin. These oligonucleotides were derived from the sequences of the corresponding genes or cDNAs (12, 17–19). Amplification products had expected sizes of 286, 329, 528, and 236 bp for β_1-, β_2-, and β_3-ARs and β-actin, respectively. They were separated on a 2% agarose gel and visualized by ethidium bromide staining. cDNA amplification was linear up to 500 ng of RNA (13). Although we did not use a quantitative PCR approach, we were thus able to compare the levels of each mRNA.

For transcription analysis, preparation of the nuclei, elongation assay, DNase I and proteinase K treatments were performed as previously described (10, 20). Equal amounts (2 × 10^7 cpm) of labeled RNA were hybridized for 40 h at 65°C in 2 ml of 0.5 M sodium phosphate, pH 6.8/7% SDS/1% BSA/1 mM EDTA to nitrocellulose filters with the immobilized DNAs. Then 500 ng of a 2-kb *Bam*HI–*Bgl* II fragment from the mouse β_3-AR gene (12), 5 μg of the pUC18 vector containing the murine β-actin cDNA, or 5 μg of pUC18 alone was spotted onto each filter. Membranes were washed twice in 2× SSC for 30 min at 55°C and treated for 30 min at 37°C in the same buffer containing RNase A at 5 μg/ml. Final washes were performed for 15 min at 55°C in 0.2× SSC/0.1% SDS.

RESULTS

Insulin Specifically Down-Regulates the Levels of β_3-AR Protein in 3T3-F442A Adipocytes. ^{125}I-CYP saturation binding experiments indicated that in control adipocytes, the density of β_3-ARs (low-affinity class) was 863 ± 131 fmol/mg of protein (98% of total β-ARs), while that of the β_1- and β_2-ARs (high-affinity class) was only 12.1 ± 3.8 fmol/mg (Table 1). After a 4-day exposure to 1 μM insulin, we observed a 3.4-fold reduction in β_3-AR population (to 255 fmol/mg), while the density of the high-affinity binding sites remained unchanged. ^{125}I-CYP competition with the β_3-selective agonist BRL37344 confirmed that insulin induced a 3.5-fold decrease in β_3-AR population (Table 2). Displacement of ^{125}I-CYP by the β_1-selective antagonist CGP20712A or the β_2-selective antagonist ICI118551 showed that the high-affinity sites for ^{125}I-CYP were exclusively of the β_1-AR subtype. The β_2-ARs were undetectable in control as well as in insulin-exposed cells.

Adenylate cyclase activity was measured on crude membranes from control and insulin-treated adipocytes. Exposure of adipocytes to insulin provoked a moderate time- and dose-dependent decline in adenylate cyclase responsiveness to a maximal dose (100 μM) of the nonselective β-adrenergic agonist isoproterenol (not shown). This effect could be detected after only 24 h following addition of insulin and was maximal after 4 days (about a 2-fold inhibition). We further investigated the modulation by insulin of β-AR expression by using β-AR subtype-selective ligands. In this respect, two compounds were of particular interest, CGP12177 and ICI201651. These two drugs exclusively stimulate the β_3-AR subtype, while they behave as antagonists at the β_1- and β_2-AR sites (14). They thus allow one to specifically address the issue of β_3-AR coupling. Insulin down-regulated adenylate cyclase activity measured in response to a maximal

Table 1. Characteristics of ^{125}I-CYP binding sites in membranes from control and insulin-treated adipocytes

	^{125}I-CYP binding sites			
	High affinity (β_1- and β_2-ARs)		Low affinity (β_3-ARs)	
Treatment	K_d, pM	B_{max}, fmol/mg	K_d, pM	B_{max}, fmol/mg
Control	39.2 ± 4.6	12.1 ± 3.8	3504 ± 304	863 ± 131
Insulin	42.6 ± 11.6	11.8 ± 2	3337 ± 350	255 ± 43*

Adipocytes maintained in a defined medium were either treated or not by 1 μM insulin for 4 days. Membranes were prepared and tested in ^{125}I-CYP saturation experiments using a wide range of concentrations (5–4000 pM) of the radioligand. Scatchard analysis of the data with the EBDA/LIGAND program was used to calculate the dissociation constants (K_d) for the high-affinity (β_1- and β_2-ARs) and low-affinity (β_3-ARs) sites for ^{125}I-CYP and the corresponding receptor densities (B_{max}).

*$P < 0.001$, insulin-treated versus control cells.

Table 2. Competition of ^{125}I-CYP with β-AR subtype-selective ligands in membranes from control and insulin-treated adipocytes

Ligand	Selectivity	Binding affinity (related β-AR subtype)	Culture conditions: No insulin K_i, nM	No insulin B_{max}, fmol/mg	Insulin at 1 μM K_i, nM	Insulin at 1 μM B_{max}, fmol/mg
CGP20712A	β_1	High (β_1-ARs)	0.36 ± 0.05	17.5 ± 8.7 (2%)	1.43 ± 0.65	20 ± 2.7 (7.5%)
		Low (β_2- and β_3-ARs)	2,757 ± 1,150	857.5 ± 8.7 (98%)	5,193 ± 3,314	247 ± 2.7* (92.5%)
ICI118551	β_2	High (β_2-ARs)	—	ND	—	ND
		Mid (β_1-ARs)	109.5 ± 25.5	21.9 ± 5.3 (2.5%)	76 ± 33	17.4 ± 8 (6.5%)
		Low (β_3-ARs)	3,822 ± 1,439	853.1 ± 5.3 (97.5%)	5,882 ± 2,230	249.6 ± 8* (93.5%)
BRL37344	β_3	High (β_3-ARs)	241 ± 86	857.5 ± 3.1 (98%)	124 ± 43	247 ± 6.7* (92.5%)
		Low (β_1- and β_2-ARs)	14,300 ± 1,556	17.5 ± 3.1 (2%)	12,203 ± 2,584	20 ± 6.7 (7.5%)

Competition experiments were performed on membranes with 300 pM ^{125}I-CYP and various concentrations of the β_1-AR-selective antagonist CGP20712A, the β_2-AR-selective antagonist ICI118551, or the β_3-AR-selective agonist BRL37344. The inhibition constant (K_i) and proportion of each binding class for a given subtype-selective ligand were derived from the analysis of the displacement curves with the EBDA/LIGAND program, taking into account the K_d values of β_1-, β_2-, and β_3-ARs for ^{125}I-CYP listed in Table 1. B_{max} values were established from the total β-AR density obtained from ^{125}I-CYP saturation experiments (see Table 1) and from the percentage of each affinity component (indicated in parentheses after the B_{max} value) determined in competition experiments. ND, nondetectable.
*$P < 0.001$, insulin-treated versus control cells.

concentration (100 μM) of the two β_3-AR agonists in a time-dependent manner. A maximal inhibition of 77% was observed after 4 days (Fig. 1A), in agreement with the degree of receptor down-regulation (see Tables 1 and 2). It was dose dependent: inhibition of CGP12177 and ICI201651 adenylate cyclase responsiveness was detectable at 0.1 nM insulin and

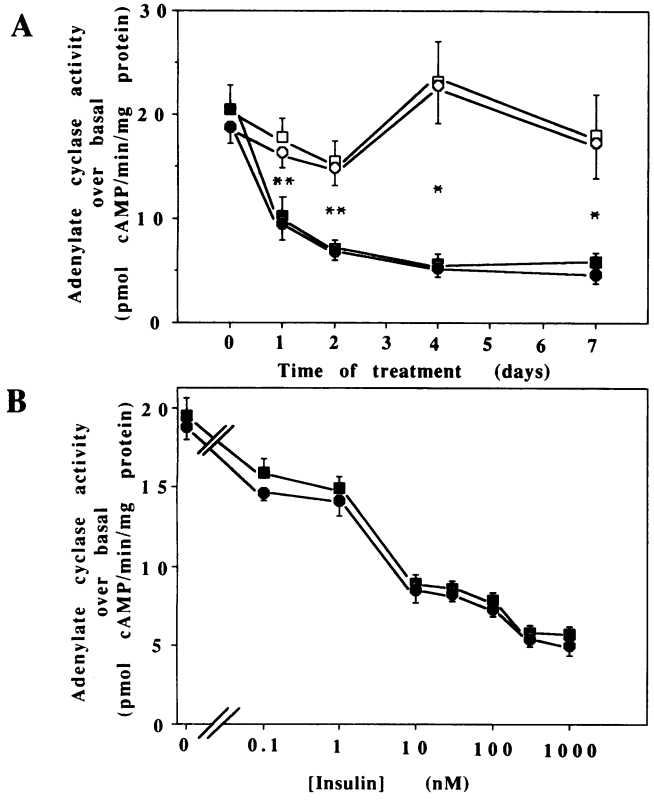

FIG. 1. Time course and dose dependence of insulin-induced decrease in CGP12177- and ICI201651-stimulated adenylate cyclase activities. (A) Insulin (1 μM) was added for various periods of time to 3T3-F442A adipocytes. Adenylate cyclase activity stimulated by CGP12177 (100 μM, squares) and ICI201651 (100 μM, circles) was measured on crude membranes from control (open symbols) and insulin-treated (closed symbols) cells. Results are expressed in pmol of cAMP formed per min per mg of protein as agonist-stimulated over basal adenylate cyclase activity. *, $P < 0.05$; **, $P < 0.01$, insulin-exposed versus control adipocytes. (B) 3T3-F442A adipocytes were treated for 4 days with various insulin concentrations. Adenylate cyclase activity stimulated by an optimal dose (100 μM) of CGP12177 (squares) or ICI201651 (circles) was determined.

was half-maximal at 2.6 ± 0.3 nM and 2.5 ± 0.2 nM for CGP12177 and ICI201651, respectively (Fig. 1B). We compared the ability of various subtype-selective β-AR agonists to stimulate adenylate cyclase in control and insulin-treated adipocytes. Upon insulin exposure, we observed a rightward shift of the dose–response curve for the β_3-AR-selective agonist BRL37344 (EC_{50} = 50 ± 20 nM and 227 ± 87 nM in control and insulin-exposed adipocytes, respectively). This was associated with a 2-fold decrease in the V_{max} values for BRL37344 in insulin-treated cells as compared with control adipocytes (not shown). Moreover, EC_{50} values for the nonselective β-AR agonist isoproterenol, the β_1-AR-selective agonist dobutamine, and the β_2-AR-selective agonist fenoterol were not significantly different between control and insulin-treated adipocytes (not shown). So, adenylate cyclase measurements and binding experiments suggested that cell exposure to insulin could specifically alter β_3-AR coupling to the adenylate cyclase system.

Insulin Down-Regulates β_3-AR Gene Expression by a Transcriptional Mechanism. The steady-state levels of β_3-AR mRNA were first quantified by Northern analysis. Hybridization with the radiolabeled β_3-AR-specific probe revealed the presence of three mRNAs: a major mRNA of 2.3 kb and two minor transcripts of 2.8 and 4.4 kb. Exposure of differentiated 3T3-F442A adipocytes to 1 μM insulin caused a rapid and steep decrease in each β_3-AR mRNA species. This effect was detectable within 1 h (about 60% inhibition) and was maximal at 3 h (93% inhibition). After a 4-day exposure to the hormone, we observed a 4.5-fold decrease in β_3-AR mRNA content. When insulin-treated cells were challenged with hormone-free medium, β_3-AR mRNA levels returned to control mRNA content within 24 h (not shown).

Down-regulation of β_3-AR mRNA by insulin was observed at a hormone concentration as low as 1 nM, but a near-maximal effect was at 10 nM, the half-maximal action being between 1 and 5 nM (Fig. 2A). Since 3T3 adipocytes rapidly degrade insulin (2), the actual insulin concentration required for this effect should be lower. Because IGF-I can bind to the insulin receptor, albeit with a lower affinity than insulin itself, we compared the effects of IGF-I and insulin on β_3-AR mRNA expression. The β_3-AR mRNA level was not modified at 10 nM IGF-I, a concentration at which insulin markedly altered the β_3-AR transcripts (Fig. 2B). Taken together, these findings strongly suggested that the insulin-induced decline of β_3-AR mRNA was mediated by the insulin receptor.

Analysis of β-AR subtype gene expression by RT-PCR showed that insulin selectively inhibited β_3-AR gene expres-

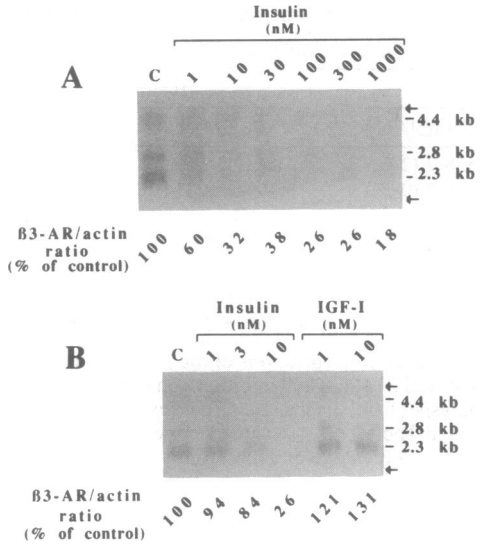

FIG. 2. Effect of insulin concentration on β_3-AR mRNA levels. 3T3-F442A adipocytes were treated for 24 h with various concentrations of insulin or insulin-like growth factor I (IGF-I). Blots of total RNA (10 μg) were hybridized to the β_3-AR-specific probe. After dehybridization, β-actin mRNA levels were measured in the same way and used for normalization. *A* and *B* show representative autoradiograms of Northern blots hybridized with a β_3-AR probe. Arrows indicate the positions of 28S and 18S rRNAs. β_3-AR/β-actin ratios measured at various concentrations of insulin or IGF-I are indicated below the autoradiograms.

sion (Fig. 3). While control adipocytes expressed high levels of β_3-AR mRNA, a 24-h exposure of the cells to 1 μM insulin induced a 4-fold decline in β_3-AR mRNA cell content. In contrast, insulin did not influence the amounts of β_1-AR and β-actin transcripts. β_2-AR mRNA was not visualized in either control or hormone-treated cells.

To determine whether insulin-induced down-regulation of β_3-AR mRNA was linked to a repressed transcription of the β_3-AR gene and/or an accelerated turnover of β_3-AR mRNA, nuclear run-on experiments and mRNA stability studies were performed in control and insulin-treated adipocytes. Transcription analysis showed that, in nuclei isolated from 3T3-F442A adipocytes treated for 30 min or 2 h with insulin, the transcription rates of the β_3-AR gene were respectively reduced by 91% and 89% as compared with the rate of control cells (Fig. 4). Furthermore, the turnover of β_3-AR mRNA

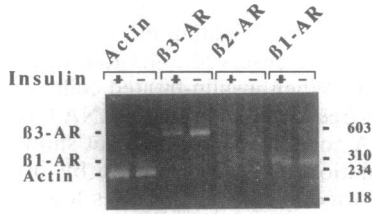

FIG. 3. RT-PCR analysis of β-AR subtype mRNAs in adipocytes exposed to insulin. 3T3-F442A adipocytes were exposed (+) or not (−) to 1 μM insulin for 24 h. Total RNA was isolated and treated with RNase-free DNase I, then with M-MLV RT. Controls without M-MLV RT were included and established that subsequent amplification products did not derive from contaminating DNA (not shown). cDNAs were amplified in the presence of *Taq* polymerase and primers specific for β_1-, β_2-, and β_3-ARs and β-actin. cDNA content in the PCR assay corresponds to initial amounts of DNase I-treated RNA of 400 ng for β_1- and β_2-ARs, 100 ng for β_3-AR, and 50 ng for β-actin. The resulting products were separated on a 2% agarose gel and visualized by ethidium bromide staining. Sizes (in bp) of molecular weight markers are indicated in the right margin, while the positions of the β_1- and β_3-AR and β-actin amplification products are indicated in the left margin.

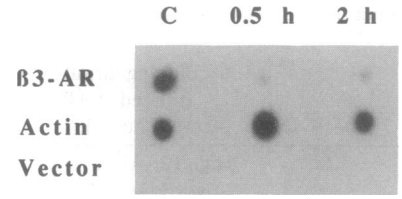

FIG. 4. Effect of insulin on β_3-AR gene transcription. 3T3-F442A cells were either not treated (control, C) or treated by 1 μM insulin for 0.5 or 2 h. Nuclei were isolated and labeled transcripts were hybridized with a β_3-AR DNA fragment spotted onto a nitrocellulose filter. β-Actin cDNA and an empty plasmid (vector) were included as controls. This representative autoradiogram corresponds to a 24-h exposure. Relative transcription rates were quantitated by densitometric scanning.

was determined in adipocytes exposed to an inhibitor of transcription (actinomycin D at 5 μg/ml) in the absence or presence of insulin. After a 10-h exposure to actinomycin, the measured half-life ($t_{1/2}$) of the β_3-AR mRNA was very short but was not affected by insulin (90 ± 15 and 80 ± 15 min in control and insulin-exposed cells, respectively) (Fig. 5).

DISCUSSION

Until now, studies of the antagonistic effect of insulin on the catecholamine-sensitive system have focused on rapid regulatory processes (3, 22–25). The first documented mechanism of which we are aware was the activation by insulin of a "low K_m" cGMP-inhibited cyclic nucleotide phosphodiesterase (3). The phosphorylation of the β_2-AR elicited by insulin could also contribute to the decreased catecholamine responsiveness of adenylate cyclase (23). Hence Engfeldt *et al.* (4) have shown that exposure of human adipocytes to insulin leads to a translocation of β-ARs from the cell surface to an internal compartment. However all these effects are immediate, transient, and overall moderate. By contrast, the insulin-induced decrease in β_3-adrenergic sensitivity reported in the present study corresponds to a delayed, long-term, and dramatic β_3-AR down-regulation.

It is now well documented that insulin can regulate gene expression at various levels: control of mRNA synthesis, processing, degradation, and/or translation efficiency (26). Our results indicate that the insulin-induced decrease in β_3-AR mRNA content is primarily due to a potent inhibitory effect on β_3-AR gene transcription. Moreover, it should be

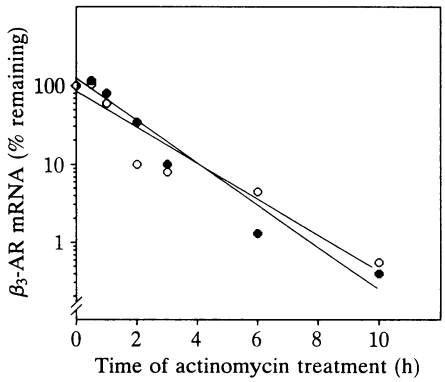

FIG. 5. Effect of insulin on β_3-AR mRNA turnover. 3T3-F442A adipocytes exposed (●) or not (○) to 1 μM insulin were treated with actinomycin D at 5 μg/ml. At the indicated times, total RNA was isolated and β_3-AR mRNA level was measured by Northern analysis. Autoradiograms were analyzed by videodensitometric scanning. Linear regression analysis of the data allowed us to calculate the related half-life values (90 ± 15 and 80 ± 15 min in control and insulin-exposed cells, respectively).

noted that the $t_{1/2}$ of the β_3-AR transcript is very short (about 90 min) and not affected by insulin. Both the rapid transcriptional repression and mRNA turnover explain the sharp decrease in β_3-AR mRNA levels observed within 1 h. Despite this rapid down-regulation of β_3-AR coupling by insulin, no data are yet available on the turnover of the receptor, so that we do not exactly know whether the potent insulin-induced decrease in β_3-AR mRNA is rapidly followed by changes in β_3-AR density. Study of β_3-AR modulation by insulin in adipose tissue of fed or fasted animals will allow us to determine whether this phenomenon occurs only in chronic hyperinsulinemia or is also efficient in response to rapid changes in the nutritional status and fluctuations of glucose homeostasis.

In adipocytes the β-AR system is the main signaling pathway involved in the production of cAMP, which in turn is a key effector of lipid metabolism. Insulin-induced down-regulation of the β_3-AR will thus exert functional consequences on cAMP-dependent cellular processes. The first one will be the well-known decreased lipolytic sensitivity of adipocytes to catecholamines after a chronic exposure to insulin (2). In addition, another major target of cAMP in adipocytes is the genetic modulation of the lipogenic pathway (27). For instance, in adipocytes, a marked down-regulation of mRNA and/or protein expression of lipoprotein-lipase (28, 29), glycerol-3-phosphate dehydrogenase (28, 30), or fatty acid synthase (31) has been observed in response to β-adrenergic agonists or cAMP analogs. As a consequence we can hypothesize that the insulin-induced decline in β_3-AR expression and cAMP accumulation will result in an increased activity and/or expression of several lipogenic enzymes. In this regard activity of the lipogenic pathway is markedly enhanced in liver and adipose tissues from obese hyperinsulinemic animals (32). Besides the known direct effects of insulin on several lipogenic enzymes (26), this insulin-induced decline in β_3-AR expression could be an indirect mechanism of control of lipid anabolism by this hormone. In addition, cAMP down-regulates gene and protein expression of the most abundant glucose transporter of adipocytes, GLUT4 (33). Thus, the decrease in adenylate cyclase responsiveness to catecholamines caused by insulin could also increase glucose availability for lipogenesis.

Otherwise, the noradrenergic pathway also plays a major role in the adaptive heat production in brown adipocytes. Nonshivering thermogenesis upon cold adaptation or facultative diet-induced thermogenesis is due to the activity of the mitochondrial uncoupling protein thermogenin, UCP. An essential feature of UCP is its enhanced expression by cAMP, through a transcriptional mechanism (34, 35). There is now increasing evidence that the β_3-AR is primarily involved in the regulation of UCP gene expression (35, 36). Through a decreased β_3-adrenergic sensitivity, insulin could diminish UCP biosynthesis and thus play a role in the impaired brown adipose tissue thermogenesis usually associated with hyperinsulinemia in animal models of obesity (15). Otherwise, cold-adapted rats have an increased thermogenesis associated with suppressed plasma insulin levels (21). These observations suggest a negative correlation between insulin and thermogenesis. However, since these two experimental situations are accompanied by a strikingly different insulin sensitivity and glucose tolerance, caution is required in assessing the exact role of insulin in the noradrenergic control of thermogenesis in the brown adipose cell.

Finally, the insulin-induced down-regulation of the β_3-AR could represent a basic mechanism of control by this hormone of cAMP-dependent cellular processes in adipocytes. These data suggest that this phenomenon may participate in the onset and maintenance of a nutritional disorder in hyperinsulinemic states. Further studies performed *in vitro* and *in vivo* will be required to ascertain the physiological and physiopathological relevance of this insulin-induced down-regulation of the β_3-AR for the noradrenergic control of lipolysis, lipogenesis, and thermogenesis.

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