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Dexamethasone-dependent expression of β^{1-24} corticotropin stimulated adenylate cyclase during adipose conversion of 3T3-F442A cells

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When 3T3-F442A preadipocytes were grown in culture media supplemented with corticosteroid poor fetal calf serum and insulin they differentiated into adipocytes. Glycerophosphate dehydrogenase, a marker of terminal differentiation, developed a 600-fold increase of activity whereas the adenylate cyclase system remained unresponsive to the synthetic ACTH(1–24) analog. In contrast, 3T3-F442A adipocytes, differentiated in the presence of dexamethasone, exhibited an adenylate cyclase activity which was stimulated 4–fold by ACTH(1–24). The stimulation of the adenylate cyclase activity by GTPγS remained unchanged (about 20–25-fold) suggesting that the G regulatory coupling protein was not functionally modified by dexamethasone. Binding studies with ^{125}I-ACTH revealed that specific cellular binding could be evidenced in dexamethasone-treated cells while control adipocytes did not exhibit any specific binding of ^{125}I-ACTH. These findings lend support to the hypothesis that the setting off of this ACTH responsiveness in 3T3-F442A cells is regulated by dexamethasone after cells are committed to adipose differentiation.

Dexamethasone; Adenylate cyclase; ACTH receptor; Differentiation; (3T3 preadipocyte)

1. INTRODUCTION

The physiological responsiveness of highly specialized and terminally differentiated cells is conditioned by environmental conditions namely concerted interactions with hormones during in vivo tissue development. Murine 3T3 preadipocyte cell lines [1–3] which exhibit an increased hormone sensitivity during their differentiation cycle [4–6] are especially suitable cell models for studying the assembly of the hormonally sensitive adenylate cyclase system during in vitro adipose conversion. While 3T3 cell subclones are not able to differentiate in defined media [5,7], the complexity of sera used for cell culture and differentiation renders it difficult to elucidate the biochemical events underlying hormonal interactions in these cells. In this paper special interest is focused on the factor(s) that regulate(s) the development of ACTH responsiveness of the adenylate cyclase system during adipose conversion of 3T3-F442A cells. We demonstrate that dexamethasone, a synthetic glucocorticoid analog, sets off the ACTH(1–24) responsiveness of the adenylate cyclase system during adipose conversion of 3T3-F442A cells in

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Abbreviations: GTPγS, guanosine 5′-(3-*O*-thio)triphosphate; ACTH(1–24), synthetic tetracosapeptide (1–24) of adrenocorticotropic hormone; G/F(G_i, G_s, G_o) the regulatory component of adenylate cyclase which appears to be a site of action of guanine nucleotides

Enzymes: adenylate cyclase or ATP pyrophosphate ly-ase (EC 4.6.1.1); creatine phosphokinase or ATP:creatine *N*-phosphotransferase (EC 2.7.3.2); glycerophosphate dehydrogenase activity (EC 1.1.1.8)

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culture medium supplemented with steroid-depleted fetal calf serum. These changes in the coupling properties of ACTH(1–24)-stimulated adenylate cyclase could be related to the modulating effect of dexamethasone on the specific cellular binding of ^{125}I-ACTH. The efficiency of the guanine nucleotide regulatory protein responsible for activation of adenylate cyclase is not modified by chronic exposure of cell cultures to dexamethasone. All these events are linked both to the cell's commitment to differentiation and to the presence of dexamethasone but are unrelated to terminal differentiation since mature adipocytes, unresponsive to ACTH(1–24), can be obtained in the absence of the drug.

2. MATERIALS AND METHODS

2.1. *Cell culture*

3T3-F442A and 3T3-C2 were generously provided by Dr Howard Green (Harvard Medical School, Boston). Cells were grown in 100 mm dishes (Falcon or CEB) in Dulbecco-Vogt modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and insulin (5 μg/ml) from the confluence. Most of the experiments were performed with fetal calf sera obtained from Boehringer (lot no.071) and Flow Laboratories (lot no.036066). These sera were chosen for their low content of corticosteroid: less than 5 ng/ml expressed in cortisol and monitored by radiocompetitive assays using human transcortine and they did not need further desteroidation before use. In some experiments, cells were grown in DH/4F medium [8] consisting of a mixture of DMEM and Ham-F12 (2:1, v/v) supplemented with 5 μg/ml insulin, 5 μg/ml transferrin, 2 μg/ml luteinizing hormone and 10 μg/ml submaxillar gland extract. At the indicated period, cell cultures were washed twice with cold phosphate buffered saline, homogenized in 1 mM dithiothreitol, 25 mM Tris-HCl buffer (pH 7.5) in a Dounce homogenizer. Homogenates were centrifuged at 10000 × g (4°C) and pellets resuspended in the homogenization buffer at a concentration of 5–10 mg protein/ml. Proteins were assayed according to Lowry et al. [9] using bovine serum albumin as a standard. Control adipocytes yielded approx. 0.33 mg of membrane protein per 10^6 cells, while dexamethasone-treated adipocytes yielded 0.25 mg of membrane protein per 10^6 cells.

2.2. *Enzyme assays*

Adenylate cyclase activity was assayed in triplicate for 10 min at 35°C in a 50 μl reaction mixture containing 0.2 mM [α-^{32}P]ATP (1 μCi), 1 mM cyclic AMP, 10 mM creatine phosphate, 0.5 unit creatine phosphokinase, 5 mM $MgCl_2$, 0.2 mM dithiothreitol, 100 mM Tris-HCl (pH 7.5). Reaction was initiated by the addition of membranes (about 25 μg protein) and cyclic [^{32}P]AMP was isolated and counted as described [5].

Glycerophosphate dehydrogenase activity was assayed by recording the initial rate of oxidation of NADH at 340 nm at 25°C [10]. The standard mixture contained 50 mM triethanolamine-HCl buffer (pH 7.5), 1 mM EDTA, 0.13 mM NADH, 1 mM dihydroxyacetone phosphate, 1 mM 2-mercaptoethanol and variable amounts of cell supernatant (10000 × g).

2.3. *ACTH receptors*

^{125}I-ACTH binding studies were performed directly in cell cultures (60 mm dishes) previously washed and kept overnight in serum-free DMEM supplemented with 1 mg/ml bovine serum albumin (BSA). Samples (about 2 to 3 × 10^6 cells) for 60 mm dishes were incubated with gentle shaking in triplicate for 30 min at 25°C in 1 ml DMEM buffered with 20 mM Hepes (pH 7.35), containing aprotinin (1 mg/100 ml), soybean trypsin inhibitor type II (10 mg/100 ml), bacitracin (100 mg/100 ml), 1% BSA and 0.2 nM (3-[^{125}I]iodotyrosyl^{23})ACTH(1–39) (spec. act. 68 TBq/mmol) (Amersham International). Non-specific binding was determined with 2 μM unlabeled ACTH(1–24) (Bachem) and accounted in about 60% of the total binding. At the end of the incubation, cells were rinsed three times with ice-cold phosphate buffered saline and collected in 0.5% SDS for the determination of radioactivity. Results were normalized to cell number; the latter was determined from parallel dishes, after harvesting the cells with trypsin and scoring in a hematocytometer.

3. RESULTS

3.1. *The development of ACTH(1–24) responsiveness of 3T3-F442A cells is dependent on dexamethasone*

Adipogenic differentiation of 3T3-F442A cells in surface cultures involves a change from a fibroblastic morphology (growing state) to an enlarged rounded cell shape (resting state); the terminal maturation process is characterized by the accumulation of abundant lipid vesicles, especially in the presence of insulin.

Recently, we demonstrated that dexamethasone could prevent terminal maturation of F442A cells and that insulin counteracted its inhibitory effect [11]. Thus, in insulin supplemented culture medium dexamethasone elicited only discrete morphological changes such as acceleration of the rounding up of the cells; cell plasma membranes were looking thicker and lipid droplets were smaller than in control cultures.

Terminal maturation of F442A adipocytes did not depend on the batch of fetal calf serum used for cell cultures; on the contrary we observed that ACTH responsiveness of the adenylate cyclase activity of the adipocytes could vary, according to the serum, within the range of 20–400% increase over basal activity (at day 8 after confluence). Thus, cells were allowed to differentiate in the presence of serum which was previously absorbed on dextran-charcoal to remove the steroids. Adipocytes differentiated in the native serum exhibited a 4-fold stimulation of their adenylate cyclase activity in the presence of ACTH(1–24) and no significant change was observed after addition of dexamethasone; otherwise their counter-

Table 1

Dexamethasone dependence of the ACTH(1–24)-stimulated adenylate cyclase activity

Serum	Dexamethasone (nM)	Adenylate cyclase activity		
		pmol cyclic AMP·min^{-1}·(mg protein)$^{-1}$		Ratio basal/ACTH(1–24)
		Basal	ACTH(1–24) 1 μM	
Native	none	2.20	9.45	4.3
	250	2.35	11.00	4.7
Deprived of steroids	0	1.50	2.30	1.5
	10	0.65	1.00	1.6
	100	1.45	3.65	2.5
	250	1.80	4.40	2.4
	1000	1.30	3.70	2.8

3T3-F442A cells were seeded in DMEM supplemented with a mixture of 9% calf serum and 1% of the mentioned fetal calf serum. 1 day before confluence insulin (5 μg/ml) was added and cultures continued in DMEM supplemented with 10% of respective sera. On day 2 after confluence dexamethasone was added at the indicated concentration. On day 10 crude membranes were prepared and assayed for adenylate cyclase activity. Fetal calf serum (batch 11 H 786) was supplied from Filorga (Paris) and deprived of steroids by dextran-charcoal absorption. The latter was performed three times with 10 mg/ml of activated charcoal (Merck) and 1 mg/ml of dextran T70 (Pharmacia) for 45 min at 50°C; at the end of each cycle the serum was clarified by centrifugation (10000 × g at 4°C for 10 min) and finally filtered through glass fiber and 0.22 μm membrane. The content of corticosteroid of the native serum was 30 ng/ml (expressed in cortisol); the deprived serum contained an undetectable level of corticosteroids

parts grown in steroid deprived serum were very poorly sensitive to the hormone (only 50% increase over basal activity (table 1)) and the addition of dexamethasone to a cell culture medium deprived of steroids allowed the cells to recover about 60% of their ACTH responsiveness as compared to control cells (table 1). Thus these initial studies suggested that dexamethasone was the agent responsible for the acquisition of ACTH responsiveness of the adenylate cyclase system. The following experiments were performed using corticosteroid-poor fetal calf sera which contained less than 5 ng of corticosteroid per ml.

The time course of the development of the adenylate cyclase activity was measured in partially purified membranes prepared from differentiating 3T3-F442A cells. No increase of adenylate cyclase activity by the ACTH(1–24) analog could be measured in membranes from undifferentiated cells (day 0) and during adipocyte development control membranes exhibited only a very poor increase of activity due to ACTH(1–24) (25% about over basal activity) (fig.1). In contrast the activation of adenylate cyclase activity by ACTH(1–24) increased markedly when measured in membranes from cells treated with 0.25 μM dexamethasone from confluence (day 0) reaching a maximum value by day 8 (fig.1). Typically 3T3-F442A adipocytes differentiated in the presence of dexamethasone possessed an adenylate cyclase system which was stimulated about 4-fold over basal activity by ACTH(1–24) at a maximal concentration of 10 μM. Otherwise adenylate cyclase of 3T3-F442A preadipocyte membranes (day 0) was highly sensitive to GTPγS alone (20-fold over basal activity) and this responsiveness remained unchanged during adipocyte development whether dexamethasone was present or not (fig.1).

The expression of a sensitive marker of terminal differentiation [12,13] indicated a 300- and 600-fold increase of enzyme activity respectively in the presence and absence of the drug (table 2). In parallel, 3T3-F442A cells grown in serum-free DH/4F medium and undifferentiating 3T3-C2 cell subclone which both retain the characteristics of fibroblasts during prolonged maintenance in culture as confluent monolayers did not exhibit any enzyme markers of adipose differentiation; furthermore no effect of dexamethasone could be evidenced (table 2).

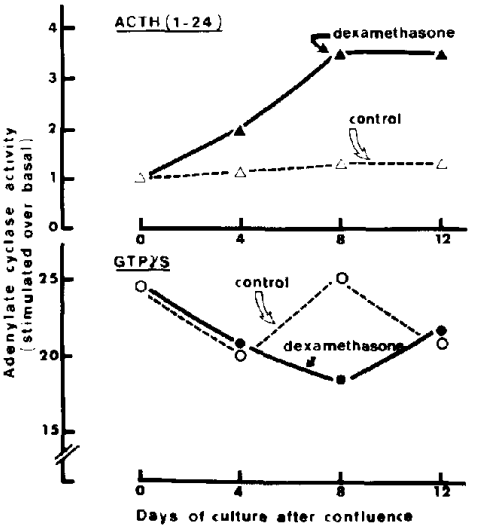

Fig.1. Development of ACTH(1–24) and GTPγS-stimulated adenylate cyclase activity of dexamethasone-treated 3T3-F442A cells. 3T3-F442A cells were grown to confluence (day 0) in DMEM supplemented with 10% fetal calf serum and insulin, 5 μg/ml. At day 0, dexamethasone (0.25 μM) was added to some cultures and crude membranes were prepared at the indicated time. Basal and stimulated adenylate cyclase activities were assayed as described in section 2 in the presence of 10 μM ACTH(1–24) for control (△—△) and dexamethasone-treated cells (▲—▲) or in the presence of 10 μM GTPγS for control (○—○) and dexamethasone-treated cells (●—●). Results are expressed as the ratio of stimulated activity over basal activity which represents the hormone receptor coupling efficiency. Values of basal adenylate cyclase activity were 3.5, 3, 5.8 and 10.4 pmol cyclic AMP·min^{-1}·(mg protein)$^{-1}$ respectively at day 0, 4, 8 and 12 for control and 2.0, 4.4 and 4.8 pmol cyclic AMP·min^{-1}·(mg protein)$^{-1}$ respectively at day 4, 8 and 12 for dexamethasone-treated cells.

3.2. *Dexamethasone regulates the phenotypic expression of adenylate cyclase activity according to the developmental stage of the cells*

The possibility has been investigated that the effect of dexamethasone could depend on its time of addition to the developmental cycle of the adipocyte. Short term exposure to dexamethasone (48 h) was performed at different stages of differentiation starting from day 0 (confluence) up to day 18 on terminally differentiated adipocytes.

Table 2

Effect of dexamethasone on the adenylate cyclase and glycerophosphate dehydrogenase activities of 3T3 cells grown in different culture conditions

Cell subclone	Culture conditions	Cell phenotype (day after confluence)	Addition[a]	Adenylate cyclase		Glycerophosphate dehydrogenase
				Basal	ACTH(1–24) 1 μM	
3T3-F442A	10% FCS[b]	preadipocyte (day 0)	none	3.5	3.0	2.0
		adipocyte (day 8)	none	5.8	6.0	1220
			dexamethasone	4.4	13.7	625
	DH/4F	preadipocyte (day 8)	none	1.3	1.4	2.6
			dexamethasone	0.9	0.8	3.0
3T3-C2	10% FCS	fibroblast (day 8)	none	2.7	2.1	0.5
			dexamethasone	3.7	3.2	0.5

[a] Dexamethasone added at confluence at a concentration of 0.25 μM
[b] Corticosteroid content of the serum was less than 5 ng/ml

Growth and differentiation of 3T3 cells were performed in DMEM supplemented with 10% fetal calf serum (FCS) and insulin (5 μg/ml) or serum-free medium (DH/4F) (see section 2). Adenylate cyclase and glycerophosphate dehydrogenase activities were determined at the indicated times and expressed in pmol cyclic AMP $\cdot$ min^{-1} $\cdot$ (mg protein)$^{-1}$ and nmol NADH oxidized $\cdot$ min^{-1} $\cdot$ (mg protein)$^{-1}$, respectively

After 2 days of exposure to the drug crude membranes were prepared; the kinetics of the appearance of ACTH(1–24) stimulated adenylate activity was biphasic (fig.2). The maximal efficiency of the dexamethasone effect was obtained by treatment of 3T3-F442A cells between day 2 and day 4 leading to a 4-fold stimulation of the adenylate cyclase activity in response to a maximal concentration of ACTH(1–24). Then there was a progressive relaxation of the dexamethasone effect; only a 60% increase of adenylate cyclase activity due to the hormone was observed when the drug was added to adipocytes cultures between day 16 and day 18 (fig.2). In the same interval of time control cultures of 3T3-F442A adipocytes, which were not exposed to dexamethasone, exhibited a very low ACTH(1–24)-stimulated adenylate cyclase activity (20–30% over basal) (fig.2).

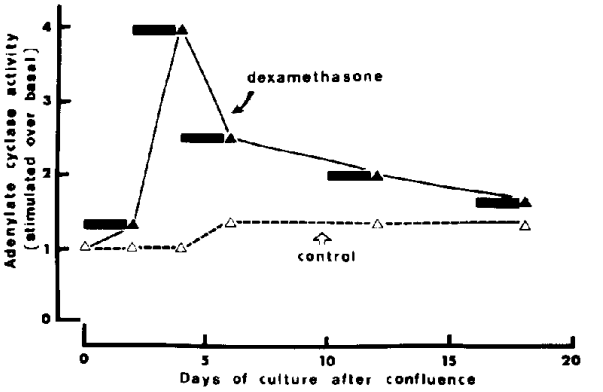

Fig.2. Variation of the ACTH(1–24) sensitivity of the adenylate cyclase system after short term exposure of F442A cells to dexamethasone at different periods of their differentiation. Starting from confluence, cultures of 3T3-F442A cells were sequentially exposed to dexamethasone (0.25 μM) for 2 days (▬) at different stages of the differentiation process. Then crude membranes were prepared and adenylate cyclase activity was assayed in the absence and presence of 10 μM ACTH(1–24) for both control (△—△) cells and dexamethasone-treated cells (▲—▲). Hormone receptor coupling is expressed by the ratio of stimulated activity over basal activity.

3.3. *Steroid specificity of the appearance of ACTH(1–24)-stimulated adenylate cyclase activity during adipose differentiation*

To examine the specificity of steroids for the acquisition of ACTH(1–24)-stimulated adenylate cyclase activity 3T3-F442A cells were differentiated in the presence of various steroids. Aldosterone and to a lesser extent progesterone were the only two non-glucocorticoids that mimicked a part of the biological response of adipocytes to dexamethasone; estradiol and testosterone were not effective at all (table 3).

3.4. *Dexamethasone promotes the increase of adrenocorticotropin receptors during adipogenic differentiation*

Binding experiments of ACTH(1–39) were conducted with specifically monoiodinated ACTH at tyrosine residue 23, leaving tyrosine 2 unmodified; this compound exhibited, like ACTH(1–24) [14], the full potency and spectrum of biological activity. Specific binding of ^{125}I-ACTH(1–39) to the 3T3-F442A fat cells was rapid at room temperature (22°C). Binding of tracer at the concentration of 0.2 nM reached saturation within 30 min and then plateaued (not shown). A displacement curve is represented in fig.3 (upper panel) giving a half-maximal concentration of inhibition of binding of

Table 3

Steroid specificity of the induction of ACTH(1–24) sensitivity of the adenylate cyclase system in 3T3-F442A cells

Steroid	Concentration (nM)	ACTH(1–24)-stimulated adenylate cyclase	
		Stimulated/basal	% of control
None	–	1.5	0
Dexamethasone	250	3.7	100
Aldosterone	10	1.4	0
	100	2.2	31
	1000	3.9	107
Progesterone	100	1.4	0
	1000	2.7	52.5
Testosterone	100	1.6	2
	1000	1.5	1
Estradiol	100	1.2	0
	1000	1.4	0

Cells were grown in DMEM supplemented with 10% of corticosteroid poor fetal calf serum (Flow Laboratories, batch 030066 containing less than 5 ng of corticosteroid per ml). Insulin, 5 μg/ml, was added at confluence and one day later medium was supplemented with the different steroids at the indicated concentration. 10 days later crude membranes were prepared and adenylate cyclase activity was determined as detailed in section 2 in the absence (basal) or presence of ACTH(1–24) at 1 μM

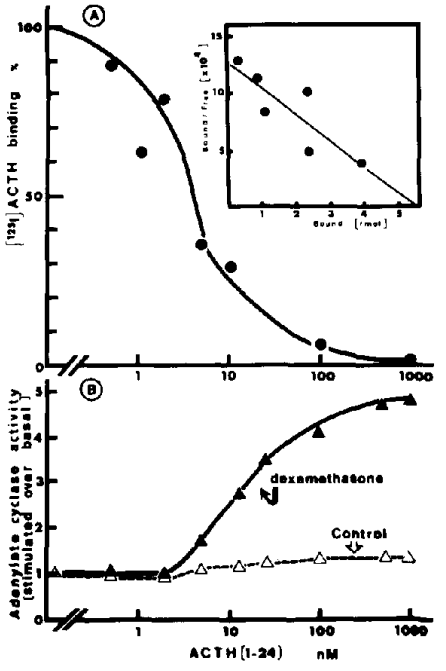

Fig.3. Correlation between ACTH binding and ACTH(1–24)-stimulated adenylate cyclase activity in dexamethasone-treated 3T3-F442A adipocytes. 3T3-F442A adipocytes were differentiated for 8 days after confluence in DMEM supplemented with 10% fetal calf serum, 5 μg/ml insulin and when specified with 0.25 μM dexamethasone. (A) Dexamethasone-treated cells (2.1×10^6 cells per 60 min dish) were incubated with ^{125}I-ACTH and various concentrations of ACTH(1–24) as described in section 2; Scatchard plot of the data was derived from a linear least squares analysis (inset). (B) Dose-response curve for ACTH(1–24)-stimulated adenylate cyclase activity of membranes from control (Δ—Δ) and dexamethasone-treated cells (▲—▲). Values for basal activities were 1.7 and 2.1 pmol cyclic AMP · min^{-1} · (mg protein)$^{-1}$ respectively in control and dexamethasone-treated cells.

4 nM. When plotted according to Scatchard (fig.3, inset) a single class of binding sites is evidenced representing 2.7 fmol ACTH bound per 10^6 cells (about 1600 receptors per cell). We could estimate a K_d value of 4.5 nM which should be compared to the value for half-maximal activation of the adenylate cyclase (K_a = 15 nM) (fig.3, lower panel). Attempts were made to characterize ^{125}I-ACTH binding sites in 3T3-F442A adipocytes differentiated in the absence of dexamethasone; no specific cellular binding was observed so that, if present, the number of ACTH receptors in these cells is probably very low (not shown), reflecting the poor sensitivity of the adenylate cyclase system of these cells (fig.1).

4. DISCUSSION

Until now, glucocorticoid manipulation by adrenalectomy of rats and/or dexamethasone administration had led to erratic and sometimes controversial results concerning the effect of these hormones on adipocyte corticotropin receptors and adipocyte responses [15–19]. Moreover, the differences observed between these studies raised questions as to whether glucocorticoid hormones had direct effects or whether some aspects of their physiological role were mediated by changes in counterregulatory hormones or metabolites.

In this respect, the use of cell models in cultures can circumvent this difficulty; thus, 3T3-F442A preadipocytes provide an attractive experimental system for studying the molecular basis of the 'permissive' effect of glucocorticoids in allowing the full expression of the ACTH-induced cellular response, namely the lipolytic one mediated via cyclic AMP production.

In the present study, we demonstrate that the stimulation of this dexamethasone modulated ACTH(1–24) sensitivity of the adenylate cyclase system requires both the cell's commitment to differentiation and the presence of dexamethasone; it does not take place in 3T3-F442A cells grown in serum free medium and dexamethasone treatment of undifferentiating 3T3-C2 cells is not efficient (table 1). Our findings demonstrate that the development of adipocyte response to corticotropin is not necessarily linked to terminal maturation of these cells since differentiated adipocytes do not exhibit any ACTH sensitivity as long as they are not exposed to dexamethasone. These results also demonstrate the independent control of the hormone-sensitive adenylate cyclase assembly and late lipogenic enzyme expression during adipose differentiation.

The guanine nucleotide-binding protein complex is a focal point in hormonal activation of the adenylate cyclase system (review [20]). GTPγS, a non-hydrolysable analog of GTP, elicits a constant activation of the enzyme all along the adipocyte development (fig.1). Thus, dexamethasone does not functionally modify the G regulatory complex of the adenylate cyclase. However we cannot totally exclude the fact that dexamethasone induces the biosynthesis of a specific, unknown macromolecular component that would selectively modify the sensitivity of adenylate cyclase to ACTH(1–24). For instance in the related 3T3-L1 preadipocyte cell line, previous studies have already indicated that changes in hormone receptors or in G_s could not account by themselves for increased adenylate cyclase response to catecholamines [6,21] so that changes in G_i and a novel G_o protein subunit have been suggested [22]. In the 3T3-F442A cell subclone, the situation seems to be less complex; the increased β-adrenergic responsiveness of the adenylate cyclase system of these cells can be solely explained by an increased number of β-adrenoreceptors linked to the adipose conversion [5].

Specific cellular binding of ^{125}I-ACTH is only observed in dexamethasone-differentiated adipocytes but remains undetectable in preadipocytes and control adipocytes. Even though our study does not deal with endocytosis and recycling of receptors which are quite possible in experiments performed at room temperature, the most obvious interpretation of our findings is that the induction of responsiveness to ACTH(1–24) in differentiating 3T3-F442A cells could be accounted for primarily by the appearance of cell receptors in response to dexamethasone. [In parallel we have demonstrated that dexamethasone prevents the increase of β-adrenergic receptor number in fully differentiated cells with insulin. Using the hydrophilic antagonist, tritiated CGP-12177, we have estimated that the cell surface density of β-receptors in dexamethasone-treated cells is 4–6-fold less than in control differentiated cells; thus a strict correlation has been found with the decrease of isoproterenol responsiveness of the adenylate cyclase

system of these cells (Fève, B. and Pairault, J., in preparation).] The number of ACTH sites reaches 1600 receptors per cell with a K_d of 4.5 nM in dexamethasone differentiated adipocytes (fig.3). The concentration of dexamethasone necessary to elicit ACTH responsiveness of 3T3-F442A adipocytes is much lower than that of aldosterone and progesterone while other steroids are inefficient (table 3). These results suggest that the regulation of ACTH receptors coupling could be mediated through glucocorticoid receptor; a 4-fold increase of glucocorticoid receptor has been recently evidenced during adipose conversion of 3T3-F442A cells [23].

As proposed by Rubin et al. [24], dexamethasone is commonly used in association with insulin and 3-isobutyl-1-methylxanthine to promote the differentiation of the related 3T3-L1 preadipocyte cell line. In these differentiating 3T3-L1 cells dexamethasone has been already shown to elicit a β_1 to β_2 switch of β-adrenergic receptor subtypes [25] and to mimic some aspects of insulin resistance including both receptor and postreceptor defects [26]. From our studies, it is probable that the appearance of ACTH receptors, evidenced in 3T3-L1 adipocytes by Grunfeld et al. [27], is linked to the presence of dexamethasone in the differentiation protocol used by these authors. Thus, in 3T3 preadipocytes, dexamethasone may have pleiotypic effects which lead to cell phenotypes exhibiting various hormonal responsiveness.

Otherwise glucocorticoid hormones have been shown to have physiological actions on multiple cellular processes; thus, the indirect, so-called 'permissive' actions may involve the modulation of the affinity and/or receptor concentrations of a variety of heterologous hormone and growth factor receptors [28–40]. So, given the data presented in this paper, ACTH could regulate its own receptor in adipocytes early during adipose tissue formation via glucocorticoid secretion from the adrenal gland; thus a dual effector mechanism of ACTH action should be retained to render an account of the biological response of the adipose cell to this hormone.

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