Inhibition of hormone-sensitive lipase gene expression by cAMP and phorbol esters in 3T3-F442A and BFC-1 adipocytes

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Hormone-sensitive lipase (HSL) catalyses the rate-limiting step in adipocyte lipolysis. Short-term hormonal regulation of HSL activity is well characterized, whereas little is known about the control of HSL gene expression. We have measured HSL mRNA content of 3T3-F442A and BFC-1 adipocytes in response to the cAMP analogue 8-(4-chlorophenylthio)-cAMP (8-CPT-cAMP) and to the phorbol ester phorbol 12-myristate 13-acetate (PMA) by Northern blot, using a specific mouse cDNA fragment. Treatment of the cells for 12 or 6 h with, respectively, 0.5 mM 8-CPT-cAMP or 1 μM PMA produced a maximal decrease of about 60 % in HSL mRNA. These effects were unaffected by the protein-synthesis inhibitor anisomycin, suggesting that cAMP and PMA actions were direct. The reduction in HSL mRNA was accompanied by a reduction in HSL total activity. The intracellular routes that cAMP and PMA follow for inducing such an effect seemed clearly independent. (i) After desensitization of the protein kinase C regulation pathway by a 24 h treatment of the cells with 1 μM PMA, PMA action was abolished whereas cAMP was still fully active. (ii) Treatment with saturating concentrations of both agents produced an additive effect. (iii) The synthetic glucocorticoid dexamethasone had no proper effect on HSL gene expression but potentiated cAMP action without affecting PMA action. cAMP inhibitory action on HSL is unexpected. Indeed, the second messenger of catecholamines is the main activator of HSL by phosphorylation. We envision that a long-term cAMP treatment of adipocytes induces a counter-regulatory process that reduces HSL content and, ultimately, limits fatty acid depletion from stored triacylglycerols.

INTRODUCTION

Hormone-sensitive lipase (HSL; EC 3.1.1.3) is the key enzyme responsible for fatty acid mobilization from adipose tissue. HSL catalyses the hydrolysis of the endogenous triacylglycerol, quantitatively the most important source of stored energy in mammals. Fatty acids liberated from adipose tissue are utilized by other tissues. The lipolysis rate is tightly controlled by hormones and neurotransmitters via the reversible phosphorylation of a single serine residue. Lipolytic agents, such as catecholamines, adenocorticotropic hormone and glucagon, activate cAMP-dependent protein kinase (PKA; EC 2.7.1.37), which phosphorylates and activates HSL [1]. The antilipolytic hormone, insulin, reduces the HSL phosphorylation level, thereby decreasing HSL activity [2]. A recent study suggests that stimulation of lipolysis is followed by a translocation of HSL from the cytosol to the lipid droplet of adipocytes, a potentially regulated process [3].

The cDNA coding for this enzyme has been cloned from rat and human adipose-tissue libraries [4,5]. The HSL gene organization has been elucidated in human and mouse [5,6]. The protein sequence is highly similar among the three species. Although numerous studies have examined the post-translational modification of the enzyme, very little information is available about the regulation of HSL gene expression. During rat ontogenesis, HSL mRNA content increases in heart, adrenal and testis but remains constant in epididymal adipose tissue, suggesting a possible age-specific and tissue-specific control of HSL gene expression [7]. In adult rats, prolonged fasting, streptozotocin-induced diabetes and late pregnancy all induce a 2- to 4-fold increase in HSL mRNA [8–10]. A recent report indicates that, in isolated rat adipocytes, the glucocorticoid analogue dexamethasone increases the HSL mRNA level [11].

The purpose of the present study is to examine the hormonal regulation of HSL gene expression in adipocytes. We have addressed this question using two adipocyte cell lines: 3T3-F442A, established from Swiss mouse embryo 3T3 fibroblasts [12], and BFC-1, isolated from mouse interscapular brown adipose tissue [13]. These two cell lines have the capacity to differentiate into adipocytes with high frequency and to express HSL among other adipose-specific markers ([14]; C. Forest, unpublished results). We demonstrate that activators of PKA and of protein kinase C (PKC; EC 2.7.1) exert a negative control on HSL gene expression (mRNA and activity levels) through two direct and independent mechanisms. These findings suggest that HSL activity may be governed partially by alterations in the degree of gene expression.

MATERIALS AND METHODS

Culture of adipose-tissue fragments and of adipocytes

Male Wistar rats (6 weeks old) were killed by decapitation. Periepididymal fat pads were quickly removed, cut with scissors into small pieces and agitated for 30 min in Dulbecco's modified Eagle's medium (DMEM) (Gibco) supplemented with 4 % BSA (Sigma). Pieces of adipose tissue (2–3 mm^3) were washed in PBS. Tissues were cultured in DMEM supplemented with 1 % BSA and containing glucose (25 mM), at 37 °C in a humidified atmosphere of 10 % CO_2. Adipocytes were isolated as described by Ong et al. [15]. Cells were cultured in DMEM containing

Abbreviations used: 8-CPT-cAMP; 8-(4-chlorophenylthio)-cAMP; DMEM, Dulbecco's modified Eagle's medium; HSL, hormone-sensitive lipase; PEPCK, phosphoenolpyruvate carboxykinase; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate.

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glucose (25 mM) and either 1 % fetal bovine serum or 4 % BSA, at 37 °C in a humidified atmosphere of 10 % CO_2.

Cell culture and treatment

3T3-F442A and BFC-1 preadipose cells were cultured in DMEM containing glucose (25 mM) at 37 °C in a humidified atmosphere of 10 % CO_2. They were grown and differentiated in 10 % fetal calf serum/penicillin (200 IU/ml)/streptomycin (50 mg/l)/biotin (8 mg/l)/pantothenate (4 mg/l). At confluence of the cells, medium was supplemented with insulin (2×10^{-8} M) to favour triacylglycerol accumulation. The medium was changed every 2–3 days for 8 days. Experiments were performed on mature adipose cells (8 days after confluence). Cells were shifted to serum-free, insulin-free medium for 24 h (or 48 h when indicated), during which treatment with effectors was performed before RNA extraction or assays of HSL activity.

Cloning of a mouse HSL cDNA fragment

A 477 bp fragment of the mouse HSL was amplified from 500 ng of mouse genomic DNA using PCR. The sense and antisense primers used were 5′-ATGGATTTACGCACGATGACACAG-3′ (nt 1–24 of the mouse coding sequence) and 5′-TAGCGTGA-CATACTCTTGCAGGAA-3′ (nt 454–477 of the mouse coding sequence) [6]. After an initial denaturation step at 92 °C for 5 min, the DNA was subjected to 30 cycles of amplification (92 °C, 1 min; 60 °C, 1.5 min; 72 °C, 1.5 min) using 2.5 units of *Taq* polymerase. The PCR product was purified and subcloned into pBluescript vector (Stratagene) using the the TA cloning procedure [16]. Dideoxysequencing of the entire fragment was in full agreement with the recently published sequence [6].

RNA extraction and analysis

RNA from tissue fragments and adipocytes was extracted by the method of Chirgwin et al. [17]. Two 60-mm-diam. dishes of 3T3-F442A BFC-1 cells were pooled, and RNA was extracted according to the method of Chomczynski and Sacchi [18]. Total RNA was electrophoresed on a 1 % agarose gel containing 2.2 M formaldehyde and blotted on to a nylon membrane. The integrity and relative amounts of RNA were assessed by Methylene Blue staining [19]. Prehybridization and hybridization of the blots were performed according to Yang et al. [20]. Membranes were hybridized overnight at 65 °C in 0.27 M NaCl/1.5 mM EDTA/15 mM sodium phosphate (pH 7.7) containing 7 % SDS, 10 % polyethylene glycol 6000, 250 μg/ml sonicated salmon sperm DNA, 250 μg/ml heparin and 10^6 c.p.m./ml cDNA labelled with [α-^{32}P]dCTP (ICN Pharmaceuticals) by random priming according to the manufacturer's recommendation. Membranes were washed twice for 15 min at room temperature in 2 × SSC [1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate (pH 7)]/0.1 % SDS and then for 30 min at 60 °C with 0.1 × SSC/0.1 % SDS. Specific cDNA probes used were the mouse HSL cDNA fragment described above and PC 116, a rat phosphoenolpyruvate carboxykinase (PEPCK; EC 4.1.1.32) cDNA fragment [21]. An oligonucleotide specific for the 18 S ribosomal RNA was ^{32}P-end-labelled and used as a control, as described previously [22]. The HSL mRNA signal was quantified by scanning densitometry and was corrected for differences in RNA loading by comparison with the signals generated by the 18 S ribosomal RNA probe.

HSL assay

The HSL assay was performed essentially as described previously [23]. Adipocyte monolayers were washed twice with PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4,$7H_2O$ and 1.4 mM KH_2PO_4, pH 7.4). Cells were then scraped with a rubber policeman and homogenized at 4 °C in 0.25 M sucrose/1 mM EDTA/1 mM dithioerythritol/protease inhibitors leupeptin and antipain (both at 20 μg/ml). Enzyme activity was determined using [^{3}H]rac-1-oleoyl-2-*O*-octadecenylglycerol as substrate. The diacylglycerol analogue was synthesized by Dr. Lennart Krabisch (Lund University, Lund, Sweden). All samples were incubated in triplicate for 30 min at 37 °C. We used a diacylglycerol analogue as substrate for enhancing assay sensitivity, since HSL has 10-fold- and 7-fold-higher activities towards diacylglycerol than towards triacylglycerol and cholesterol esters respectively. The diacylglycerol lipase activity is not modified by PKA-mediated phosphorylation [24–26]. Moreover, since this substrate only has one hydrolysable ester bond at the 1(3) position, neither monoacylmonoalkylglycerol nor its hydrolysis products are substrates for monoacylglycerol lipase, which is abundant in the adipose tissue. Furthermore, under conditions of the assay, i.e. pH 7.0 and no apolipoprotein CII present, very low lipoprotein lipase activity was measured [23]. For all these reasons, it is likely that changes in HSL activity directly reflect differences in amount of HSL protein and not in the extent of phosphorylation of the enzyme. One unit of enzyme activity is defined as 1 μmol of fatty acid released/min at 37 °C. Lipase activity was normalized to the protein concentration of the infranatant, which was measured according to Lowry, using BSA as a standard [27].

RESULTS

Comparison of HSL gene expression in adipose-tissue fragments, primary adipocytes and the 3T3-F442A adipose cell line

With the goal in mind to study HSL gene regulation in adipocytes, we first followed the expression of this gene in adipose-tissue fragments, in primary adipocytes and in the 3T3-F442A cell line during several hours of culture. After 18 h of culture, the HSL mRNA concentration was drastically reduced in adipose-tissue fragments and in adipocytes (Figure 1). Similar observations were made whether adipocytes were cultured with serum or not (results not shown). In contrast, in 3T3-F442A cells, HSL mRNA was not expressed at the early stage of the differentiation process (i.e. at confluence) and was present in differentiated adipocytes at a concentration similar to that of adipose-tissue fragments before culture (Figure 1) [28]. This situation is reminiscent of the *in vivo* situation, where, in adipose tissue, only adipocytes express HSL. Moreover, the HSL mRNA level remained stable with time once the cells were terminally differentiated, even after 24 h of serum deprivation (results not shown). Therefore we decided to use the adipocyte cell line as a stable model to investigate regulation of HSL gene expression in response to various effectors.

cAMP and isoprenaline decreased the HSL mRNA level in 3T3-F442A adipocytes

The effects that 8-(4-chlorophenylthio)-cAMP (8-CPT-cAMP; cAMP) and the β-adrenergic agonist isoprenaline exerted on HSL gene expression were tested in differentiated 3T3-F442A cells. A representative Northern blot is presented in Figure 2. After 6 or 18 h of treatment with 8-CPT-cAMP (cAMP), the HSL mRNA level was severely decreased. The action of isoprenaline was similar to that of cAMP, although reduction in the HSL mRNA level was less drastic. In order to potentiate the response to isoprenaline, we used the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine. 3-Isobutyl-1-methylxanthine alone produced a decrease in the HSL mRNA level that was more pronounced at 18 h than at 6 h (Figure 2). In association with 3-

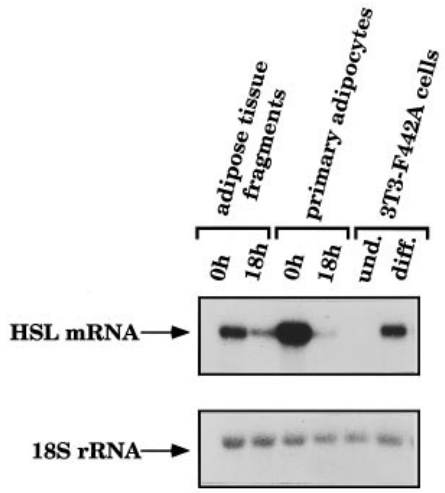

Figure 1 Evolution of the HSL mRNA steady-state level in adipose-tissue fragments, primary adipocytes and the 3T3-F442A cell line during culture

Adipose-tissue fragments and primary adipocytes were isolated as described in the Materials and methods section. Part of the biological material was immediately frozen in liquid nitrogen (0 h) before RNA extraction and analysis. The other part was cultured for 18 h, then frozen in liquid nitrogen (18 h) before RNA extraction and analysis. HSL mRNA and 18 S rRNA were revealed by sequential hybridization of the same blot with the corresponding probes. The autoradiogram shown is representative of two experiments with identical results. und., undifferentiated; diff., differentiated.

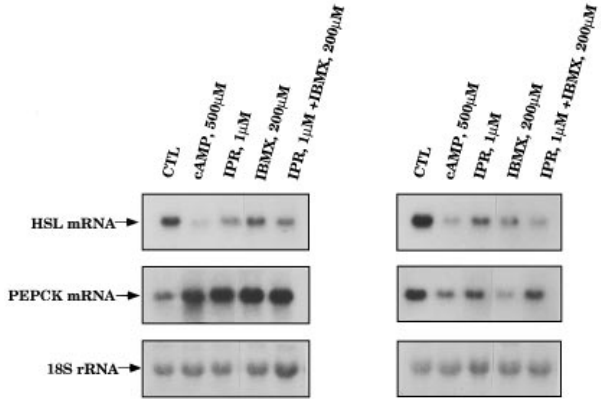

Figure 2 Modulation of the HSL mRNA level by 8-CPT-cAMP and isoprenaline in 3T3-F442A adipocytes

Effectors were added to the medium at the indicated concentrations for 6 h (left panel) or 18 h (right panel) before cell harvesting and RNA preparation. HSL and PEPCK mRNAs and 18 S rRNA were revealed by sequential hybridization of the same blot with the corresponding probes. For each time point, the autoradiogram shown is representative of two experiments with similar results. CTL, control; IPR, isoprenaline; IBMX, 3-isobutyl-1-methylxanthine.

isobutyl-1-methylxanthine, isoprenaline was as potent as cAMP (Figure 2, right panel). Such a decrease in response to cAMP or isoprenaline was specific to the HSL mRNA level, because, as expected from previous studies [29], PEPCK mRNA reacted in a manner opposite to HSL mRNA at 6 h (Figure 2, left panel). The increase in the PEPCK mRNA level was no longer observed at 18 h (Figure 2, right panel).

The time course of cAMP action was studied next (Figure 3). The HSL mRNA level was decreased by 40 % at 6 h and reached a maximum of about a 60 % decrease at 12 h. This effect

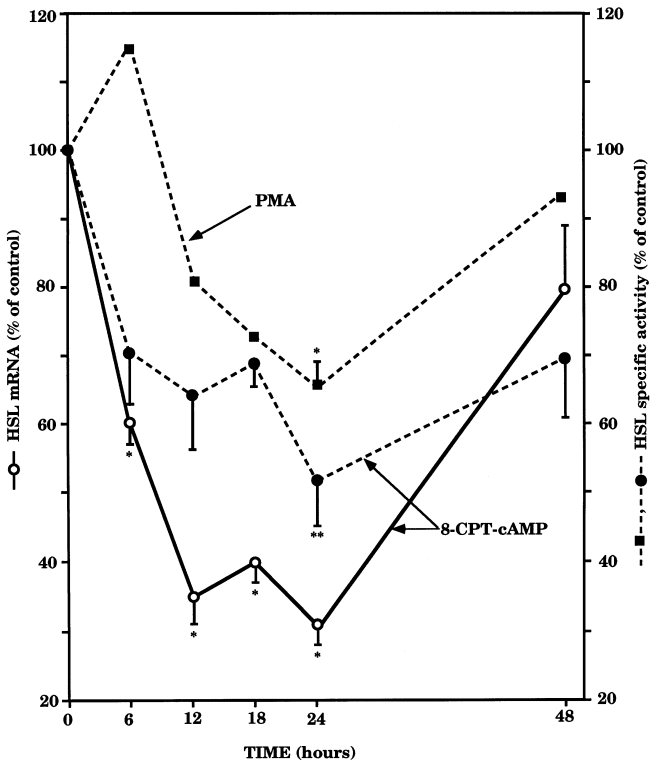

Figure 3 Time course of 8-CPT-cAMP and phorbol 12-myristate 13-acetate (PMA) action on the HSL mRNA level and HSL specific activity in 3T3-F442A adipocytes

Cells were treated with either 8-CPT-cAMP (500 μM) or PMA (1 μM) for the indicated times before RNA extraction and analysis. Data, obtained by scanning densitometry, were normalized for differences in RNA loading by using the 18 S rRNA signal. Each value is the mean $\pm$ S.E.M. of data collected from at least three independent experiments. HSL specific activity was measured as described in the Materials and methods section and normalized by measuring the protein concentration. Data are expressed as the percentage of the HSL signal from control, untreated cells. $^{*}P < 0.001$ and $^{**}P < 0.01$ (Student's t test for unpaired data) when compared with the control.

remained maximal for 12 h, and had almost totally disappeared 24 h later. We selected a treatment time of 6 h for determining the concentration dependence of the cAMP effect (Figure 4). The decrease in the HSL mRNA level was dose dependent, with a maximum reached at 200 μM.

Glucocorticoids are believed to act as modulatory hormones for transmembrane signalling events propagated via G-protein-linked receptors [30]. We analysed the action that glucocorticoids might exert on HSL gene expression in 3T3-F442A adipocytes in association or not with cAMP. The effect of the synthetic glucocorticoid dexamethasone alone on HSL mRNA was not significant. Indeed, Student's t test for unpaired data rejected the hypothesis that values from control and dexamethasone-treated cells were different ($P < 0.508$) (Table 1). In contrast, dexamethasone significantly ($P < 0.001$) potentiated cAMP action. Indeed, the association of cAMP and dexamethasone resulted in a drastic 80 % decrease in the HSL mRNA level (Table 1).

Phorbol esters decreased the HSL mRNA level in 3T3-F442A adipocytes

Signal transduction through PKC is a potential pathway for gene regulation. Phorbol esters are well-known activators of PKC. We used PMA to test the hypothesis that phorbol esters might

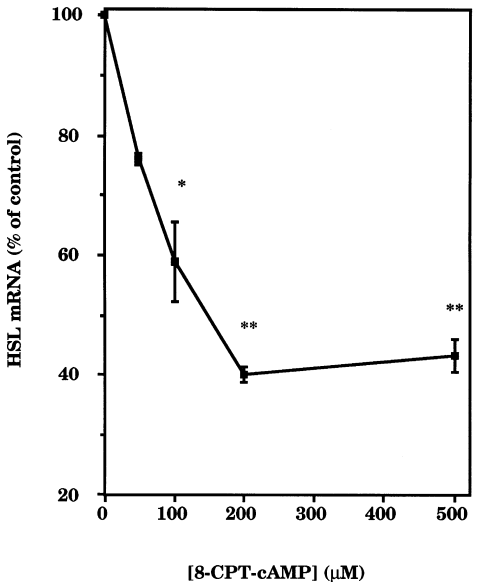

Figure 4 Effect of 8-CPT-cAMP concentration on the HSL mRNA level in 3T3-F442A adipocytes

Cells were treated for 6 h with the indicated concentrations of 8-CPT-cAMP before RNA extraction and analysis. Data, obtained by scanning densitometry, were normalized for differences in RNA loading by using the 18 S rRNA signal and expressed as a percentage of the HSL signal from control, untreated cells. Each value is the mean ± S.E.M. of data collected from three independent experiments. $^{*}P < 0.001$ and $^{**}P < 0.0009$ (Student's *t* test for unpaired data) when compared with the control.

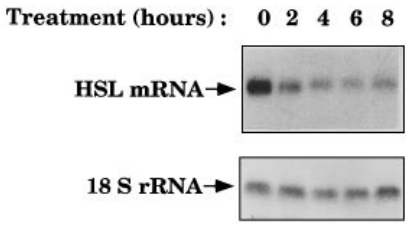

Figure 5 Time course of PMA action on the HSL mRNA level in 3T3-F442A adipocytes

Cells were treated with 1 μM PMA for the indicated times before RNA extraction and analysis. Results of the autoradiograms were obtained with the same blot hybridized sequentially with the HSL and 18 S probes and are representative of two experiments with identical results.

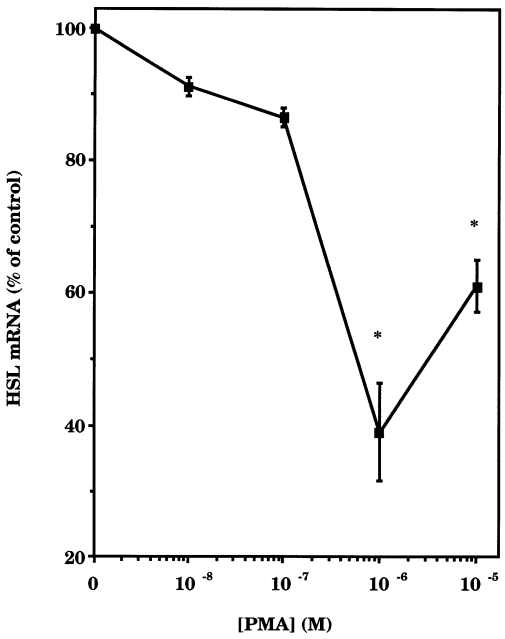

Figure 6 Effect of PMA concentration on the HSL mRNA level in 3T3-F442A adipocytes

PMA, at the indicated concentrations, was added to the medium for 6 h before RNA extraction and analysis. Data, obtained by scanning densitometry, were normalized for differences in RNA loading by using the 18 S rRNA signal and expressed as a percentage of the HSL signal from control, untreated cells. Each value is the mean ± S.E.M. of data collected from three independent experiments. $^{*}P < 0.001$ (Student's *t* test for unpaired data) when compared with the control.

modulate HSL gene expression. As shown in Figure 5, a treatment of 3T3-F442A adipocytes with 1 μM PMA drastically decreased the HSL mRNA level. This effect was specific, because the PEPCK mRNA level was unchanged by short-term treatment with PMA (results not shown). PMA action on the HSL mRNA level was rapid. The decrease was already apparent at 2 h of treatment and was maintained for at least 8 h (Figure 5). A treatment time of 6 h was chosen to investigate the dose dependence of the PMA effect (Figure 6). At concentrations ranging from 1 nM to 100 nM, PMA was inefficient, whereas at 1 μM, it induced a 60 % maximal decrease.

cAMP and PMA appeared to follow distinct signalling pathways and acted independently of protein synthesis

Evidence of cross-talk between PKA and PKC signalling pathways has been described previously [31]. We investigated whether

Table 1 Effect of long-term PMA treatment on 8-CPT-cAMP action on the HSL mRNA level in 3T3-F442A adipocytes

Cells were pretreated with PMA (1 μM) for 24 h or not pretreated, before addition of 500 μM 8-CPT-cAMP (cAMP), 1 μM PMA and/or 100 nM dexamethasone (Dex). RNA was extracted from cells 18 h later and subjected to blot-transfer analysis. Data, obtained by scanning densitometry, were normalized for differences in RNA loading by using the 18 S rRNA signal and were expressed as a percentage of the HSL signal from control, untreated cells. Each value is the mean ± S.E.M. of data collected from three independent experiments. $^{*}P < 0.001$ (Student's *t* test for unpaired data) when compared with cAMP (500 μM). $^{**}P < 0.002$ (Student's *t* test for unpaired data) when compared with cAMP (500 μM) and $P < 0.001$ (Student's *t* test for unpaired data) when compared with PMA (1 μM). ND, not determined.

PMA pretreatment	HSL mRNA (% of control)					
	PMA	cAMP	Dex	cAMP + Dex	cAMP + PMA	Dex + PMA
No	50.2 ± 4.1	41.3 ± 2.7	116.6 ± 3.7	22.7 ± 2.7*	23.5 ± 3.6**	62.2 ± 2.5
Yes	88.3 ± 4.1	51.2 ± 2.1	ND	ND	ND	ND

Table 2 Effect of 8-CPT-cAMP, dexamethasone and PMA on the HSL mRNA level and HSL activity in BFC-1 adipocytes

Cells were treated with 500 μM 8-CPT-cAMP (cAMP), 100 nM dexamethasone (Dex) and/or 1 μM PMA for 18 h. HSL mRNA and activity were analysed as described in the Materials and methods section. Total RNA (20 μg per lane) was subjected to blot-transfer analysis. Data, obtained by scanning densitometry, were normalized for differences in RNA loading by using the 18 S rRNA signal and expressed as a percentage of the HSL signal from the control. Values of HSL specific activity are normalized to the protein concentration and are expressed as a percentage of a HSL signal from control, untreated cells. Each value is the mean of data collected from at least two independent experiments. *$P < 0.0009$ (Student's t test for unpaired data) when compared with the control. **$P < 0.054$ (Student's t test for unpaired data) when compared with cAMP.

	cAMP	Dex	cAMP + Dex	PMA
HSL mRNA (% of control)	15.5 ± 1.7*	123.2 ± 5.3	8.4 ± 3.0**	65.3 ± 12.9
HSL specific activity (% of control)	29.5	98.0	26.5	68.5

such a cross-talk occurred for the regulation of HSL gene expression. For that purpose, different strategies were used. First, we treated 3T3-F442A adipocytes for 18 h with supra-maximal concentrations of cAMP and PMA in conjunction (Table 1). Under these conditions, the HSL mRNA level was decreased by about 80 %, whereas treatment with cAMP or PMA alone induced, respectively, a 60 % or 50 % decrease. Thus cAMP and PMA effects were additive. Secondly, we preincubated 3T3-F442A adipocytes with 1 μM PMA for 24 h before PMA or cAMP treatment as above. Such a long exposure to phorbol esters was previously shown to induce a desensitization of the PKC signalling pathway [32]. As expected, PMA was no longer active on the HSL mRNA level when applied to desensitized cells (Table 1). In contrast, cAMP remained fully active (Table 1). Thirdly, we wondered whether dexamethasone, which potentiated the cAMP effect, modulated the PMA effect. Cells treated with either PMA alone or PMA plus dexamethasone presented similar levels of HSL mRNA (Table 1). All these arguments support the hypothesis that cAMP and PMA act through independent signalling pathways to decrease the HSL mRNA level in adipocytes.

In order to determine whether PMA and cAMP actions were direct, we used the inhibitor of protein synthesis anisomycin. Cells were preincubated for 30 min with anisomycin, then cAMP or PMA was added to the cells for, respectively, 18 h or 6 h to induce maximal effects. Anisomycin did not prevent cAMP or PMA action. Indeed, the HSL mRNA level from anisomycin-treated cells reached 54.5 ± 3.3 or 49.6 ± 3.8 % of control after, respectively, cAMP or PMA treatment. These values were not significantly different from those obtained from cells treated with cAMP or PMA alone ($P < 0.001$; Student's t test for unpaired data). This lack of effect of anisomycin was not due to a failure of drug action, because it was able to prevent the dexamethasone-induced decrease in the PEPCK mRNA level under identical conditions (S. Franckhauser and C. Forest, unpublished work). Therefore inhibition of HSL gene expression by cAMP or PMA does not appear to be dependent upon protein neosynthesis.

cAMP and PMA reduced HSL total activity

To determine whether the decrease in the HSL mRNA level had a repercussion on the HSL level, we measured HSL total activity, which is an indirect estimation of the quantity of this protein [23]. In the conditions of the assay, enzyme activity determination is a reliable evaluation of the HSL protein amount. In response to cAMP or PMA, we observed a transient decrease in HSL total activity, which followed the HSL mRNA level with a delay of several hours, being maximal at 24 h (Figure 3). The extent of decrease in the HSL level was lower than that of the mRNA level and reached about 50 % for cAMP and 30 % for PMA at 24 h of treatment (Figure 3).

BFC-1 adipocytes responded to cAMP and PMA in a manner similar to that of 3T3-F442A adipocytes

To ascertain that the regulation demonstrated in 3T3-F442A adipocytes was not restricted to this cell line, we tested another independent adipocyte cell line, BFC-1 [13]. At concentrations as low as 100 μM, 8-CPT-cAMP induced more than a 80 % decrease in the HSL mRNA level at 18 h of treatment (Table 2). Dexamethasone alone had no effect but slightly potentiated the cAMP-induced decrease, which reached 90 %. PMA was less efficient and produced a 35 % decrease in the HSL mRNA level (Table 2). As already described for 3T3-F442A adipocytes (Figure 3), HSL total activity of BFC-1 adipocytes followed HSL mRNA content, although the extent of the decrease was lower (Table 2).

DISCUSSION

The strong inductive action that cAMP-elevating agents exert on phosphorylation of HSL and on adipocyte lipolysis is well documented. A large number of experiments aimed at studying hormonal control of lipolysis have been carried out *in vitro*, on preadipose cell lines having the capacity to differentiate into adipocytes under well-defined culture conditions [14]. Among these cell lines, 3T3-F442A and BFC-1 represent good models [14,33,34]. They have been isolated totally independently [12,13]. Therefore the observation that similar regulation occurs in both lines validates the results. Moreover, HSL mRNA is absent from preadipocytes but is strongly expressed in mature adipocytes in both cell lines (Figure 1; E. Plée-Gautier and C. Forest, unpublished work). Thus the 3T3-F442A and BFC-1 cell lines appear to constitute appropriate models for investigating the hormonal regulation of HSL gene expression in adipocytes.

Our discovery that β-adrenergic agonists and cAMP potently decrease the HSL mRNA and protein levels in 3T3-F442A and BFC-1 adipocytes is, at first view, unexpected. It can be assumed that, in contrast to the rapid onset of cAMP action on HSL activity [23], several hours are required for cAMP to induce a maximal decrease in HSL gene expression (Figure 2 and Table 2). This effect is independent of protein neosynthesis, and therefore directly related to alterations of either gene transcription or mRNA stability. The significance of such an effect is not clear. An explanation could be that such a decrease might represent a counter-regulatory adaptative process that the cell develops to offset the action of lipolytic agents on a long-term basis. Examples of counter-regulatory processes are frequently observed in physiology. Commonly, reaction end-products exert a negative feedback and control their own synthesis, by an action either on enzyme activity or on the synthesis of a key modulator involved in the reaction. For instance, cholesterol restricts its own formation from acetate by an inhibition of 3-hydroxy-3-methylglutaryl-CoA synthase gene expression [35]. In that respect, we wondered whether the lipolysis reaction products, namely fatty acids, would actually be mediators of the effect of cAMP on HSL gene expression. This hypothesis can be now ruled out, because we showed that oleate, a long-chain fatty acid present to a large extent in stored triacylglycerols, does not affect

the HSL mRNA level in 3T3-F442A adipocytes ([36]; E. Plée-Gautier and C. Forest, unpublished work). This does not exclude a potential role of intermediary lipid metabolites in HSL gene regulation, such as the one described for the inhibition of HSL activity [37]. Nevertheless, we face here a different type of counter-regulatory mechanism of the action of cAMP, which rapidly stimulates HSL activity and inhibits HSL synthesis on a longer-term basis.

The observation that cAMP and PMA decrease the HSL mRNA level and HSL activity only transiently (Figure 3) argues that these drugs do not induce a toxic state that could be irreversible. These fully reversible effects are not due to a desensitization process but rather to a degradation of the drugs, because their further addition in the culture medium at 24 h allows the maintenance of a low HSL mRNA signal for at least 24 h (results not shown).

What may be the physiological significance of cAMP-induced negative regulation of HSL mRNA in adipose tissue? *In vivo* experiments are required to address this question. Sztalryd and Kraemer [8] recently observed that 1 day fasting, which is correlated with a large increase in intracellular cAMP, induces about a 2-fold decrease in the HSL mRNA level from adipose cells isolated from epididymal fat pads. Longer fasting (3–5 days) increases the HSL mRNA level about 2-fold [8]. This result is consistent with our findings that the cAMP-induced decrease in HSL gene expression is maximum at 24 h of treatment of 3T3-F442A adipocytes.

Among the other potential modulators of adipocyte lipolysis that we have tested (phorbol esters, glucocorticoids, insulin, growth hormone and retinoic acid), phorbol esters and glucocorticoids only are regulators of HSL gene expression in BFC-1 and 3T3-F442A adipocytes. The mechanisms of action of cAMP, phorbol esters and glucocorticoids appear to be different. Like cAMP, the phorbol ester PMA, a well-known activator of PKC, strongly decreases the HSL mRNA level in 3T3-F442A and BFC-1 adipocytes (Figures 5 and 6 and Table 2), whereas it has been described as an activator of lipolysis [30]. Following a 6 h PMA treatment of 3T3-F442A cells, Fève et al. [32] have described a decrease in the level of the β_3-adrenergic receptor, which contributes to the reduction in lipolytic sensitivity of adipocytes to catecholamines. This result is in agreement with our finding that PMA reduces HSL and is therefore potentially involved in the limitation of lipolysis. The physiological factor responsible for the down-regulation of HSL gene expression via PKC activation in adipocytes is not identified. Insulin modulates gene expression in adipocytes at least partially through the PKC signalling pathway [32]. Insulin is a well-known antilipolytic hormone and could regulate HSL gene expression. However, under our experimental conditions, insulin has no effect on this gene (results not shown).

Well-known differences in cAMP and phorbol ester signalling pathways have been observed, but potential cross-talk between the two signalling pathways must be considered [31]. In the case of HSL gene expression, it is unlikely that the latter eventuality occurs, because (i) maximally effective concentrations of both agents show additive effects (Table 1); and (ii) cAMP remains efficient on PKC-desensitized cells (Table 1). Moreover, glucocorticoids exhibit differential actions *vis-a-vis* cAMP and PMA. Dexamethasone has no effect by itself but potentiates the cAMP inhibitory effect on the HSL mRNA level, whereas it does not modulate the PMA response (Tables 1 and 2).

The absence of a dexamethasone effect in 3T3-F442A adipocytes is in contrast to the positive action that this hormone exerts on a long-term basis on the HSL mRNA level in isolated adipocytes [11]. The large differences between the two cell systems could be the basis for such a discrepancy. Our discovery of a potentiating action of dexamethasone on the cAMP-induced decrease in HSL gene expression is, however, not altogether surprising, because glucocorticoids have been shown already to potentiate the lipolytic action of growth hormone [38] and to facilitate cAMP-mediated lipolysis in white adipose tissue [39]. Therefore a link between glucocorticoids and cAMP actions has been already clearly established. The mechanism of the glucocorticoid effect on adipocyte lipolysis might be extremely complex, resulting from (i) cAMP-dependent modulation of HSL gene expression, (ii) a decrease in β_3- and β_1-adrenergic receptors associated with an increase in β_2-adrenergic receptors [40–42], and (iii) a decrease in activity of the low-K_m cAMP phosphodiesterase [43,44]. Moreover, our observation that glucocorticoids help to decrease HSL synthesis is consistent with the fact that administration of dexamethasone to rats results in a greater lipid deposition in adipose tissue [30].

In conclusion, this study presents evidence in support of an additional level of regulation of the key enzyme of lipolysis in adipocytes, HSL. It is largely agreed that a rapid change in the phosphorylation state activates the enzyme [1,5]. More recently, it has been shown that translocation of HSL to the lipid droplet is a step necessary to its activation [3]. This latter step is potentially regulated. Our results demonstrate another level of control, implying that a modification of the HSL mRNA and protein levels is likely to occur.

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