Insulin and insulin-like growth factor 1 antagonize the stimulation of *ob* gene expression by dexamethasone in cultured rat adipose tissue

Bénédicte A. REUL, Lumbe N. ONGEMBA, Anne-Marie POTTIER, Jean-Claude HENQUIN and Sonia M. BRICHARD*

Endocrinology and Metabolism Unit, University of Louvain, Faculty of Medicine, UCL 55.30, Avenue Hippocrate 55, B-1200 Brussels, Belgium

The *ob* gene, specifically expressed in fat cells, encodes leptin, a hormone that induces satiety and increases energy expenditure. In this study, we investigated the interactions between glucocorticoids and insulin on *ob* gene expression in cultured explants of rat adipose tissue. Only low levels of *ob* mRNA were detected when adipose tissue from fasted rats was cultured for 12–24 h in minimal essential medium. However, the addition of dexamethasone to the medium increased *ob* gene expression in a concentration-dependent manner (EC_{50} 10 nM). With 1 μ M dexamethasone, *ob* mRNA levels were similar to those in fresh fat pads from fed rats, reaching a maximum after 12 h. The effect of dexamethasone was blocked by actinomycin D, which indicates an action on transcription. This effect was increased when a minimum amount of fuel (glucose or a mixture of lactate and pyruvate) was supplied in the medium. Unlike dexamethasone, insulin, even when combined with high glucose concentrations, did not induce *ob* expression, although it strongly increased the accumulation of mRNA species for fatty acid synthase (FAS),

the insulin-sensitive glucose transporter GLUT4 and the γ isoform of peroxisome proliferator-activated receptor (PPAR γ). Unexpectedly, insulin dose-dependently inhibited dexamethasone-induced *ob* mRNA accumulation. This effect was observed at low concentrations of insulin (IC_{50} 1 nM) and was delayed in onset, beginning after 6–9 h of culture. It was mimicked by insulin-like growth factor 1 (IGF-1) (100 nM). The inhibition by insulin was only detectable when fuels were present and/or when a critical level of *ob* expression was reached. As this inhibitory effect was reversed by cycloheximide, this suggests that it required ongoing protein synthesis. In conclusion, unlike dexamethasone, insulin had no direct stimulatory effect on *ob* gene expression. On the other hand, insulin (and IGF-1) even inhibited the dexamethasone-induced accumulation of *ob* mRNA. The underlying mechanism involved ongoing synthesis of an inhibitory protein by insulin, which is in keeping with its delayed effect. Moreover, the expression of genes for FAS, GLUT4 and PPAR γ may be inversely related to that of *ob*.

INTRODUCTION

The *ob* gene is specifically expressed in fat cells [1]. It encodes leptin, a hormone that induces satiety and increases energy expenditure, thereby playing a key role in weight maintenance [2]. Although leptin secretion might be acutely modified by hormones [3], circulating leptin levels also seem to be regulated at the pretranslational level [4,5]. Unravelling the mechanisms involved in the control of *ob* gene expression is therefore essential for our understanding of energy homeostasis.

The hormonal regulation of the *ob* gene is still unclear. Glucocorticoids and insulin have been proposed as potential stimulatory candidates. Glucocorticoids increased *ob* expression *in vivo* when administered at pharmacological doses to normal rats [6], and *in vitro* in primary cultures of adipocytes [7,8] but not in the 3T3-L1 cell line [9]. Insulin might be stimulatory *in vivo*; however, this issue is still debated. *ob* mRNA and protein levels were decreased by fasting and re-induced by refeeding, concomitantly with the changes in insulinaemia [4,10–12]. High insulin levels up-regulated *ob* expression during euglycaemic-hyperinsulinaemic clamps in rats [12–14]. Hyperinsulinaemia imposed in man also resulted in increased plasma leptin levels in some studies [15] but not all [16–18]. Moreover, insulin treatment only partially restored (40–60 % of control levels) the depressed expression of *ob* mRNA in streptozotocin-diabetic rats [10,19]. Although insulin could acutely increase the secretion of leptin by isolated adipocytes [3], experiments *in vitro* have not solved the controversy over the potential direct effects of the hormone on leptin synthesis and *ob* gene expression. Both an increase [9,12,20]

and no change [7,8,17,19] in *ob* mRNA have been reported, perhaps because of the use of different culture models.

To assess the direct role of hormones on the expression of fat genes, cultured explants of adipose tissue have decisive advantages over clonal cell lines or stromal cells differentiated by long-term exposure to pharmacological doses of ‘adipogenic cocktails’. The approach is more physiological and the model is highly responsive to insulin [21]; moreover, interactions between cells are preserved.

In the present study we therefore characterized the multi-hormonal control of *ob* gene expression in cultured explants of adipose tissue from normal rats. We more particularly focused on the interactions between insulin and glucocorticoids. For comparison, we also measured the expression of other genes whose products play a key role in adipose tissue metabolism [i.e. those for fatty acid synthase (FAS), the insulin-sensitive glucose transporter GLUT4 and a member of the peroxisome proliferator-activated receptor subfamily of nuclear hormone receptors (PPAR γ)].

MATERIALS AND METHODS

Adipose tissue culture

Untreated male Wistar rats ($\pm$ 180 g) were killed in the morning after 24 h of fasting, when *ob* expression is expected to be depressed [10]. Epididymal fat pads were quickly removed under sterile conditions, cut with scissors into small pieces (approx. 2–3 mm³) and agitated for 30 min in minimal essential medium with Earle’s salts (MEM) supplemented with 1 % (w/v) BSA, as

Abbreviations used: FAS, fatty acid synthase; G0, G5, G20, 0 mM, 5 mM and 20 mM glucose respectively; GLUT4, insulin-sensitive glucose transporter; IGF, insulin-like growth factor; MEM, minimal essential medium; PPAR γ , peroxisome proliferator-activated receptor γ isoform.

* To whom correspondence should be addressed.

described [21]. The pieces of adipose tissue were then washed in PBS and incubated in 100 mm Petri dishes containing 9 ml of MEM supplemented with 1 ml of fetal calf serum, 100 i.u./ml penicillin, 100 μ g/ml streptomycin and 0.25 μ g/ml amphotericin B. One (unilateral) fat pad was cultured per dish and a given experimental condition was always tested with fat pads isolated from different rats. Unless otherwise indicated, the final concentration of glucose in the medium was 5 mM. The basal concentrations of cortisol and insulin were extremely low (approx. 0.5 nM and 3 pM respectively). Different hormones, substrates or pharmacological agents were added in various combinations in accordance with the experimental protocols. BRL 35135 was provided by SmithKline Beecham. Non-esterified fatty acids were dissolved in 0.1 M NaOH in a closed plastic tube placed in a bath of boiling water, then added slowly and during continuous agitation to the experimental solutions. The dishes were cultured for up to 24 h at 37 °C in an air/CO₂ (19:1) atmosphere. At the end of the experiment the pieces of adipose tissue were again rinsed in PBS, collected, frozen in liquid nitrogen and stored at -70 °C for subsequent RNA extraction.

RNA extraction and Northern blot analysis

Total RNA was isolated with an acid guanidinium thiocyanate/phenol/chloroform mixture as previously described [10]. The concentration of RNA was determined by absorbance at 260 nm. For Northern blot analysis, 30 μ g of total RNA was denatured in a solution containing 2.2 mM formaldehyde and 50% (v/v) formamide by heating at 95 °C for 2 min. RNA was then size-fractionated by 1% (w/v) agarose-gel electrophoresis, transferred to a Hybond-N membrane (Amersham, Little Chalfont, Bucks., U.K.) and cross-linked by ultraviolet irradiation. The integrity and relative amounts of RNA were assessed by Methylene Blue staining of the blot.

The cDNA probes for FAS [22] and GLUT4 [23] were kindly supplied by Dr. A. G. Goodridge and Dr. D. E. James respectively. The *ob* cDNA probe was prepared as described [10]. Hybridizations with the different radiolabelled probes and subsequent washing of the membranes were performed as reported [10]. The PPAR γ [24] cDNA probe was prepared as follows. A product of 391 bp was obtained after reverse transcriptase-PCR on total RNA from rat adipose tissue (sense primer 5'-TATG-GAGCCTAAGTTTGAGT-3'; anti-sense primer 5'-GCAATC-AATAGAAGGAACAC-3'). The filters were then exposed to Kodak X-OMAT AR films for 4–72 h at -70 °C with intensifying screens. The same filters were hybridized successively with the different probes. Intensities of the mRNA bands on the blots and of 18 S RNA on the membranes were quantified by scanning densitometry (Sharp Scanner JX 325 combined with Image Master Software; Pharmacia, Uppsala, Sweden) and expressed as attenuation units (D). Ribosomal 18 S RNA levels were not influenced by the different experimental conditions.

Presentation of the results

Results are means $\pm$ S.E.M. for the indicated numbers of Petri dishes. Comparisons between groups were performed by unpaired Student's *t* test or analysis of variance followed by the Newman-Keuls test for multiple comparisons when appropriate. Differences were considered statistically significant at *P* < 0.05.

RESULTS

The time course of the effects of dexamethasone on *ob* gene expression in cultured white adipose tissue is shown in Figure 1A. *ob* mRNA levels were compared with, and expressed as

Figure 1 Time course and concentration-dependence of the effects of dexamethasone on *ob*-gene expression

(A) Time-course of the effects of dexamethasone on *ob* gene expression in cultured white adipose tissue. Epididymal adipose tissue from fasted rats was cultured in MEM with (●) or without (○) 1 μ M dexamethasone (Dexa) for up to 24 h. In the experiments indicated by ★, the tissue was cultured for 24 h in the presence of 1 μ M dexamethasone and 2.5 μ g/ml actinomycin D. (B) Concentration dependence of dexamethasone effects on *ob*, PPAR γ , GLUT4 and FAS mRNA species in cultured white adipose tissue. Epididymal adipose tissue from fasted rats was cultured for 12 h in MEM containing various concentrations of dexamethasone. Values are means $\pm$ S.E.M. for four to ten (A) or three or four (B) individual fat pads. mRNA levels were quantified by scanning densitometry of autoradiographic signals from Northern blots and are expressed as percentages of values obtained in freshly isolated epididymal fat pads pooled from fed control rats.

percentages of, values obtained in freshly isolated epididymal fat pads from fed control rats. Time 0 corresponds to the values measured in adipose tissue from fasted rats (after the pads had been dissected into small pieces and agitated for 30 min, i.e. just before culturing). As expected [10], *ob* expression was low in rats fasted for 24 h and amounted to only approx. 30% of the values measured in fed rats. It declined further to nearly undetectable levels when the tissue was cultured in the absence of dexamethasone. This might perhaps explain why *ob* expression was reported to be low (only approx. 1% of the values measured in freshly isolated mouse adipose tissue) in 3T3-L1 adipocytes cultured in standard medium [19]. In contrast, 1 μ M dexamethasone induced *ob* expression to a maximum of approx. 2.5-fold starting values after 12 h. Actinomycin D (indicated by the star in Figure 1A) prevented the stimulatory effect of the glucocorticoid on *ob* expression after 24 h of culture, suggesting that the effects of the hormone could be mediated by transcriptional mechanisms.

Table 1 Effects of insulin and/or glucose on *ob*, FAS, GLUT4 and PPAR γ gene expression

Epididymal adipose tissue from fasted rats was cultured for 24 h in MEM containing G20 with or without 100 nM insulin (Ins). Levels of mRNA were quantified by scanning densitometry of autoradiographic signals from Northern blots. Values are means $\pm$ S.E.M. for the numbers of fat pads shown in parentheses, and are expressed as percentages of results obtained in freshly isolated epididymal fat pads from fed control rats. * $P < 0.001$ for the effect of insulin.

Gene	Adipose tissue cultured with ...	mRNA levels (% of fed controls)	
		G20	G20 + Ins
<i>ob</i>		8 $\pm$ 1 (5)	12 $\pm$ 2 (12)
FAS		9 $\pm$ 2 (5)	54 $\pm$ 7* (12)
GLUT4		10 $\pm$ 3 (5)	53 $\pm$ 7* (12)
PPAR γ		64 $\pm$ 6 (5)	86 $\pm$ 12 (5)

Figure 2 Time course of the inhibitory effect of insulin on dexamethasone-induced stimulation of *ob* gene expression

Epididymal adipose tissue from fasted rats was cultured for up to 12 h in MEM with 50 nM dexamethasone (Dexa, D) in the absence (○) or in the presence (■) of 100 nM insulin (Ins, I). Values are means $\pm$ S.E.M. for four individual fat pads. mRNA levels were quantified by scanning densitometry of autoradiographic signals from Northern blots (such as that shown in the inset) and are expressed as percentages of values obtained after 12 h of culture with 50 nM dexamethasone alone.

The concentration dependence of dexamethasone stimulation of *ob* gene expression was examined after 12 h of culturing (Figure 1B). The glucocorticoid was effective at concentrations above 1 nM. With 1 μ M dexamethasone, *ob* mRNA levels were almost similar (83 $\pm$ 1 %) to those in fresh fat pads from fed rats, and the stimulation produced by 10 nM dexamethasone reached 47 $\pm$ 13 % of these fed control levels (Figure 1B). Under the same culture conditions, there were no significant changes in mRNA species for FAS and GLUT4, which remained depressed, or in mRNA for PPAR γ , which ranged from 25 to 45 % of control levels (Figure 1B).

In the absence of dexamethasone, the *ob* mRNA level remained similarly low (less than 10 % of fed control levels) whether the culture medium contained 5 mM (G5) or 20 mM (G20) glucose (compare Figure 1A with Table 1). Unlike dexamethasone, insulin either combined with basal (G5) (results not shown) or high (G20) glucose concentrations and did not re-induce *ob* expression, although it strongly increased the accumulation of mRNA species for FAS and GLUT4 (approx. 50 % of control levels; $P < 0.001$ compared with G20 alone) (Table 1). Insulin also tended to increase PPAR γ expression but this change was not statistically significant (Table 1). Similar results were

Figure 3 Concentration dependence of the effects of insulin (■) or IGF-1 (○) on *ob*, FAS, GLUT4 and PPAR γ gene expression

Epididymal adipose tissue from fasted rats was cultured for 12 h in MEM containing 50 nM dexamethasone and various concentrations of insulin or IGF-1. Values are means $\pm$ S.E.M. for four to eight individual fat pads. mRNA levels were quantified by scanning densitometry of autoradiographic signals from Northern blots and are expressed as percentages of values obtained in the presence of 50 nM dexamethasone alone.

obtained when cultures were performed with adipocytes obtained from fed rats (results not shown).

Unexpectedly, insulin inhibited dexamethasone-induced *ob* mRNA accumulation. As shown in Figure 2, this inhibitory effect was delayed in onset, beginning between 6 and 9 h of culture. After 12 h the effect of dexamethasone was inhibited by 54 % by 100 nM insulin ($P < 0.05$).

As shown in Figure 3, this inhibitory effect of insulin was detectable at a low concentration of the hormone ($P < 0.01$ at 1 nM, which roughly corresponded to IC_{50}). This inhibition was maximal at 100 nM (Figure 3). In contrast, insulin stimulated expression of the genes for FAS, GLUT4 and PPAR γ but these effects were significant or detectable only at higher concentrations of the hormone (10 nM; $P < 0.01$ or less for the three genes).

The inhibitory effect of insulin on dexamethasone-stimulated *ob* expression was mimicked by the insulin-like agent vanadate

Table 2 Influence of glucose or lactate/pyruvate on the inhibition by insulin of dexamethasone-induced stimulation of *ob* gene expression

Epididymal adipose tissue from fasted rats was cultured for 12 h in MEM containing 50 nM dexamethasone (Dexa), with or without 100 nM insulin. The culture medium contained G0, G5 or G20, or no glucose but 10 mM lactate/1 mM pyruvate (L/P). FAS mRNA concentrations are given for comparison. mRNA levels were quantified by scanning densitometry of autoradiographic signals from Northern blots. Values are means $\pm$ S.E.M. for the numbers of fat pads shown in parentheses, and are expressed as percentages of values obtained in G5 without insulin. * $P < 0.05$ or less for the effect of insulin; † $P < 0.01$ compared with G5.

Condition	Adipose tissue cultured with Dexa and ...	<i>ob</i> mRNA level (% of that with 50 nM Dexa and G5)			
		G0	G5	G20	L/P
No insulin		65 $\pm$ 7† (8)	100 $\pm$ 11 (8)	104 $\pm$ 3 (8)	104 $\pm$ 5 (8)
Insulin (100 nM)		57 $\pm$ 11 (8)	52 $\pm$ 13* (4)	60 $\pm$ 2* (4)	54 $\pm$ 8* (8)
FAS mRNA level (% of that with 50 nM Dexa and G5)					
No insulin		0 (8)	100 $\pm$ 10 (8)	0 (8)	0 (8)
Insulin (100 nM)		640 $\pm$ 120 (8)	6497 $\pm$ 1380* (8)	9840 $\pm$ 470* (4)	1580 $\pm$ 308* (8)

(50 μ M Na₃VO₄ yielded 76% of the inhibition by 50 nM dexamethasone without Na₃VO₄; $P < 0.001$) (results not shown). It was also mimicked by insulin-like growth factor 1 (IGF-1) but higher concentrations of IGF-1 than of insulin were required to obtain a significant effect (47% inhibition at 100 nM; $P < 0.05$ compared with 50 nM dexamethasone without IGF-1) (Figure 3). IGF-1 (100 nM) also increased the levels of FAS and GLUT4 mRNA species (19-fold and 6-fold respectively compared with basal values; $P < 0.01$ or less for both genes), but the magnitude of this stimulation was much smaller than that of the stimulation produced by insulin (Figure 3). PPAR γ mRNA was not clearly affected by IGF-1 (Figure 3).

We next examined whether fuels are required for the inhibitory effect of insulin on glucocorticoid induction of *ob* mRNA. In the presence of dexamethasone, 5 mM glucose alone increased the expression of *ob* [$P < 0.01$ compared with 0 mM glucose (G0)], but this effect was not augmented further by 10 mM glucose (results not shown) or G20 (Table 2). The combination of 10 mM lactate/1 mM pyruvate was as effective as glucose. Insulin was ineffective in G0, but decreased *ob* expression when dexamethasone was combined with glucose or the mixture of lactate/pyruvate. Thus the inhibitory effect of insulin was detectable only when fuels were present or when a critical level of *ob* expression was reached. As already shown (Figure 3) [21], insulin strongly increased FAS mRNA levels in the presence of G5. This stimulation was further accentuated by higher concentrations of glucose ($P < 0.001$ for G20 plus insulin, compared with G5 plus insulin) (Table 2) [21], which was not true of PPAR γ mRNA (results not shown). In the absence of the insulin and/or in G0, FAS mRNA levels were almost undetectable (Table 2). These levels were only very weakly stimulated by the combination of the hormone with lactate/pyruvate (Table 2).

To elucidate further the mechanisms by which insulin inhibits dexamethasone-induced *ob* gene expression, we evaluated the possible effects of cAMP, PMA and non-esterified fatty acids. Increasing cAMP levels in fat cells by different cAMP analogues (*N*,*O*'-dibutyl cAMP, bromo-cAMP), isobutyl methylxanthine or a specific agonist of the β 3-adrenoceptor (alone or in combination) did not affect the low levels of *ob* mRNA in the absence of dexamethasone, but inhibited the stimulatory effect of the glucocorticoid (Table 3). Conversely, nicotinic acid, which is known to decrease intracellular cAMP levels in adipose tissue [25], tended to increase *ob* gene expression in the presence of dexamethasone [up to 150 $\pm$ 17% ($n = 4$) of that in 50 nM dexamethasone alone]. Activation of protein kinase C by the phorbol ester PMA had no effect on *ob* gene expression whether alone or in combination with the hormone (Table 3). Saturated

Table 3 Effects of cAMP, PMA and non-esterified fatty acids on dexamethasone-induced stimulation of *ob* gene expression in cultured adipose tissue

Epididymal adipose tissue from fasted rats was cultured for 12 h in MEM with the indicated agents. Values are means $\pm$ S.E.M. for the numbers of fat pads shown in parentheses, and are expressed as percentages of results obtained in the presence of 50 nM dexamethasone (Dexa) alone within the same experiment. Statistical comparisons were restricted to test and control groups from the same experiments, thus with similar values of n . * $P < 0.01$ for the effect of the test agent. Abbreviations: (Bu)₂-cAMP, dibutyl cAMP; IBMX, isobutylmethylxanthine; BRL 35135, a specific agonist of β 3-adrenoceptor; n.d., not done.

Addition	Adipose tissue cultured with ...	<i>ob</i> mRNA level (% of that with Dexa)	
		No Dexa	Dexa
None		7 $\pm$ 1 (28)	100 $\pm$ 5 (49)
(Bu) ₂ -cAMP (1 mM)		0 $\pm$ 0 (4)	3 $\pm$ 2* (11)
Bromo-cAMP (1 mM)		0 (2)	4 $\pm$ 4* (4)
IBMX (0.5 mM)		14 (2)	21 $\pm$ 6* (8)
BRL 35135 (1 μ M)		8 $\pm$ 5 (4)	29 $\pm$ 11* (8)
IBMX + BRL		n.d.	0 $\pm$ 0* (6)
PMA (25 nM)		0 $\pm$ 0 (3)	76 $\pm$ 11 (4)
C _{16:0} (250 μ M)		7 $\pm$ 4 (5)	122 $\pm$ 9 (15)
C _{18:0} (250 μ M)		10 $\pm$ 3 (4)	123 $\pm$ 21 (5)
C _{18:1} (250 μ M)		0 $\pm$ 0 (4)	93 $\pm$ 7 (4)

or unsaturated fatty acids had no influence on basal or dexamethasone-stimulated *ob* expression (Table 3). They also did not alter the inhibitory effect of insulin on the stimulation by dexamethasone. Thus *ob* mRNA levels (as percentages of levels obtained with 50 nM dexamethasone) were 45 $\pm$ 4 ($n = 21$), 48 $\pm$ 6 ($n = 9$), 47 $\pm$ 3 ($n = 4$) and 33 $\pm$ 7 ($n = 4$), when no free fatty acids or 250 μ M C_{16:0}, C_{18:0} or C_{18:1} respectively were co-cultured with 50 nM dexamethasone and 100 nM insulin.

We next examined whether the inhibitory effect of insulin on dexamethasone-induced accumulation of *ob* mRNA could be prevented by cycloheximide, an inhibitor of protein synthesis. As well as dexamethasone, cycloheximide by itself was able to reinduce *ob* expression (to approx. 60% of the levels obtained with 50 nM dexamethasone) (Table 4). Cycloheximide did not influence dexamethasone-induced *ob* mRNA accumulation, but prevented the inhibitory effect of insulin (Table 4). In contrast, cycloheximide did not reverse the inhibitory effect of cAMP on dexamethasone-stimulated *ob* mRNA (Table 4). The 90-fold and 1.7-fold increases in FAS and PPAR γ mRNA species produced

Table 4 Reversal by cycloheximide of the inhibition by insulin of *ob* gene expression in adipose tissue cultured in the presence of dexamethasone

Epididymal adipose tissue from fasted rats was cultured for 12 h in MEM with or without 50 nM dexamethasone in the presence or in the absence of 10 nM insulin (Ins) and/or 10 μ M cycloheximide (CHX). The effects obtained in the presence of 1 mM dibutylcAMP [(Bu)₂cAMP] are shown for comparison. Levels of *ob* mRNA were quantified by scanning densitometry of autoradiographic signals from Northern blots. Values are means $\pm$ S.E.M. for the numbers of fat pads shown in parentheses, and are expressed as percentages of results obtained in the presence of 50 nM Dexa alone. * $P < 0.05$ or less compared with all other conditions without Dexa; † $P < 0.001$ compared with all other conditions with Dexa; ‡ $P < 0.001$ compared with all other conditions with Dexa except for the combination (Bu)₂cAMP + CHX. Abbreviation: n.d., not done.

Addition	Adipose tissue cultured with ...	<i>ob</i> mRNA level (% of that with Dexa)	
		No Dexa	Dexa
None		0.4 $\pm$ 0.4 (4)	100 $\pm$ 1 (16)
CHX		60 $\pm$ 12* (7)	115 $\pm$ 11 (4)
Ins		0 (5)	66 $\pm$ 6† (8)
Ins + CHX		n.d.	108 $\pm$ 9 (6)
(Bu) ₂ -cAMP		0 (4)	1 $\pm$ 1‡ (7)
(Bu) ₂ -cAMP + CHX		n.d.	0 (3)

by insulin in the presence of 50 nM dexamethasone were fully prevented by the inhibitor of protein synthesis (results not shown).

DISCUSSION

This study shows that *ob* gene expression rapidly fades out when explants of white adipose tissue are cultured in control MEM medium. Transcription of the gene is strongly stimulated by dexamethasone but is unaffected by insulin alone. However, insulin dose-dependently prevented the dexamethasone-induced accumulation of *ob* mRNA. This inhibitory effect required the presence of a fuel in the medium, was mimicked by IGF-1 and vanadate, was delayed in onset (more than 6 h) and was reversed by cycloheximide.

The stimulatory effect of dexamethasone on *ob* gene expression was observed at low concentrations of the hormone (EC50 approx. 10 nM), which can be considered to be within a physiological range (if dexamethasone concentrations are converted into cortisol concentrations on the basis of their respective glucocorticoid potencies of 30:1). After exposure to 1 μ M dexamethasone for 12 h, the abundance of *ob* mRNA was similar to that in fresh fat pads from fed rats. We thus confirm the rapid and potent stimulatory effect of dexamethasone on *ob* gene expression previously reported in primary cultures of adipocytes [7,8], although not in the 3T3-L1 cell line [9]. We further show that this effect was blocked by actinomycin D and thus could be mediated by transcriptional mechanisms. Glucocorticoids are therefore likely to modulate *ob* expression through the specific glucocorticoid response element that has recently been identified in the regulatory region of the human *ob* gene [26].

Raising glucose from euglycaemic (5 mM) to hyperglycaemic (20 mM) levels did not alter basal or dexamethasone-stimulated expression of the *ob* gene. This is in agreement with results *in vivo* from hyperglycaemic clamps [12] and some results *in vitro* [8]. However, increasing glucose concentrations from 0 to 5 mM increased by one-third the expression of *ob* stimulated by dexamethasone. This effect was mimicked by lactate/pyruvate. Thus a minimum amount of fuel seems to be required for full expression of the *ob* gene at a given concentration of glucocorticoids. This latter observation might explain why, in some

reports, the intraperitoneal injection of glucose to mice previously fasted was found to be stimulatory [27].

Unlike dexamethasone, insulin added for up to 24 h to the medium had no effect on *ob* gene expression, in agreement with all [7,8,17] except for one [12] study on rodent or human primary cultures of adipocytes. This suggests that the stimulatory effect of the hormone on *ob* expression *in vivo* [12–15] is mediated indirectly. In differentiated 3T3-L1 or 3T3-F442A cell lines, insulin was found to increase *ob* mRNA [9,20]. However, the absolute levels of both basal and insulin-stimulated *ob* mRNA in these cell lines were extremely low [7,9,19,20].

Unexpectedly, insulin prevented the dexamethasone-induced accumulation of *ob* mRNA while having its expected stimulatory effect on GLUT4 and FAS mRNA species. This inhibitory effect was dose-dependent and was detectable at low, physiological, concentrations of insulin (IC₅₀ approx. 1 nM). It was mimicked by the insulin mimicker vanadate and by IGF-1. However, IGF-1 was effective only at concentrations one or two orders of magnitude higher than those of insulin, which suggests that IGF-1 might act through the insulin receptor.

Several mechanisms could contribute to this novel inhibitory effect of insulin. First, a lowering of intracellular cAMP concentrations is known to be involved in some of the effects of insulin in adipose tissue [28]. This cannot account for the present findings because increasing the cAMP concentration in fat cells by cAMP analogues, isobutylmethylxanthine or a selective agonist of the β_3 -adrenoceptor also decreased *ob* expression, in agreement with a recent report [8]. In addition, nicotinic acid, which is known to inhibit cAMP synthesis [25], tended to increase *ob* mRNA. Secondly, insulin might decrease cytosolic non-esterified fatty acid concentrations by inhibiting lipolysis through cAMP-dependent and cAMP-independent mechanisms [29]. Such a decrease is, however, unlikely to explain the modulation of *ob* expression because, in contrast with a previous report in 3T3-L1 cells [9], saturated or unsaturated fatty acids had no influence on basal, dexamethasone-stimulated or insulin-inhibited *ob* gene expression in primary cultures. Thirdly, protein kinase C might be involved in the action of insulin [28], whose effects can partly be mimicked by phorbol esters [30]. This pathway does not seem to have a role in the regulation of the *ob* gene because PMA was without effect. Fourthly, the reversal of the inhibitory effect of insulin by cycloheximide indicates that the synthesis of an inhibitory protein is involved. This conclusion is also in keeping with the delay in the insulin effect. Moreover, cycloheximide alone increased *ob* gene expression in the absence of dexamethasone. This suggests that a constitutively expressed protein either inhibits the transcription of the *ob* gene or destabilizes the messengers. As cycloheximide and dexamethasone had no additive effects on *ob* gene expression, we cannot rule out the possibility that the glucocorticoid effect might be mediated by a decrease in the synthesis of this inhibitory protein. However, this mechanism would not explain why cycloheximide alone only partly reproduced the stimulatory effect of dexamethasone. In contrast, cAMP inhibited *ob* gene expression in the presence of both dexamethasone and cycloheximide, which clearly indicates an independent mode of action. Thus at least two or three distinct signalling pathways might ultimately modulate the *ob* gene.

As suggested by experiments with cycloheximide, a negative tone is exerted on *ob* gene expression under basal conditions. This might be teleologically meaningful. This inhibitory tone and the resultant attenuation of circulating leptin concentrations could allow the storage of some excess energy, thereby providing a selective advantage for the survival of a species when subjected to calorie deprivation.

In liver, insulin inhibits the glucocorticoid-induced transcriptional stimulation of at least three different genes: those for IGF-binding protein 1, phosphoenolpyruvate carboxykinase (a key gluconeogenic enzyme) and the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2/FBPase-2) [31–35]. The hormonal regulation of the *ob* gene is the first example of a dominant inhibitory role of insulin over a stimulation induced by glucocorticoids in adipose tissue. Nevertheless some differences in this regulation might exist between liver and adipose tissue: for instance, the inhibition by insulin of the dexamethasone-induced stimulation of the gene for PFK-2/FBPase-2 does not require the presence of fuel (glucose), or ongoing protein synthesis [35]. Unravelling the molecular mechanisms that govern the antagonism between the two hormones in fat tissue will provide further insights into the signalling pathways by which the adipocyte controls energy homeostasis.

Unlike the *ob* gene, the genes coding for FAS [21], GLUT4 [36] and PPAR γ – a nuclear receptor that controls the expression of several fat-specific genes and triggers adipocyte differentiation [37] – were positively regulated by insulin, whether or not dexamethasone was added to the medium. The increase in PPAR γ mRNA levels by insulin is in agreement with the regulation of this gene *in vivo*. Thus PPAR γ mRNA concentrations were decreased in fasted and in insulin-deficient (streptozotocin) diabetic mice, and partly restored by treatment with insulin [38]. Our results in cultured adipose tissue more directly support a role of the hormone in the control of PPAR γ expression, and indicate that ongoing protein synthesis is necessary for the stimulation of this gene by insulin, as it is for the FAS gene [39], and this study).

Because PPAR γ is an adipocyte-specific and lipid-activated transcription factor, it has been proposed to regulate the fat sensor *ob* [40]. In this study, changes in *ob* mRNA were inversely related to those of PPAR γ mRNA. As definitive conclusions cannot be drawn from the sole gene expression, further studies are needed to assess whether the *ob* gene promoter is regulated by PPAR γ .

In conclusion, unlike dexamethasone, insulin had no direct effect on *ob* gene expression. Unexpectedly, insulin even inhibited the dexamethasone-induced accumulation of *ob* mRNA, whereas it strongly stimulated the expression of the genes for FAS, GLUT4 and PPAR γ . The inhibition by insulin was mimicked by IGF-1 and vanadate, was only detectable when a minimum amount of fuel was supplied and required ongoing protein synthesis, which is consistent with its delayed onset. Unravelling the molecular mechanisms that govern the antagonism between the two hormones and their interplay in *ob* gene regulation will provide important insights into the signalling pathways by which the adipocyte controls energy homeostasis.

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