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Analysis of Gene Expression During Adipogenesis in 3T3-F442A Preadipocytes: Insulin and Dexamethasone Control

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In the present study, we have investigated dexamethasone and insulin regulation of the expression of adipose-specific mRNA, namely, glycerophosphate dehydrogenase (G3PDH) and adipsin, at different stages of differentiation. During adipose conversion, insulin promotes an accumulation of G3PDH mRNA which is linked to cell differentiation; in fully differentiated cells, insulin is not required to maintain G3PDH gene expression. Differentiating cells in serum deprived medium already exhibit, at day 1, a maximal amount of mRNA encoding for adipsin, which is tenfold decreased by 10 nM of insulin; insulin also exerts a negative effect on the abundance of adipsin mRNA in mature cells. This result indicates that adipsin appears to be a very early marker of adipose conversion, the gene expression of which is down-regulated by the presence of insulin. Dexamethasone (DEX) decreases the G3PDH message at all stages of adipose conversion, while it promotes the accumulation of adipsin mRNA mainly in differentiating cells. In DEX-treated adipocytes, the transcription efficiency of the G3PDH gene is not altered, and reduction to 50% of the message is due essentially to an approximately twofold decrease in its half-life.

Key words: differentiation, insulin, glucocorticoids, glycerophosphate dehydrogenase, adipsin mRNA's

Under appropriate culture conditions, preadipocyte subclones derived from mouse 3T3 cells differentiate into adipocytes in a manner resembling the process which occurs during adipose tissue development [1]. Thus, the 3T3-F442A cell line is an excellent model system for investigating agents and mechanisms that govern the conversion of progenitor cells into terminally differentiated cells which perform highly specialized functions. Differentiated cells exhibit both morphological and biochemical changes which are closely related to reprogramming of cellular metabolism; thus, the protein composition of these cells is extensively altered [2,3] in accordance with increases in

Abbreviations used: G3PDH, glycerophosphate dehydrogenase; DMEM, Dulbecco's modified Eagle's medium; DRB, 5,6 dichloro 1-β-D ribofuranosyl benzimidazole; DEX, dexamethasone.

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many of the enzymes required for triglyceride synthesis and/or mobilization [1]. One example of these numerous changes is that of two adipose-specific mRNA's encoding for glycerophosphate dehydrogenase (G3PDH) and adipsin, which have been shown to increase over 100-fold during differentiation [4].

The mechanisms regulating the transition from the preadipocyte to the mature adipocyte remain obscure. Nevertheless, it is clear that hormonally regulated processes are essentially involved. The role which specific hormones play in controlling the differentiation of adipocytes is presently being elucidated [5–7]. In particular, terminal maturation has been shown to depend upon physiological concentrations of insulin which modulate the expression of lipogenic enzymes in 3T3-F442A cells, leading to increased lipid deposition within the cells [8–10].

Among other hormonal and pharmacological agents which have been reported to accelerate the differentiation of such cells, glucocorticoids seem to play a complex role. In mouse 3T3 cell subclones, assays used to evaluate their triggering of adipocyte differentiation have essentially relied on morphological and biochemical conversion to the adipogenic phenotype [11]. The activation by glucocorticoids of adipose-related genes has been evidenced in the stable adipogenic cell line TA1 derived from 5-azacytidine-treated 10T1/2 mouse embryo fibroblast [12], but these glucocorticoid inducible genes thus far remain unidentified [13–15]. Similarly, in 3T3-L1 cells, expression of the differentiation-induced gene for fatty acid-binding protein has been reported to be activated by dexamethasone (DEX) [16]. Conversely, we have recently demonstrated that DEX could slow down terminal differentiation of 3T3-F442A cells [8,9].

To understand adipocyte development in this context, we have examined the properties of DEX and insulin by studying gene expression of two adipose-specific mRNA's (G3PDH and adipsin mRNA) at different stages of adipose conversion of 3T3-F442A cells. Furthermore, since essential differentiation factors for optimal adipose conversion are present only in fetal calf serum [17], we have analyzed the ordered and coordinated expression of these adipose-specific genes in primed cells allowed to express the differentiated phenotype in a serum-free medium previously described [8,18].

The present study demonstrates that insulin responsiveness of the G3PDH gene is dependent upon its developmental activation, whereas a negative effect of insulin on adipsin mRNA occurs both in differentiating and fully differentiated cells. Moreover, evidence is given that DEX exerts an opposite effect upon the expression of G3PDH and adipsin mRNA's during the course of adipogenic differentiation: the synthetic glucocorticoid reduces the former, while it promotes the accumulation of the latter within the cells. Finally, using a serum-free medium, we have reassessed the temporal expression of both messenger RNA's and have demonstrated that in the absence of insulin, the emergence of adipsin mRNA can precede that of G3PDH mRNA.

MATERIALS AND METHODS

Materials

Petri dishes were from Corning, tissue culture products were from Gibco, and chemicals and hormones from Sigma. Radiolabelled nucleotides and nick-translation components were purchased from Amersham.

Cell Cultures

3T3-F442A cells [19], kindly provided to us by Dr H. Green (Harvard Medical School, Boston) were allowed to differentiate according to two procedures:

In protocol I, cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (regular medium). At confluence, cells were grown with 10 nM of insulin for 5 days and then deprived of this hormone for 2 days. They were further treated for 24 h with insulin (10 nM) and/or dexamethasone (10 nM). When these hormone effects were tested on fully differentiated cells (15 days postconfluence in insulin-supplemented medium), cells were deprived of insulin for 24 h and treated for 1, 2, or 3 days by insulin (10 nM) and/or dexamethasone (10 nM). Accordingly, a 24 h insulin deprivation has been reported to be sufficient to promote insulin responsiveness with respect to stimulation of glucose transport in these cells [9].

In protocol II, cells were grown in DMEM supplemented with 5% calf serum and 5% fetal calf serum for 4 days until confluence. Cell cultures were then fed for 3 days (phase of induction) with DMEM supplemented with 10% fetal calf serum, 0.1 mM isobutyl methylxanthine, and 0.25 μM dexamethasone; at that time (day 0), cell cultures were rinsed once with fresh DMEM and fed with serum-free medium [8]. The latter, referred to as DH/3F medium, was a mixture of DMEM and Ham F12 supplemented with 1 mg/ml bovine serum albumin, 5 μg/ml transferrin, 2 μg/ml luteinizing hormone (LH), and 10 μg/ml submaxillary gland rat extract prepared according to Bottenstein et al. [20]. Insulin (10 nM) and/or dexamethasone (10 nM) were added to the medium during the latter phase of expression.

Cell Extracts and Enzyme Determination

At the indicated time, cell monolayers were washed with phosphate-buffered saline and homogenized in 0.25 mM sucrose at pH 7.4 containing 1 mM dithiothreitol and 1 mM EDTA; G3PDH activity (EC.1.1.1.8) was assayed in the 100,000g supernatant according to the Wise and Green method [21]. Proteins were determined by Lowry's method [22] using bovine serum albumin as a standard.

RNA Isolation and Northern Blot Analysis

Total RNA was isolated by extraction with guanidine isothiocyanate as previously described [23]. Total RNA was electrophoresed in 1.5% agarose, 2.2 M formaldehyde gels [24], transferred to Hybond nylon membranes (Amersham), and covalently linked by UV cross-linking. Membranes were prehybridized in 45% formamide; 4×SSC; 0.1 M sodium phosphate, pH 6.5; 5×Denhardt's; 0.1% SDS; and 75 μg/ml denatured salmon sperm DNA for 4 h at 42°C, then hybridized in the same solution supplemented with 10% dextran sulfate containing about 2×10^6 cpm/ml [α^{32}P]-labelled cDNA probe at 42°C for 16 h. Labelled cDNA probes were synthesized by the nick translation procedure [25] using [α^{32}P]-dCTP (Amersham). After hybridization, membranes were washed in 2×SSC, 0.1 SDS for 1 h at 60°C and in 0.2×SSC, 0.1% SDS for 30 min at 60°C. Membranes were autoradiographed using KODAK X AR-5 film at −80°C between two intensifying screens for 5–48 h. Plasmids pGPD1, pAd20, and pβactin cDNA clones, generously provided to us by Dr. Green, have been shown to recognize a single mRNA species with an apparent size of 3.5 kb, 1 kb, and 2.15 kb for G3PDH, adipsin, and actin, respectively [4].

Turnover of mRNA in 3T3-F442A Adipocytes

Fully differentiated 3T3-F442A cells were treated overnight (14 h) with DEX and/or insulin. At that time, mRNA synthesis was inhibited by addition to the media of 25 μg/ml of DRB (5,6 dichloro 1-β-D ribofuranosyl benzimidazole) [26]. Total RNA was extracted at 0, 1, 2, 4, 8, 10, and 24 h after the addition of the drug and analyzed by Northern blotting as indicated above.

Nuclear Run on Transcription

Nuclei were isolated from 5 dishes (90 mm) according to Zechner et al. [27] and immediately frozen and stored at −80°C in 250 μl of 50 mM Tris-HCl, pH 7.8, containing 5 mM $MgCl_2$ and 0.1 mM EDTA. DNA was quantified by the Burton assay [28]. For the transcription reaction, 20 μl of nuclear suspension (50 μg of DNA containing about 4.10^6 nuclei) were incubated with 20 μl of 120 mM Tris-HCl, pH 7.8, containing 50 mM NaCl, 350 mM $(NH4)_2 SO_4$, 4 mM $MnCl_2$, 0.24 mM EDTA; 5 μl heparin (20 μg/ml); and 5 μl each of 20 mM ATP, 20 mM GTP, 20 mM UTP, and 100 μCi [αP^{32}]-CTP in a total volume of 100 μl for 45 min at 32°C. The reaction was then terminated according to Zechner et al. [27]. The mixture was then extracted with phenol-chloroform and precipitated with an equal volume of ice-cold 20% trichloroacetic acid. The pellet was dissolved in 10 mM Tris-HCl, pH 7.5, and 1 mM EDTA and labelled nuclear RNA was ethanol-precipitated. Equal amounts of labelled nuclear RNA were then hybridized to filters containing immobilized plasmids in a hybridization mixture similar to that used in Northern blots except for salmon DNA, which was omitted and replaced by yeast tRNA (200 μg/ml).

RESULTS

Hormonal Control of Adipogenesis in Serum-Based Differentiation

As judged by examination of cell morphology, lipid accumulation, and emergence of glycerophosphate dehydrogenase, it was previously shown that insulin counteracted the inhibitory effects of dexamethasone upon adipocyte terminal differentiation [8,9]. Since evidence is still lacking at the molecular level, we focused on insulin and DEX regulation of G3PDH and adipsin expression during adipogenesis of 3T3-F442A cells.

For this purpose, cells were first grown for 5 days in regular medium supplemented with 10 nM insulin; after 2 days of insulin deprivation, they were further treated for 24 h with various hormone regimens. Total RNA which originated from such differentiating cells were analyzed by Northern blot analysis using pGPD1 (G3PDH) and pAd20 (adipsin) cDNA probes. To correct for differences both in the amounts of RNA loaded into each lane and in transfer efficiency, results were normalized to signals generated by probing with the mouse cDNA βactin clone and densitometry scanning of autoradiographs.

Figure 1A shows that in the absence of insulin, treatment of cell culture by 10 nM of DEX resulted in a 50% decrease in G3PDH mRNA expression compared to control cells. The opposite situation was encountered for adipsin mRNA content, which was increased up to 300% over control levels in the same interval (Fig. 1B). Conversely, the addition of insulin (10 nM) increased the G3PDH signal by 50%, while decreasing that of the adipsin hybridizable species by 70% compared with control cells (Fig. 1A,B). Treatment with a combination of dexamethasone and insulin did not result in a

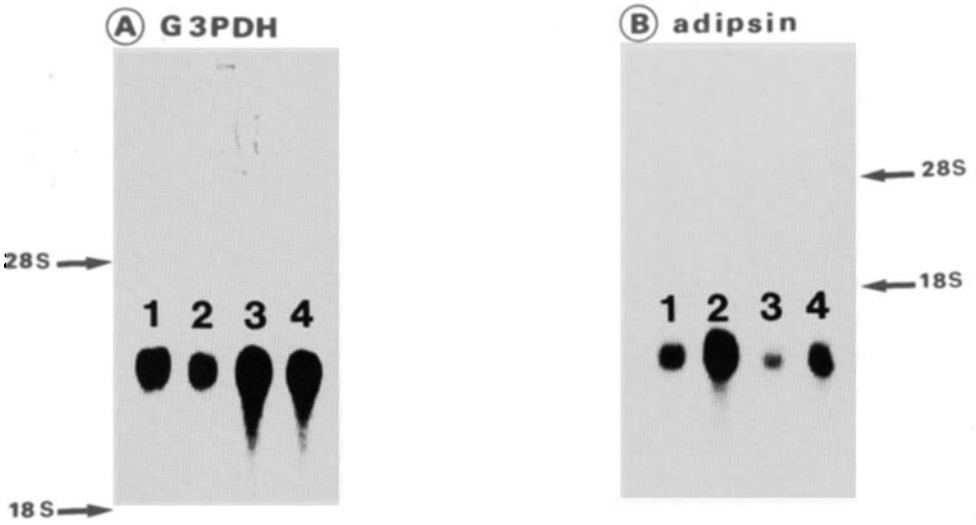

Fig. 1. Hormonal control of G3PDH (**A**) and adipsin (**B**) mRNA content in differentiating 3T3-F442A cells. Confluent 3T3-F442A cells were grown for 5 days in DMEM supplemented with fetal calf serum (10%) and insulin (10 nM); after 48 h deprivation of insulin, they were further exposed for 24 h to the indicated hormones. **Lane 1** (control), **lane 2** (10 nM DEX), **lane 3** (10 nM insulin), **lane 4** (DEX + insulin).

significant change in G3PDH mRNA expression compared to that obtained with insulin alone (Fig. 1A). By contrast, in insulin-treated cells, dexamethasone was able to enhance the expression of adipsin mRNA. Thus, insulin and DEX exerted opposite effects upon these two specific mRNA's.

Alternate Expression of G3PDH and Adipsin mRNA in Primed Cells Following Expression of Adipogenesis in Serum-Free Medium

Experiments in serum-supplemented medium which indicated the opposite effects of insulin and DEX on adipsin gene expression led us to hypothesize that the emergence of the adipsin signal was not necessarily a late event in adipose conversion. To test this hypothesis, we decided to study further the temporal expression of both G3PDH and adipsin mRNA in primed cells grown in serum-free hormone-defined medium and submitted to the adipogenic signal of insulin (protocol II). Primed cells were obtained by 3-day exposure of confluent cells to the priming mixture (isobutylmethyl-xanthin, DEX, and fetal calf serum). Starting at that time (day 0), the cells were further grown in serum-free medium supplemented or not with insulin. As expected, there was a fourfold decrease in actin mRNA content in primed cells compared to confluent 3T3-F442A preadipocytes (Fig. 2). Otherwise, in the absence of insulin, cells were not able to express the G3PDH gene. By contrast, insulin provoked a net accumulation of G3PDH at day 4 which was consistent with the measurement of G3PDH specific activity (Fig. 2, insert). This positive effect of insulin on G3PDH mRNA accumulation could not be observed after day 1 of treatment, suggesting a minimum 24 h lag period in G3PDH gene expression.

The pattern of adipsin gene expression was completely opposite that of the G3PDH counterpart. Probing of membranes with nick-translated adipsin cDNA revealed early expression of adipsin mRNA (at day 1) in cells grown in the absence of

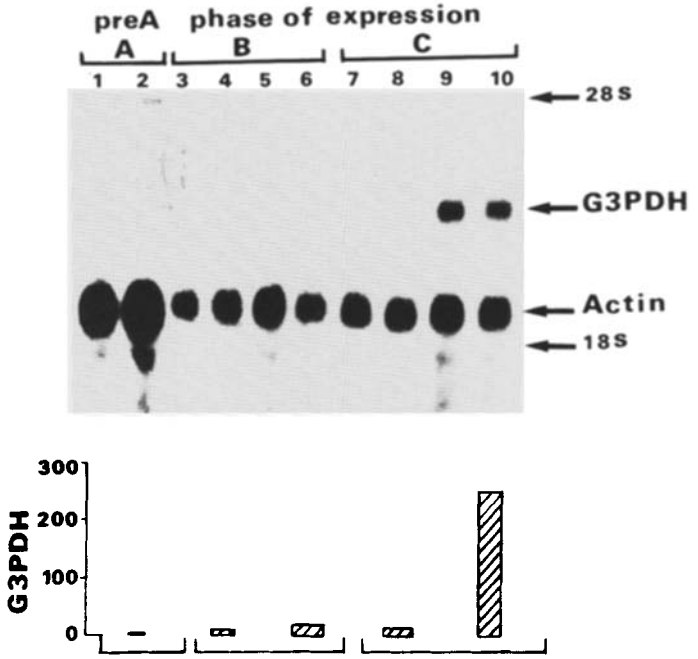

Fig. 2. Insulin control of G3PDH mRNA in primed cells grown in serum-free medium. Confluent 3T3-F442A preadipocytes (**A**) were exposed to the priming mixture (fetal calf serum (10%) + methyl isobuthyl xanthin (0.1 mM) + DEX (250 mM)). Thus, cells were fed with serum-free medium and total RNA analyzed in duplicate at day 1 (**B**) and day 4 (**C**) of the phase of expression. **Top:** G3PDH and actin mRNA's in the absence of insulin—**lanes 3, 4** (day 1) and **lanes 7, 8** (day 4)—and in the presence of insulin (10 nM)—**lanes 5, 6** (day 1) and **lanes 9, 10** (day 4). **Bottom:** G3PDH-specific activity expressed as nmol/min/mg protein.

insulin (Fig. 3). At day 1, the amount of adipsin mRNA represented 90% of that measured at day 4 (not shown), indicating that no substantial increase of this mRNA occured within 4 days of the expression phase. Strong inhibition of the adipsin mRNA level (90%) was observed when insulin was present. As observed for cells grown in the presence of serum, dexamethasone also elicited a 2.5-fold increase in the adipsin signal after 24 h of treatment of the cells, whether insulin was present or not (results not shown). Taken together, these findings clearly demonstrate that adipsin gene expression could be turned on very early during the course of adipose differentiation.

Hormonal Control of Expression of G3PDH and Adipsin mRNA in Fully Differentiated Cells

To characterize further the fate of these adipose-specific mRNA's, we examined whether G3PDH and adipsin genes were specifically responsive to glucocorticoids and insulin once cells were fully matured. With this in mind, cells were allowed to achieve their terminal differentiation under optimal conditions (regular medium plus 10 nM insulin) for 2 weeks. Thereafter, adipocytes were deprived of insulin for 1 day and further exposed or not for 24 h to DEX (10 nM) and/or insulin (10 nM). Figure 4 shows that the G3PDH mRNA level was slightly enhanced by the presence of insulin (about 25%). When these cells were exposed to dexamethasone, there was a 50% decrease in G3PDH

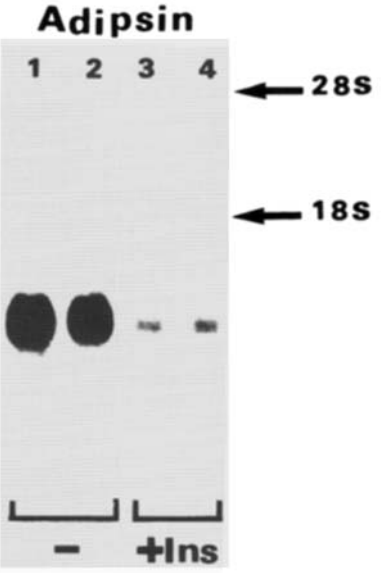

Fig. 3. Insulin control of adipsin mRNA in primed cells grown in serum-free medium. The cells were grown as indicated in Figure 2. Total RNA was analyzed at day 1 of the phase of expression. **Lanes 1, 2:** Control (no insulin). **Lanes 3, 4:** + insulin (10 nM).

mRNA content; this negative effect of DEX was counteracted by the presence of insulin. These findings could be related to the values of enzyme-specific activity according to the different regimens of cell culture which mimicked the changes in G3PDH mRNA levels after a 24 h lag period (Fig. 4, lower panel).

On the other hand, when cells were exposed to insulin for 24 h, the adipsin mRNA content was sharply decreased (70%) (Fig. 5). In the absence of insulin, glucocorticoids did not modify adipsin mRNA content in adipocytes. By contrast, in the presence of insulin, dexamethasone elicited a 70% increase in adipsin gene expression in these cells whereas corticosterone did not have any effect. The fact that diminished G3PDH mRNA content could be observed in DEX-treated cells did not allow us to determine whether glucocorticoids exerted hormonal control upon the gene expression, at a transcriptional or at a post-transcriptional level. Thus, we performed run-on assays on nuclei extracted from control and DEX-treated cells. The slot-blot illustrated in Figure 6 clearly indicates that the presence of dexamethasone did not affect elongation of nascent G3PDH mRNA chains. Since the rate of RNA degradation may greatly influence mRNA abundance, we also estimated the half-life of mRNA in adipocytes using DRB as an inhibitor of RNA synthesis. Subsequently, we measured the loss of these adipose-specific mRNA's by Northern blot.

As shown in Table I, the half-life of G3PDH mRNA was estimated to be 7.2 h under basal condition. The presence of DEX accelerated G3PDH mRNA degradation, while insulin stabilized the message (4.5 and 20 h in DEX- and insulin-treated cells, respectively). In the presence of the two hormones, results show that insulin mainly antagonized the destabilizing effect of DEX. Conversely, adipsin mRNA had a greater half-life than that of G3PDH (17 h) and was not modified by DEX treatment. A stabilizing effect of insulin (30 h) could also be observed.

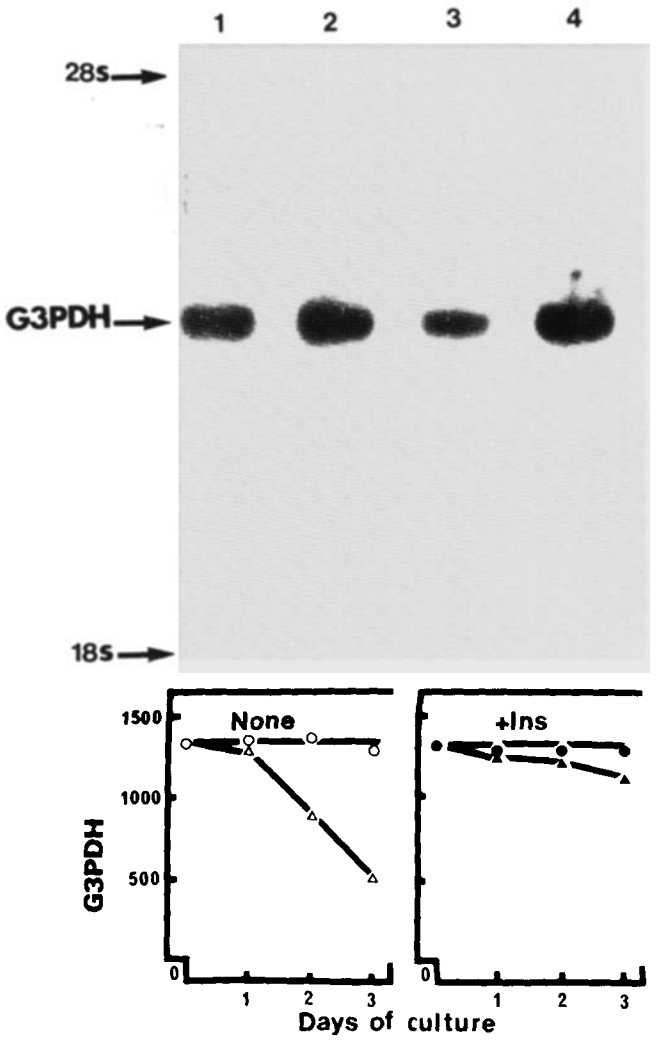

Fig. 4. Effect of dexamethasone and insulin on G3PDH mRNA and specific activity in 3T3-F442A adipocytes. **Top:** G3PDH mRNA levels. Fully differentiated cells were deprived of insulin for 24 h and further treated for 24 h with the indicated hormones. **Lane 1:** (control), **lane 2** (insulin (10 nM), **lane 3** (DEX, 10 nM), and **lane 4** (insulin + DEX). **Bottom:** Time course of G3PDH-specific activity (nmole/min/mg protein) in mature adipocytes treated with DEX (10 nM) in the absence or presence of insulin. Control (○, ●) and DEX (△, ▲).

DISCUSSION

Although internal signals thus far remain to be identified, the ability to activate the system hormonally provides the opportunity to investigate hormonally regulated gene products that play an active role in the process of adipose differentiation. In this study, we provide evidence for the existence of an orchestrated set of biochemical processes that instruct primed preadipocytes to undergo final adipogenic conversion.

We report that insulin triggers G3PDH gene expression in primed cells and thus acts as an authentic lipogenic signal. Conversely, in differentiated cells, insulin slightly

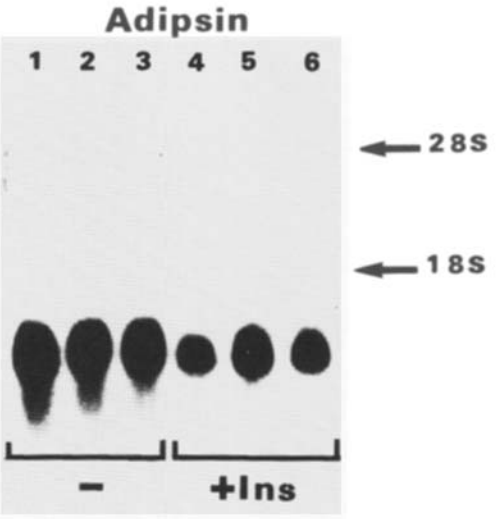

Fig. 5. Adipsin mRNA content in corticosteroid and insulin treated mature adipocytes. A condition similar to Figure 4. Lanes 1–3 (no insulin): **1** (control), **2** (DEX, 10 nM), **3** (corticosterone, 100 nM). Lanes 4–6 (+insulin, 10 nM): **4** (control), **5** (DEX), **6** (corticosterone).

modulates the G3PDH mRNA content through a stabilizing effect (Table I) and thus plays a minor role in the control of G3PDH gene expression. Our studies support evidence that insulin is able precociously to activate the expression of a key regulatory gene for lipogenesis; furthermore, its presence is not required to maintain continuous expression of this gene. When cells were differentiated in the presence of serum and insulin, the emergence of adipsin mRNA was a relatively late event in adipose conversion [4]. In this report, we clearly demonstrate that the expression of adipsin mRNA behaves as an early marker of adipocyte differentiation: indeed, primed cells began to accumulate adipsin mRNA in a serum-free, insulin-free medium which is known to be inadequate for terminal adipocyte differentiation [8]. This experimental condition was previously shown to be associated with the early, insulin-independent emergence of lipoprotein lipase activity [18]. While insulin in itself hastens the morphological appearance of the

Fig. 6. Nuclear run on assay of transcription of the G3PDH gene in 3T3-F442A adipocytes. Nuclei were prepared from cells treated overnight without (CONTROL) or with dexamethasone 10 nM (DEX). Nuclei run on assay and extraction of ^{32}P-labelled RNA are described in Methods. Slot-blot of DNA probes: control plasmid, pBR322 (**lane 1**), β-actin cDNA (**lane 2**), and G3PDH cDNA (**lane 3**).

TABLE I. Turnover of G3PDH and Adipsin mRNA in 3T3-F442A Adipocytes*

RNA species	Apparent half-time of degradation (h)			
	Control	DEX	Ins	DEX + Ins
G3PDH	7.2 (± 0.4)	4.5 (± 0.5)	20	15
Adipsin	17	23 (± 5)	30 (± 1)	20.5

*3T3-F442A fully differentiated cells were treated overnight with dexamethasone and/or insulin. At that time, mRNA synthesis was inhibited by addition to the medium of 25 μg/ml of DRB. Total RNA was extracted at 0, 1, 2, 4, 8, 10, and 24 h after addition of the drug and analyzed by Northern blotting. Blots were quantified by densitometry scanning. Half-lives were calculated after linear regression of the semi-log plot of the scanning data as a function of time (mean ± SEM, n = 3).

adipocyte phenotype, we herein demonstrate that it dramatically down-regulates the expression of adipsin mRNA (Fig. 3). It is noteworthy that insulin also exerts its negative effect upon adipsin expression in cells after their terminal maturation, in spite of its stabilizing effect upon adipsin mRNA (30 h vs. 17 h, Table I). Thus, our results suggest that the major point of insulin regulation of adipsin gene expression took place at the transcriptional level, as recently reported by Dani et al. [29].

Several groups studying rodent adipogenic cell lines have reported acceleration of differentiation by DEX when analyzed morphologically by lipid accumulation and by induction of enzymatic activities [11]. However, interpretation of these observations is complicated because DEX was used during a short period of time and always concomitantly with insulin, which clearly hastens the differentiation process. In contrast, we previously reported an inhibitory effect of DEX on the specific activity of lipogenic enzymes involved in terminal differentiation; this effect was antagonized by the presence of insulin [8,9].

Previous studies on rat adipose tissue have shown that in addition to its inhibitory effect on glucose uptake [30], DEX is also able to decrease lipogenic enzymes, namely, fatty acid synthetase and acetyl Coa carboxylase [31]; lipoprotein lipase is also decreased in some cases by long-term exposure of adipose tissue to DEX [32]. Our data show that, in cultured mature adipocytes, DEX is also able to decrease the mRNA encoding for a lipogenic enzyme. In agreement with this, the same effect of DEX has been described for gene expression of another lipogenic enzyme, namely, phosphoenolpyruvate carboxykinase, in 3T3 cells: this effect was only partly linked to inhibition of transcription and was postulated to be essentially due to a post-transcriptional event [33]. In our study, since the transcriptional efficiency of G3PDH gene was not altered in fully differentiated cells (Fig. 6), the decrease in G3PDH mRNA expression elicited by DEX may essentially reflect a destabilizing effect of the drug due to shortening of the half-life of this mRNA (Table I). Why glucocorticoids increase G3PDH mRNA degradation is unknown. A possible explanation could be an induction or an activation by DEX of enzymes such as RNAases that break down mRNA [34,35]. Conversely, as reported above, insulin counteracts the negative effect of DEX by increasing the half-life of G3PDH mRNA from 4.5 to 15 h. Thus, according to our studies, the lack of a DEX effect upon G3PDH recently reported by Dobson et al. in 3T3 adipocytes [36] could be attributed to the simultaneous presence of high doses of insulin in culture media.

Major features of the effects of glucocorticoids have been previously evidenced in the TA1 preadipose cell line: during the course of adipocyte differentiation of these cells, the drug clearly accelerates the expression of some unidentified genes [12–15]. In the present study, evidence is presented that expression of adipsin mRNA, an mRNA species which is induced over 100-fold during adipose conversion, is much more accelerated by DEX in differentiating than in fully differentiated cells. Furthermore, the latter did not respond to corticosterone (Fig. 5); thus, this adipose-specific gene is unlike a typical glucocorticoid inducible gene. Our results are in agreement with a recent study which reported that no superimposed glucocorticoid regulation of adipsin mRNA content was observed in cultured 3T3 and primary rat fat cells [37]. Finally, DEX effect on adipsin gene expression is probably not mediated by glucocorticoid receptors since RU38486 antiglucocorticoid has been recently shown also to elicit an increase in adipsin mRNA [38].

Taken together, these findings demonstrate that glucocorticoids may exhibit pleotypic effects. These observations are consistent with the well-known effects of glucocorticoids in lipid metabolism as evidenced by redistribution of adipose stores in the hypercorticoid state. Recent data have suggested that glucocorticoids could be involved in the depressed adipsin expression of obese animals [37]: as suggested by these authors, if the effect of glucocorticoids in vivo is not direct, one may presume that insulin plays an important role in this defect since obese animals are known to be in a hyperinsulinemic state. In particular, we have demonstrated a decreased adipsin mRNA content in adipose tissue of Zucker rats which display hyperinsulinemia, whereas their corticosteronemia is normal [39]. Thus, our present data emphasize the fact that insulin may contribute significantly to defective adipsin expression in obese mice.

The ordered, coordinated expression of cell-specific proteins and functions during differentiation is believed to reflect the activation of networks of functionally altered genes [40]. By studying the expression of two adipose-specific mRNA's, evidence is herein given that the temporal and functional integration of such a gene can be controlled by insulin and glucocorticoids acting in a contradictory manner or in combination. Likewise, the present study emphasizes that expression of adipsin gene in cultured adipose cells represents a useful model for studying glucocorticoid counter-regulation of insulin down-regulated gene expression. This argues that the insulin-to-glucocorticoid ratio is an important determinant in the fat storage process and could thus be an etiologic factor in obesity.

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