Down-regulation of β3-adrenergic receptor expression in rat adipose tissue during the fasted/fed transition: evidence for a role of insulin

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The β3-adrenergic receptor (β3-AR) exerts a central role in the transduction of catecholamine effects in white and brown adipose tissue (WAT and BAT). A recent report has documented that insulin strongly down-regulates β3-AR expression and catecholamine responsiveness in 3T3-F442A adipocytes [Fève, El Hadri, Quignard-Boulangé and Pairault (1994) Proc. Natl. Acad. Sci. U.S.A. **91**, 5677–5681]. In the present report we show that the rise in plasma insulin levels elicited by the fasted/fed transition is associated with a reduction in β3-AR mRNA levels and β-adrenergic responsiveness in WAT and BAT. β3-AR transcripts are also decreased in adipose tissue from animals subjected for 6 h to euglycaemic hyperinsulinaemic glucose clamps. Moreover, insulin acts directly on cultured rat white and brown adipocytes to decrease β3-AR gene expression and adenylate cyclase activity in response to β3-AR-selective agonists. These results suggest that there is a close relationship between food intake, plasma insulin levels and β3-AR expression.

INTRODUCTION

The interplay between insulin and noradrenergic pathways is of fundamental physiological relevance for hormone action in adipose tissue. Insulin, through its pleiotropic effects on metabolism in adipose cells, promotes lipid accumulation [1]. Conversely, activation of β-adrenergic receptors (β-ARs) by catecholamines increases thermogenesis [2] and reduces fat deposits by stimulation of lipolysis and inhibition of lipogenesis [3]. Acute antilipolytic effects of insulin in adipose cells are related to activation of a cGMP-inhibited cyclic nucleotide phosphodiesterase [4] and/or sequestration of β-ARs from the cell surfaces [5]. In contrast, the mechanisms by which long-term insulin treatment reduces β-adrenergic sensitivity are less well documented [6]. We have shown previously [7] that long-term insulin exposure of 3T3-F442A adipocytes induces a strong down-regulation of the β3-AR component.

The β3-AR plays a central role in the regulation of lipolysis and thermogenesis in rodent white and brown adipose tissue (WAT and BAT) [8–10], and this has been highlighted recently by the phenotype of mice that display a targeted disruption of the β3-AR gene [11]. Thus the chronic effect of insulin on this receptor in 3T3 adipocytes could represent a newly characterized and potent mechanism to modulate all cAMP-dependent biological processes. However, the physiological relevance of such a mechanism has not yet been demonstrated *in vivo*. The aim of the present study has been to determine whether, in rodents, changes in nutritional and/or insulin status are accompanied by variations in β3-AR responsiveness and gene expression. Here we demonstrate that the fasted/fed transition is accompanied, within a few hours, by a clear decrease in β3-AR mRNA levels and β3-AR responsiveness of the adenylate cyclase system. Experiments performed in euglycaemic-hyperinsulinaemic-glucose-clamped rats, together with the direct inhibitory effect of insulin on β3-AR expression in isolated white or brown adipocytes, strongly suggest that this modulation results from a direct effect of insulin on the adipocyte β3-AR. These rapid changes in β3-AR expression and sensitivity following variation in insulin status could represent an essential adaptive mechanism to control lipid storage and mobilization according to nutritional conditions.

EXPERIMENTAL

Animals

Six-week-old Wistar rats were housed in temperature-controlled conditions (23 ± 1 °C) with free access to regular food (57 % carbohydrate, 22 % fat, 21 % protein, by calorific value; UAR, Villemoisson-sur-Orge, France) and tap water, and a light (07:00–19:00 h)/dark cycle. Experiments were undertaken according to the Guidelines for the Care and Use of Experimental Animals. After decapitation, epipidymal or peri-renal WAT and interscapular BAT were removed under sterile conditions. Blood was collected and serum glucose and plasma insulin concentrations were measured by means of a glucose analyser (Yellow Springs Instruments) or by RIA (Insulin-CT, CIS Bio-international, Gif/Yvette, France) respectively, using rat insulin as standard.

Studies *in vivo*

We first compared fasted and fed male rats according to their circadian rhythm of food intake [12]. Fasted rats were maintained without food for 10 h (9:00–19:00 h) and killed at the end of the light period. To ensure a rapid rise in plasma insulin levels, fed rats were given a high-carbohydrate diet (70 % carbohydrate,

Abbreviations used: β-AR(s), β-adrenergic receptor(s); BAT, brown adipose tissue; BRL37344, sodium 4-{2-[2-hydroxy-2-(3-chlorophenyl)-ethylamino]propyl}phenoxyacetate sesquihydrate (*RR.SS* diastereoisomer); CGP12177, (±)-4-(3-t-butylamino-2-hydroxypropoxy)benzimidazol-2-one; GTP[S], guanosine (5′→O^3)-1-thiotriphosphate; ISO, (−)-isoproterenol; MMLV-RT, Moloney murine leukaemia virus-reverse transcriptase; RT, reverse transcriptase; WAT, white adipose tissue.

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10 % fat, 20 % protein; by calorific value) *ad lib.* and were killed 3 h after the beginning of the dark period, which corresponded with their spontaneous nocturnal refeeding phase.

β3-AR gene expression in perirenal adipose tissue from female rats was also compared between fasted animals and animals subjected to euglycaemic hyperinsulinaemic glucose clamps. Three days before the experiments, indwelling catheters were inserted into the left jugular vein and the right carotid artery under light diethyl ether anaesthesia so that the rats were conscious during further experimentation [13]. Euglycaemic hyperinsulinaemic clamping was applied for 6 h as described previously [14]. Briefly, human insulin (Actrapid Novo, Copenhagen, Denmark) was infused through the jugular vein at a constant rate of either 0.6 units/h per kg of body weight to reach a plasma insulin concentration of 315 μ-units/ml, or 3 units/h per kg of body weight to reach 4820 μ-units/ml. Blood glucose was maintained at euglycaemic levels by a variable infusion of glucose (30 %, w/v). Glucose infusion was started 1 min after the beginning of insulin infusion and was set at 1.8 mmol/min per kg of body weight, based on the results of previous experiments. The glucose infusion was periodically adjusted to maintain euglycaemia. Blood samples (10 μl) were withdrawn from the carotid artery every 5 min during the first hour, then every 30 min until the end of the experiments. Blood glucose concentration was determined extemporaneously using a glucose analyser (Yellow Springs Instruments). Additional samples (100 μl) were taken hourly for plasma insulin and glucose determinations. The samples were immediately centrifuged at 4 °C and the plasma was removed and frozen at −20 °C. At the end of the experiments, the rats were killed by an overdose of pentobarbital and the fat pads were removed, quickly frozen in liquid N_2 and stored at −80 °C until processed.

Adipocyte isolation and culture

Adipose tissues from 6-week-old rats were digested with collagenase. Isolation of white adipocytes was performed as described previously [15]. Isolated brown adipocytes were obtained using a similar procedure with slight modifications (5 mg/ml of collagenase for 30 min). Brown and white adipocytes were cultured in 50 ml Falcon tubes in Dulbecco's modified Eagle's medium containing 22 mM glucose, 20 mM *N*-(2-hydroxyethyl)-piperazine-*N*′-(2-ethanesulphonic acid), 10 % (v/v) fetal calf serum and antibiotics at 37 °C in an atmosphere of 5 % CO_2 in a humidified incubator. The medium was renewed after 24 h and the cells were either exposed or not exposed to insulin. Preliminary experiments indicated a maximal repression of β3-AR mRNA levels after 6 h of treatment with insulin (results not shown). Consequently, this exposure time was chosen to study the effects of insulin on β3-AR transcripts. The optimal effect of insulin on β3-AR coupling to the adenylate cyclase system was tested after 48 h of exposure to insulin.

Membrane extraction and adenylate cyclase assay

Whole tissues or isolated cells were homogenized and crude membranes were prepared according to the method of Granemann and MacKenzie [16]. The protein content was measured by the method of Bradford [17]. Adenylate cyclase (EC 4.6.1.1) activity was measured on membranes (20–30 μg/assay) as described previously [18,19], either in the absence or in the presence of the following effectors: (−)-isoproterenol (ISO), guanosine (5′→O^3)-1-thiotriphosphate (GTP[S]) and forskolin (Sigma). Sodium 4-{2-[2-hydroxy-2-(3-chlorophenyl)ethylamino]propyl}phenoxyacetate sesquihydrate (*RR,SS* diastereoisomer) (BRL37344) and (±)-4-(3-t-butylamino-2-hydroxypropoxy)benzimidazol-2-one (CGP12177) were generously provided by Beecham Pharmaceuticals and Ciba–Geigy respectively. BRL37344 is a β3-AR-selective agonist but also activates β1- and β2-ARs with a lower potency [20–22]. CGP12177 is a β3-AR agonist but is also a β1-/β2-AR antagonist [22], and thus allows specific coupling of β3-AR to the adenylate cyclase system.

RNA analysis

Total RNA was extracted from cells and tissues by the procedure of Chomczynski and Sacchi [23] and reverse transcriptase (RT)-PCR analysis was carried out as described previously [24,25]. Briefly, after digestion with RNase-free DNase I (RQ1 DNase; Promega), RNA samples were reverse-transcribed with Moloney murine leukaemia virus (MMLV)-RT (Life Technologies Inc.) according to the manufacturer's instructions. To ensure that subsequent DNA amplification did not derive from contaminant genomic DNA, a control without MMLV-RT was included for each RNA sample. cDNAs were denatured for 5 min at 94 °C and submitted to 25 cycles of amplification (1 cycle: 94 °C, 30 s; 60 °C, 1 min; 72 °C, 1 min) followed by a final extension of 5 min at 72 °C in a DNA thermal cycler 9600 (Perkin Elmer). PCR was performed in a reaction sample volume of 25 μl containing 1 unit of *Taq* polymerase (Bioprobe, Montreuil-sur-Bois, France), 125 μM of each dNTP, 5 % (w/v) formamide, 125 nM of both sense and antisense oligonucleotides for the β3-AR and β-actin. The buffer consisted of 20 mM Tris/HCl (pH 8.55)/16 mM $(NH_4)_2SO_4$/2.5 mM $MgCl_2$, and 150 mg/ml BSA. Sequences of the sense and antisense oligonucleotides were: 5′-ATGGCTCCGTGGCCTCAC-3′ and 5′-CCCAACG-GCCAGTGGCCAGTCAGCG-3′ for the β3-AR; 5′-GAGA-CCTTCAACACCCC -3′ and 5′-GTGGTGGTGAAGCTGT-AGCC-3′ for β-actin. These oligonucleotides were derived from the sequences of the corresponding genes and cDNAs [26,27]. Amplification products had the expected sizes of 308 and 236 bp for β3-AR and β-actin respectively. They were separated on a 2 % agarose gel and visualized by ethidium bromide staining. Where indicated, amplification products were transferred after electrophoresis to nylon Nytran-plus membranes (Schleicher and Schuell). Hybridization with specific β3-AR or β-actin [α-^{32}P]dCTP random-primed DNA probes was carried out in sodium phosphate buffer [28]. Final washes were performed under stringent conditions [0.2 × SSC, 0.1 % (w/v) SDS for 15 min at 65 °C; 1 × SSC: 150 mM NaCl/15 mM sodium citrate]. Preliminary experiments were carried out with various amounts of cDNA to determine non-saturating conditions of PCR amplification for both β3-AR and β-actin. Thus cDNA amplification was performed in comparative and semi-quantitative conditions. Gels or autoradiograms were analysed by video-densitometric scanning (Vilber Lourmat Imaging, Marne-la-Vallee, France) and β3-AR signals were normalized to those of β-actin.

Statistical analyses

Results are presented as means ± S.E.M. The level of significance between groups was assessed using either paired or unpaired Student's *t* tests.

RESULTS

Changes in plasma insulin levels are associated with variations in β3-AR expression

To evaluate the relationships between plasma insulin levels and β3-AR expression in adipocytes, molecular and functional

Table 1 Influence of food intake on β3-AR mRNA expression in WAT and BAT

Total RNA was extracted from epididymal WAT and interscapular BAT of fasted or fed rats. Blood samples were collected to monitor glycaemia and insulinaemia. Total RNA was digested with DNase I and treated or not with MMLV-RT. PCR products were separated on a 2% agarose gel, transferred to a nylon membrane and hybridized with β3-AR- or β-actin-specific DNA probes. For quantification, autoradiograms were analysed by video-densitometric scanning. β-Actin mRNA levels were used to standardize β3-AR mRNA content. The results for fed animals are expressed as the percentage of β3-AR mRNA levels detected in WAT or BAT from fasted rats and represent the means ± S.E.M. of four individual determinations in each group. *, $P < 0.05$; **, $P < 0.01$, fed versus fasted animals.

	Plasma glucose (g/l)	Plasma insulin (μ-units/ml)	β3-AR mRNA levels (%) WAT	BAT
Fasted	1.13 ± 0.07	21.8 ± 3.1	100 ± 32	100 ± 36
Fed	1.70 ± 0.11**	150.4 ± 36.7**	58 ± 22*	39 ± 7*

Table 2 Variations in β3-AR coupling to the adenylate cyclase system in adipose tissue from fasted or fed animals

Membranes from fasted or fed rats ($n = 4$ per group) were prepared from WAT or BAT and adenylate cyclase activity was measured in response to an optimal concentration (100 μM) of each β-AR ligand. The results are expressed as means ± S.E.M. of effector-stimulated adenylate cyclase activity over basal levels. For each β-AR effector, the values in parentheses for fed animals represent the percentage of stimulated-adenylate cyclase activity measured in membranes from fasted rats. The basal adenylate cyclase activity was 39.8 ± 6.8 and 45 ± 3.9 pmol cAMP/min per mg of protein in WAT from fasted and fed animals, and 40.3 ± 6.5 and 57.2 ± 2.7 pmol cAMP/min per mg of protein in BAT of fasted and fed rats respectively. *, $P < 0.05$; **, $P < 0.02$, fed versus fasted animals.

	Adenylate cyclase activity (above basal levels) (pmol cAMP/min per mg protein)			
	WAT		BAT	
Effector (100 μM)	Fasted	Fed	Fasted	Fed
ISO	221.7 ± 22.8	96.5 ± 4.6**(43.5%)	221.4 ± 12.2	137.2 ± 4.0** (62%)
BRL37344	183.9 ± 22.2	78.7 ± 2.7*(43%)	181.7 ± 10.0	113.2 ± 5.2** (62%)
CGP12177	156.4 ± 23.0	53.4 ± 6.8**(35%)	91.1 ± 16.9	35.6 ± 7.8** (39%)

studies were performed on adipose tissue from rats subjected to conditions known to affect insulinaemia.

We first tested the effect of the fasted/fed transition on β3-AR expression in WAT and BAT. Food intake enhanced blood glucose levels (50% increase) (Table 1), and caused a 7-fold increase in plasma insulin. Since limited amounts of total RNA were available, we chose to study gene expression by RT-PCR analysis. Preliminary experiments were performed to ensure that amplification was carried out in the linear range for the β3-AR and β-actin (see the Experimental section). Under these conditions, we were able to compare the relative amounts of specific mRNAs. Compared with fasted rats, intake of a high-carbohydrate diet produced a rapid reduction in β3-AR mRNA in adipose tissue. In fed rats, we observed a 42% and a 61% decrease in β3-AR mRNA content in WAT and BAT respectively (Table 1). Adenylate cyclase activity in response to β-AR effectors was also measured in membrane extracts from WAT and BAT (Table 2). Reduction in β3-AR mRNA levels was paralleled by a decreased β-AR responsiveness of the adenylate cyclase system. In WAT from fed animals compared with fasted rats, adenylate

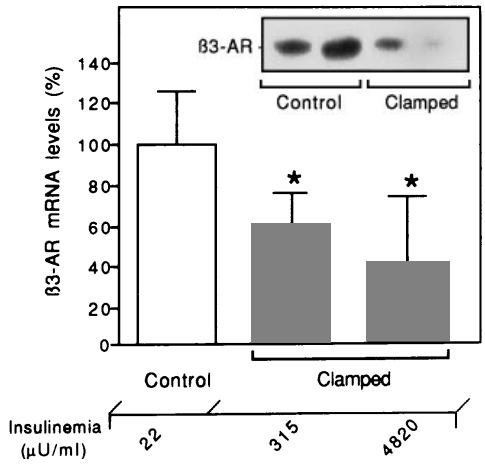

Figure 1 Modulation of β3-AR mRNA levels in WAT from 6 h-clamped rats

Total RNA was extracted from perirenal WAT after a 6-h euglycaemic hyperinsulinaemic glucose clamp performed with the indicated physiological or pharmacological plasma insulin levels ($n = 4$ in each group). RT-PCR analysis was carried out as described in the caption to Table 1. Results for 4 individual clamped animals are expressed as the percentage ± S.E.M. of β3-AR mRNA levels observed in fasted control animals. The plasma insulin levels of each group are shown beneath the columns. The inset shows the results of a typical RT-PCR experiment in 2 fasted (control) rats and 2 animals perfused with a high insulin concentration. *, $P < 0.02$, clamped versus control animals.

cyclase activity in response to ISO and to the β3-AR-selective agonist, BRL37344, was reduced by 56% and 57% respectively. Similarly, in BAT, ISO- and BRL37344-stimulated enzyme activity was reduced by 38% after feeding. Interestingly, both in WAT and BAT, inhibition of β-AR coupling to the adenylate cyclase system during the fasted/fed transition was most pronounced (61–65% inhibition) when tested in the presence of CGP12177, a partial β3-AR agonist with antagonistic properties at the β1- and β2-AR sites [22].

The effect of insulin on β3-AR gene expression in adipose tissue was also measured with the aid of euglycaemic hyperinsulinaemic glucose clamps. Euglycaemic clamps over a period of 6 h allowed an increase in plasma insulin concentration which was within the physiological (315 μ-units/ml) or pharmacological (4820 μ-units/ml) range, while maintaining plasma glucose at euglycaemic levels (5.3 ± 0.2 mM). Compared with fasted rats, β3-AR mRNA steady-state levels were down-regulated by 40% and 60% in perirenal WAT from mildly and severely hyperinsulinaemic animals respectively (Figure 1).

Insulin inhibits β3-AR gene expression and β3-AR responsiveness in isolated white and brown adipocytes

To address the direct effect of insulin on β3-AR gene expression, adipocytes isolated from epididymal fat pads were treated with varying concentrations of the hormone. Figure 2 shows that down-regulation of β3-AR mRNA levels by insulin was dose-dependent. The inhibitory effect of insulin was detectable at a concentration of 1 nM and was maximal at 100 nM (~ 2-fold inhibition). The concentration of insulin giving half-maximal action was in the 1–10 nM range. To determine the functional consequences of this insulin-induced decrease in β3-AR mRNA levels, response of adenylate cyclase activity to β-AR effectors was measured on membranes from epididymal adipose cells which had or had not been exposed to insulin (Table 3). Insulin

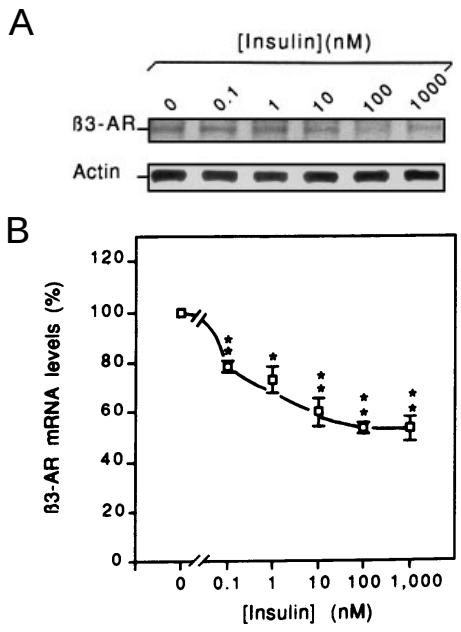

Figure 2 Dose-dependent effect of insulin on β3-AR mRNA levels in cultured rat adipocytes

Epididymal adipocytes were isolated as described in the Experimental section and cultured for 6 h in the absence or in the presence of the indicated insulin concentrations. RT-PCR experiments were performed as described in the caption to Table 1. PCR products were resolved on a 2% agarose gel stained with ethidium bromide and analysed by videodensitometric scanning. (**A**) Represents a typical PCR experiment. The positions of β3-AR and β-actin PCR products are shown on the left. (**B**) Results for insulin-treated cells are represented as the percentage of the level detected in control adipocytes. The data represent the means $\pm$ S.E.M. of four independent cultures. *, $P < 0.02$; **, $P < 0.005$, insulin-treated versus control adipocytes.

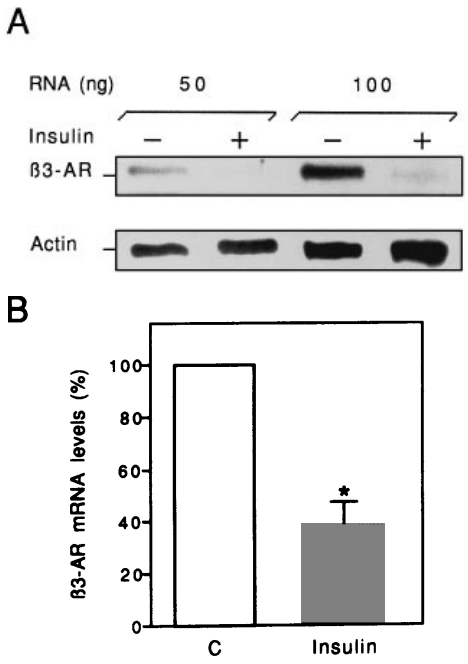

Figure 3 Effect of insulin on β3-AR mRNA levels in rat cultured brown adipocytes

Brown adipocytes were isolated and cultured as described in the Experimental section, and were exposed (+) or not (–, C) to 100 nM insulin for 6 h. Total RNA was extracted and analysed by RT-PCR as described in the caption to Table 1. (**A**) shows a typical ethidium bromide-stained gel of PCR products. The positions of β3-AR and β-actin PCR products are shown on the left. cDNAs derived from various amounts (ng) of total RNA were used in the PCR reaction and assessed that amplification was performed in non-saturating conditions. (**B**) Results for insulin-treated cells are represented as the percentage of the level detected in control adipocytes. The data represents the means $\pm$ S.E.M. of three separate cultures. *, $P < 0.02$, insulin-exposed versus control (C) adipocytes.

Table 3 Insulin selectively inhibits adenylate cyclase activity stimulated by β3-AR agonists in isolated white and brown adipocytes

Membranes were extracted from white or brown adipose cells cultured for 48 h in the absence or in the presence of 100 nM insulin and adenylate cyclase activity was measured in the presence of an optimal concentration (100 μM) of each effector. The results are expressed as effector-stimulated adenylate cyclase activity over basal levels and represent the means $\pm$ S.E.M. of four and eight independent cultures of brown and white adipose cells respectively. For each β-AR effector, the values in parentheses for insulin-treated cells represent the percentage of stimulated adenylate cyclase activity detected in control cells. Basal adenylate cyclase activity was 91.5 $\pm$ 3.6 and 85.7 $\pm$ 4.3 pmol cAMP/min per mg of protein in control and insulin-treated white adipose cells, and 37.6 $\pm$ 4.0 and 42.6 $\pm$ 3.6 pmol cAMP/min per mg of protein in control and insulin-exposed brown adipocytes respectively. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, insulin-treated versus control adipocytes.

	Adenylate cyclase activity (above basal levels) (pmol cAMP/min per mg of protein)			
	WAT		BAT	
Effector (100 μM)	Control	Insulin	Control	Insulin
ISO	104.3 $\pm$ 1.5	84.0 $\pm$ 7.1*** (80.5%)	126.3 $\pm$ 10.1	77.8 $\pm$ 1.4* (61.5%)
BRL37344	72.6 $\pm$ 3.8	57.2 $\pm$ 6.6*** (79%)	80.4 $\pm$ 6.3	38.8 $\pm$ 1.8** (48%)
CGP12177	42.5 $\pm$ 6.9	19.2 $\pm$ 4.9*** (45%)	53.1 $\pm$ 7.7	19.7 $\pm$ 2.1** (37%)

induced a significant reduction of 19% and 21% in adenylate cyclase activity stimulated by ISO and BRL37344 respectively. As mentioned above, during the experiments *in vivo* this effect was more marked (55% inhibition) with the β3-AR agonist/β1–β2-AR antagonist, CGP12177. To determine whether insulin regulates catecholamine responsiveness essentially through the modulation of β-ARs, adenylate cyclase activity was also measured in control or insulin-treated adipose cells in response to G-protein or adenylate cyclase effectors. Chronic exposure to insulin had no significant effect on adenylate cylase activated by an optimal concentration (100 μM) of GTP[S] or forskolin. GTP[S]-stimulated adenylate cyclase activity was 352 $\pm$ 15 and 327 $\pm$ 9 pmol cAMP/min per mg of protein in control and insulin-exposed adipocytes respectively ($n = 4$; $P = 0.16$). Forskolin-stimulated adenylate cyclase activity was 1920 $\pm$ 102 and 2089 $\pm$ 422 pmol cAMP/min per mg of protein in control and insulin-treated cells respectively ($n = 4$; $P = 0.74$). Thus in insulin-treated white adipose cells, adenylate cyclase activity in response to GTP[S] and forskolin represented 93 $\pm$ 3% and 109 $\pm$ 20% of the activities measured in control cells respectively.

Similarly, exposure of cultured brown adipocytes to insulin caused a 2.6-fold reduction in β3-AR mRNA steady-state levels (Figure 3). In functional studies, the extent of the insulin-induced inhibition of β-AR coupling to the adenylate cyclase system was also in the rank order: CGP12177 > BRL37344 $\geqslant$ ISO, consistent with the selectivity of these drugs for the β3-AR (Table 3). Furthermore, there was a strict parallelism between the decrease in β3-AR mRNA levels and the reduction in CGP12177-stimulated adenylate cyclase activity measured in brown adipocyte membranes (2.7-fold decrease) (Figure 3 and Table 3).

DISCUSSION

In adipocytes, cross-talk between insulin and adrenergic pathways is of fundamental importance for the modulation of energetic homoeostasis. Beside the previously known acute effects of insulin on several steps of the β-AR signalling pathway [4,5], we have shown that insulin selectively down-regulates β3-AR gene and protein expression in mouse 3T3-F442A adipocytes at a transcriptional level [7]. The present data derived from rat studies both *in vivo* and *in vitro* strongly suggest the physiological relevance of the heterologous regulation of the β3-AR by insulin.

Our results indicate that the insulin-induced decrease in the levels of β3-AR transcripts is followed by parallel alterations in β3-AR coupling both in cultured white and brown adipose cells, whereas insulin has no effect on GTP[S]- and forskolin-stimulated adenylate cyclase activities. Moreover, we and others [7,29–33] have generally observed a good correlation between the variation in β3-AR mRNA and protein levels. Taken together, these findings strongly suggest that the insulin-elicited decrease in β3-AR responsiveness is a receptor- rather than a G-protein- or adenylate cyclase-related event.

In this work we show that the fasted/fed transition in the rat results in a β3-AR down-regulation at both molecular and functional levels. Can variations in β3-AR trancripts and sensitivity during the fasted/fed transition be related solely to changes in insulin status? Other hormonal or metabolic factors could also contribute to receptor regulation in response to food intake. Thus the potential involvement of the sympathetic nervous system in the post-prandial regulation of this adrenergic receptor subtype can be questioned. Since β-AR agonist administration to rodents decreases β3-AR expression [30,34,35], it is conceivable that the diet-induced activation of the sympathetic nervous system could control adipose tissue β3-AR levels. Several groups have reported that the β3-AR is much more resistant to agonist-promoted desensitization *in vivo* [36] or in cellular systems [31,33,37,38]. However, in the present work several lines of evidence support the hypothesis that insulin may be the essential mediator for a decrease in β-adrenergic responsiveness after food intake. First, the use of euglycaemic hyperinsulinaemic glucose clamping allows us to circumvent the possible interference of metabolic alterations associated with the fasted/fed transition. When plasma insulin concentration was raised within the physiological or pharmacological range during clamping, a clear-cut down-regulation of adipose β3-AR mRNA levels was observed, which was independent of variation in blood glucose levels. Since hyperinsulinaemia *per se* increased plasma noradrenaline levels during hyperinsulinaemic euglycaemic clamping [39], however, we cannot exclude that an insulin-induced activation of the sympathetic nervous system could modulate β3-AR expression in adipose tissue during the clamp procedure. Second, in agreement with our previous observation in 3T3-F442A adipocytes [7], physiological concentrations of insulin strongly inhibit β3-AR gene expression and β3-AR coupling to the adenylate cyclase system when cultured white and brown adipocytes are exposed to the hormone. Finally, when tested in the presence of CGP12177, which stimulates the β3-AR exclusively, the extent of inhibition of adenylate cyclase activity is similar between measurements during the fasted/fed transition of animals and those obtained after exposure of isolated fat cells to insulin *in vitro*. In conclusion, the decrease in adipocyte β3-AR expression observed during the fasted/fed transition and euglycaemic hyperinsulinaemic glucose clamping, together with the direct down-regulatory effect of insulin on this adrenergic receptor subtype in cultured adipocytes, and the strong parallelism between molecular and functional approaches support a causal relationship between food intake, insulin levels and β3-AR gene expression.

Since in rodent WAT or BAT the β3-AR is an essential component of the β-adrenergic system [8], the insulin- or food intake-induced down-regulation of this receptor has potential consequences on all cAMP-dependent processes. By decreasing the β3-AR density, insulin could thus diminish the lipolytic activity of catecholamines and thereby, after a few hours, could relay the acute antilipolytic effects initiated by activation of a cGMP-dependent phosphodiesterase [4] or sequestration of cell surface β-ARs [5]. Catecholamines and cAMP also exert a negative control on several enzymes of the lipogenic pathway. For instance, fatty-acid synthase [40], lipoprotein lipase [41,42], and acetyl-CoA carboxylase [43] gene and/or protein expression are decreased by β-AR agonists or cAMP analogues. The insulin-induced decline in β3-AR expression and cAMP production can thus limit the inhibitory β-adrenergic control of lipogenesis and could reinforce the direct insulin action on several lipogenic enzymes [44]. Through decreased lipolytic sensitivity and a reduced response of lipogenesis to catecholamines, down-regulation of the β3-AR could provide a basic mechanism by which insulin could promote lipid storage and prevent lipid mobilization after food intake.

Numerous studies have focused on the role of insulin in diet-induced thermogenesis [45]. Although insulin obviously has some direct effects on the metabolism of BAT, it is generally accepted that its effects on thermogenesis are mediated by central activation of the sympathetic nervous system [46]. However, the present study shows that the fasted/fed transition is associated with a decreased β3-AR expression and sensitivity in BAT, and that insulin acts directly on cultured brown adipocytes to modulate this β3-AR component. Consequently, the insulin-induced decrease in BAT catecholamine responsiveness could alter the noradrenergic control of uncoupling protein, and hence could limit an excessive diet-induced thermogenesis and energy dissipation.

The β3-AR plays a pivotal role in rodent WAT and BAT but is expressed at much lower levels in humans [47–49]. Nevertheless, recent studies *in vivo* have indicated that the β3-AR is able to mediate lipolysis in subcutaneous fat in humans [50,51]. It has also been reported that a missense mutation of the β3-AR is associated with earlier onset of non-insulin-dependent diabetes, insulin resistance, decreased energy expenditure and obesity [52–54]. These findings suggest that the β3-AR might also be involved in the control of energy balance in humans. However, no data concerning the hormonal and/or nutritional regulation of the β3-AR are available for humans. Though it is tempting to speculate that the modulation of the human β3-AR during physiological or physiopathological variations of insulinaemia might regulate lipolysis and lipogenesis, it is not yet possible to extrapolate the results presently observed in a rodent model to humans.

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