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11β-hydroxysteroid Dehydrogenase 1 in Adipocytes: Expression is Differentiation-dependent and Hormonally Regulated

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11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1) catalyses the reversible metabolism of physiological glucocorticoids (cortisol, corticosterone) to inactive metabolites (cortisone, 11-dehydrocorticosterone), thus regulating glucocorticoid access to receptors. 11β-HSD-1 expression is regulated during development and by hormones in a tissue specific manner. The enzyme is highly expressed in liver, where it may influence glucocorticoid action on fuel metabolism, processes also important in adipose tissue. Here we show that 11β-HSD-1 is expressed in white adipose tissue, in both the adipocyte and stromal/vascular compartments, and in the adipocyte cell lines 3T3-F442A and 3T3-L1. In these cells, 11β-HSD-1 expression is induced upon differentiation into adipocytes and is characteristic of a 'late differentiation' gene, with maximal expression 6–8 days after confluence is reached. In intact 3T3-F442A adipocytes the enzyme direction is predominantly 11β-reduction, activating inert glucocorticoids. The expression of 11β-HSD-1 mRNA is altered in fully differentiated 3T3-F442A adipocytes treated with insulin, dexamethasone or a combination of the hormones, in an identical manner to glycerol-3-phosphate dehydrogenase (GPDH) mRNA (encoding a key enzyme in triglyceride synthesis and a well-characterised marker of adipocyte differentiation). The demonstration of 11β-HSD-1 expression in adipocytes and its predominant reductase activity in intact 3T3-F442A adipocytes suggests that 11β-HSD-1 may play an important role in potentiating glucocorticoid action in these cells. 3T3-F442A and 3T3-L1 represent useful model systems in which to examine the factors which regulate 11β-HSD-1 gene expression and the role of 11β-HSD-1 in modulating glucocorticoid action in adipose tissue.

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INTRODUCTION

11β-hydroxysteroid dehydrogenase (11β-HSD) enzymes interconvert active glucocorticoids (cortisol, corticosterone) and their inert 11-keto metabolites (cortisone and 11-dehydrocorticosterone, respectively), thereby playing a key role in regulating glucocorticoid access to both types of intracellular receptor, glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) (reviewed in Refs. [1, 2]). Two isozymes of 11β-HSD have been identified, 11β-HSD-1 and 11β-HSD-2, which differ in their biological properties and tissue distributions. 11β-HSD-2 is a high affinity NAD^+-dependent enzyme which appears to function exclusively as a dehydrogenase (inactivating glucocorticoids). 11β-HSD-2 is expressed predominantly in aldosterone-target tissues, where it plays a well-characterised role in conferring mineralocorticoid specificity upon otherwise non-selective MR [3, 4], and in placenta, where it protects the developing foetus from the effects of maternal glucocorticoids [5]. By contrast, 11β-HSD-1 is a lower affinity NADP(H)-dependent enzyme, widely

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expressed, with particularly high levels in liver [6], where its expression is hormonally regulated [7–11]. Although the enzyme reaction catalysed by 11β-HSD-1 *in vitro* is bidirectional, recent evidence suggests that in most intact cell types, reductase activity predominates, reactivating inert glucocorticoids [7, 12–15]. This activity is likely to be of particular importance in tissues such as liver and adipose tissue and brain, where it may maintain high intracellular levels of glucocorticoids. Glucocorticoids play a central role in the control of energy metabolism, in particular in maintaining glucose homeostasis. Attenuation of hepatic 11β-HSD-1 in rats is associated with decreased expression of the gene encoding phosphoenolpyruvate carboxykinase (PEPCK), the rate limiting enzyme in gluconeogenesis [16]. Furthermore, mice homozygous for a targeted disruption of the 11β-HSD-1 gene show reduced fasting plasma glucose levels compared to their wild-type littermates [17], consistent with a decreased level of gluconeogenesis and reflecting the important role of glucocorticoids in regulating hepatic glucose metabolism.

Glucocorticoids play a key role both in the regulation of adipose tissue metabolism and in the differentiation of pre-adipocytes into adipocytes [18–20]. Glucocorticoid excess in Cushing's syndrome results in the redistribution of fat depots, with increased abdominal and reduced peripheral adipose deposits [21] and is associated with enhanced activity of lipoprotein lipase (LPL) in abdominal fat [21]. The role of glucocorticoids in the induction of adipocyte differentiation has largely been elucidated in cell lines capable of undergoing adipocyte conversion *in vitro*, which include the mouse 3T3-L1 and 3T3-F442A cell lines. These cells faithfully reproduce preadipocyte differentiation *in vitro* when cultured in the presence of inducing agents, including dexamethasone, a synthetic glucocorticoid [22]. The differentiation of these cells is accompanied by the sequential activation of adipose-specific genes and acquisition of differential sensitivity to hormones [23]. In fully differentiated 3T3 adipocytes, glucocorticoids also modulate the expression of many genes involved in adipocyte energy metabolism, including those encoding glycerol-3-phosphate dehydrogenase (GPDH) [24], PEPCK [25], β-adrenergic receptors [26, 27] and insulin receptor substrate-1 [28, 29]. However, the enzymatic modulation of glucocorticoid levels in these cells has not been investigated. In mammary adipose tissue, cortisol and corticosterone are oxidized to cortisone and 11-dehydrocorticosterone indicating the presence of 11β-HSD, at least at this site [30]. Furthermore, 11β-HSD-1, without 11β-HSD-2, expression has recently been reported in human adipose stromal cells [31], but the presence or absence of 11β-HSD in adipocytes was not addressed. Clearly 11β-HSD in adipose tissue might modify glucocorticoid action in a site- and developmentally-specific manner.

We have therefore (i) examined the expression of 11β-HSD enzyme activity and 11β-HSD-1 mRNA in isolated primary rat adipocytes, (ii) examined whether 11β-HSD-1 is expressed in the clonal cell lines, 3T3-F442A and 3T3-L1 and (iii) investigated whether hormonal manipulation alters 11β-HSD-1 expression in these cells.

MATERIALS AND METHODS

Animals

Male rats (Wistar 200–250 g) were maintained under conditions of controlled lighting (lights on 07.00 to 19.00 h) and temperature, with water and food *ad libitum*. After cervical dislocation, epididymal adipose tissue was excised and adipocytes isolated as previously described [32]. Briefly, epididymal adipose tissue was washed several times in PBS, trimmed of large blood vessels and minced. Tissue was incubated in Krebs–Ringer buffer (KRB), containing collagenase II (2 mg/ml) (Sigma, U.K.) at 37°C for 40 min. The digested material was passed through a 250 μm nylon filter and briefly centrifuged. The separated adipocyte (floating) and stromal vascular (pellet) fractions were collected for RNA extraction and 11β-HSD bioassay.

Cell culture

3T3-F442A, 3T3-L1 and 3T3-C2 cells (kindly provided by Dr Pairault, Henri Modor Hospital, Creteil, France and Dr Spiegelman, Dana Faber Cancer Institute, Boston, MA) were maintained in 'basal medium' consisting of Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% newborn calf serum, 200 IU/ml penicillin and 50 μg/ml streptomycin. To differentiate 3T3-F442A cells, 'basal medium' was removed from confluent cells and replaced with 'differentiation medium' (DMEM supplemented with 10% fetal calf serum, 200 IU/ml penicillin, 50 μg/ml streptomycin and 5 μg/ml insulin); confluent cells were maintained in this for 11 days with the medium changed every 48 h. Addition of 'differentiation medium' to confluent 3T3-F442A cells induced over 90% of the cells to differentiate from fibroblasts into fully-mature adipocytes, as determined by visible lipid accumulation (data not shown). Confluent 3T3-L1 cells were differentiated by replacing 'basal medium' with 'differentiation medium' additionally supplemented with 0.5 μM dexamethasone and 0.25 μM methylxanthine. To establish basal conditions for all cells before hormonal manipulations, both undifferentiated and fully differentiated 3T3-F442A cells (day 10 post confluence) were transferred to fresh 'basal medium' for at least 24 h prior to hormone treatment. Hormones

were then added to 'basal medium' for a further period of 48 h before harvesting cells for RNA isolation.

Assay of 11β-HSD activity

11β-HSD activity was determined, both in intact cells and in homogenates, by measuring the rate of conversion of [^{3}H]-corticosterone to [^{3}H]-11-dehydrocorticosterone, as previously described [10, 13]. For homogenates, cells were washed with PBS (pH 7.4), harvested and homogenised in Krebs–Ringer buffer (118 mM NaCl, 3.8 mM KCl, 1.19 mM KH_2PO_4, 2.54 mM $CaCl_2$, 1.19 mM $MgSO_4$, 25 mM $NaHCO_3$, 25 mM Hepes, 5 mM glucose, pH 7.4). Protein concentrations in homogenates were measured using the Bio-Rad protein assay solution (Bio-Rad, Hemel Hempstead, Herts, U.K.). 11β-HSD activity in homogenates was assayed by incubating 125 μg protein with 200 μM cofactor (NADP or NAD; Sigma, U.K.) and 12 nM [1, 2, 6, 7] [^{3}H]-corticosterone (specific activity 88 Ci/mmol, Amersham International, Herts, U.K.) in a total volume of 250 μl Krebs–Ringer buffer for 15 min at 37°C. Preliminary experiments established that these conditions gave steroid conversion within the linear portion of the relationship between enzyme activity and enzyme concentration. For intact cells, medium was removed and replaced with 'basal medium' containing 25 nM [^{3}H]-corticosterone (7 Ci/mmol) or 25 nM 11-dehydrocorticosterone (7 Ci/mmol), prepared as previously described [13]. 3, 8 and 24 h after the addition of steroids, 500 μl of medium was sampled from each culture dish. Steroids were extracted with either 5 vol. ethyl acetate (for homogenates) or 2 vol. ethyl acetate (for intact cells), the organic layer separated and evaporated to dryness and steroids resuspended in the mobile phase (60% H_2O:40% methanol). Mobile phase was injected into a Berthold HPLC system and eluted tritiated steroids detected by a scintillation counter. The percent conversion of tritiated steroids was calculated as an index of enzyme activity.

RNA analysis

Total RNA was extracted either from tissue or from cultured cells by the guanidinium thiocyanate method, as previously described [10]. Equal aliquot of total RNA was electrophoresed through a 1.2% agarose-formaldehyde gel and blotted overnight onto a nylon membrane (Hybond N, Amersham International). Pre-hybridization was carried out for 2 h at 42°C in either 50% formamide, 5× Denhardt's solution, 5× SSPE, 0.1% SDS, 100 μg/ml sonicated single-stranded DNA or 0.2 M NaH_2PO_4, 5 mM EDTA, 6% SDS, 100 μg/ml sonicated single-stranded DNA. Hybridization was performed at 42°C overnight in the same buffer with the addition of cDNA probes labelled with [α^{32}P]-dCTP (3000 Ci/mmol, Amersham International) to a specific activity of 10^9 cpm/μg DNA using a random prime kit (Boehringer Mannheim, U.K.). cDNA probes used encode rat 11β-HSD-1 [6], mouse glycerol-3-phosphate dehydrogenase (GPDH) [33], mouse adipocyte P2 (aP2) [34] or mouse 7S RNA [35] (to control for RNA loading). Membranes were washed to a final stringency of 0.1× SSPE, 0.1% SDS at 65°C and exposed to Kodak XAR film at −70°C.

RESULTS

11β-HSD-1 is expressed in adipose tissue and isolated adipocytes

11β-HSD-1 mRNA is expressed in rat white (epididymal) adipose tissue, at similar levels in both adipocytes and the stromal vascular fraction (Fig. 1). In each case a single 11β-HSD-1 mRNA of approxi-

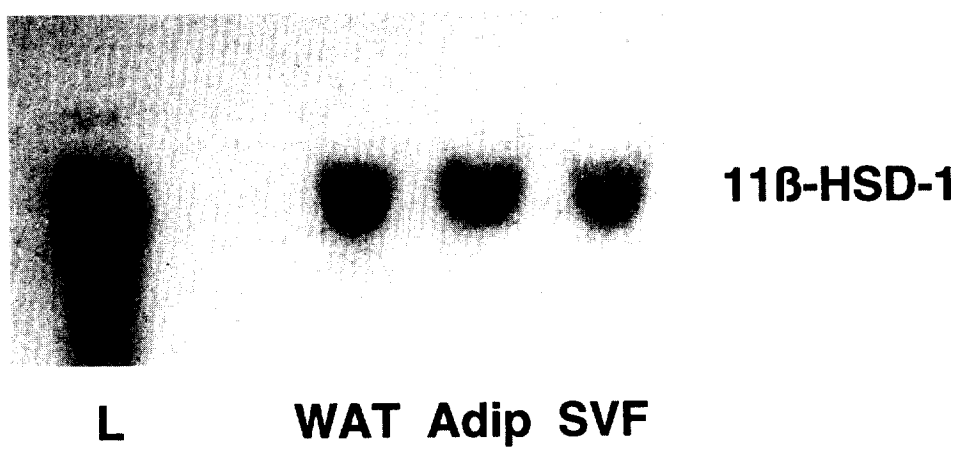

Fig. 1. 11β-HSD-1 gene expression in rat epididymal adipose tissue, isolated adipocytes and stromal/vascular fraction. Autoradiograph of a representative Northern blot hybridized with [^{32}P]11β-HSD-1 cDNA showing the presence of 11β-HSD-1 mRNA in total RNA from rat liver (L) (10 μg), white adipose tissue (WAT) (30 μg), isolated adipocytes (adip) (30 μg) and stromal/vascular fraction (SVF) (30 μg). Equivalence of loading was confirmed by ethidium bromide staining of RNA.

mately 1.6 kb (of similar size to hepatic 11β-HSD-1 mRNA) was detected, albeit at lower levels than in liver. The presence of 11β-HSD activity in homogenates of isolated rat adipocytes confirmed that this mRNA is translated into functional protein (data not shown).

11β-HSD-1 is a reductase in 3T3-F442A cells and is induced by adipocyte differentiation

The 3T3 cell lines represent useful models of adipocyte differentiation and function [36, 37] and respond to glucocorticoids in a similar manner to adipose tissue *in vivo* [25, 38]. We therefore examined 11β-HSD-1 expression in these cells. The differentiation of 3T3-F442A cells into adipocytes led to a dramatic increase in the level of 11β-HSD activity in cell homogenates, when assayed in either the dehydrogenase [Fig. 2(A)] or reductase directions [Fig. 2(B)]. Maximal 11β-HSD activity was obtained in the presence of added co-factor, with NADP eliciting a larger increase in dehydrogenase activity than NAD, as previously demonstrated for 11β-HSD-1 in liver [39]. In contrast, intact 3T3-F442A cells, whether undifferentiated or differentiated, showed no 11β-HSD activity in the dehydrogenase direction over a period of 24 h [Fig. 2(C)], whereas differentiated 3T3-F442A cells could readily convert 11-dehydrocorticosterone to corticosterone, with 100% conversion of 11-dehydrocorticosterone into corticosterone within 8 h [Fig. 2(C)]. These results clearly demonstrate that in intact 3T3-F442A adipocytes, 11β-HSD-1 encodes an enzyme which is predominantly a reductase. 11β-reductase activity was also clearly detectable in undifferentiated cells, but was present at much lower levels than in differentiated cells [Fig. 2(C)].

We next examined the time course of expression of 11β-HSD-1 mRNA during 3T3-F442A cell differentiation and found that 11β-HSD-1 mRNA has the characteristics of a late differentiation gene, since 11β-HSD-1 transcripts became detectable by Northern analysis only 6–8 days after differentiation was induced (Fig. 3). Moreover, the time course of appearance of 11β-HSD-1 mRNA paralleled that of GPDH mRNA, a late marker of adipocyte differentiation (Fig. 3). Although 11β-HSD-1 mRNA was not detectable in undifferentiated 3T3-F442A cells by Northern analysis, it was nonetheless readily detectable by RT-PCR (data not shown), consistent with a low level of expression of 11β-HSD-1 mRNA and enzyme activity in undifferentiated 3T3-F442A cells.

11β-HSD-1 is expressed in 3T3-L1 adipocytes

To assess whether 11β-HSD-1 gene expression was unique to 3T3-F442A adipocytes or was a general property of differentiated adipocyte cell lines and also to determine which hormonal milieu would effect the upregulation of the gene in adipocyte differentiation, a different subclone of Swiss 3T3 mouse fibroblasts, 3T3-L1 cells, was tested, using different conditions for differentiation (see Fig. 4 legend for details). As shown in Fig. 4, only 'differentiation medium' containing insulin (5 μg/ml) throughout the post-confluent period was able to fully differentiate 3T3-L1 cells and induce 11β-HSD-1 gene expression (at day 7 post-confluence, when these cells show ≥90% adipocyte phenotype). Insulin given only on the day of confluence was much less effective in inducing adipocyte differentiation and expression of 11β-HSD-1 mRNA (Fig. 4). As with 3T3-F442A cells, 11β-HSD-1 mRNA expression in 3T3-L1 cells paralleled the expression of the late adipocyte differentiation markers, GPDH and aP2.

Hormonal regulation of 11β-HSD-1 MRNA in 3T3-F442A adipocytes

3T3-adipocyte cell lines have been widely used to study the hormonal control of adipocyte differentiation and function, since these cells are responsive to a number of metabolically-important hormones and factors [22, 23]. To test whether 11β-HSD-1 gene expression is hormonally regulated, fully differentiated 3T3-F442A adipocytes were exposed, in basal medium, to insulin (5 μg/ml) and/or dexamethasone (10^{-7} M) for 48 h. As shown in Fig. 5, insulin treatment reduced the ratio of 11β-HSD-1 mRNA:7S RNA by approximately 50% (determined by quantitative densitometry of autoradiographs). Similarly, dexamethasone treatment resulted in an approximate 80% decrease in the ratio of 11β-HSD-1 mRNA:7S RNA [Fig. 5(A)]. However, when added together, the action of insulin predominated over the effect of dexamethasone, and the level of 11β-HSD-1 mRNA expression was similar to that in the presence of insulin alone [Fig. 5(A)]. In all cases, hormonal treatment elicited a similar effect on mRNA encoding GPDH [Fig. 5(A)].

As 5 μg/ml represents a supraphysiological dose of insulin, which potentially may activate IGF-1 receptors, in a further experiment fully differentiated 3T3-F442A cells were exposed to physiological levels of insulin (20 ng/ml) or to IGF-1 (20 ng/ml), added to basal medium for 48 h. As shown in Fig. 5(B), the lower dose of insulin had little or no effect on 11β-HSD-1 gene expression, whereas treatment of 3T3-F442A adipocytes with IGF-1 or high levels of insulin decreased expression of 11β-HSD-1 mRNA to a similar extent [Fig. 5(B)], suggesting the reduction in 11β-HSD-1 expression seen with the supraphysiological dose of insulin is mediated via IGF-1 receptors.

DISCUSSION

Glucocorticoids potently influence carbohydrate, lipid and protein metabolism, playing a central role in the regulation of gluconeogenesis in the liver and

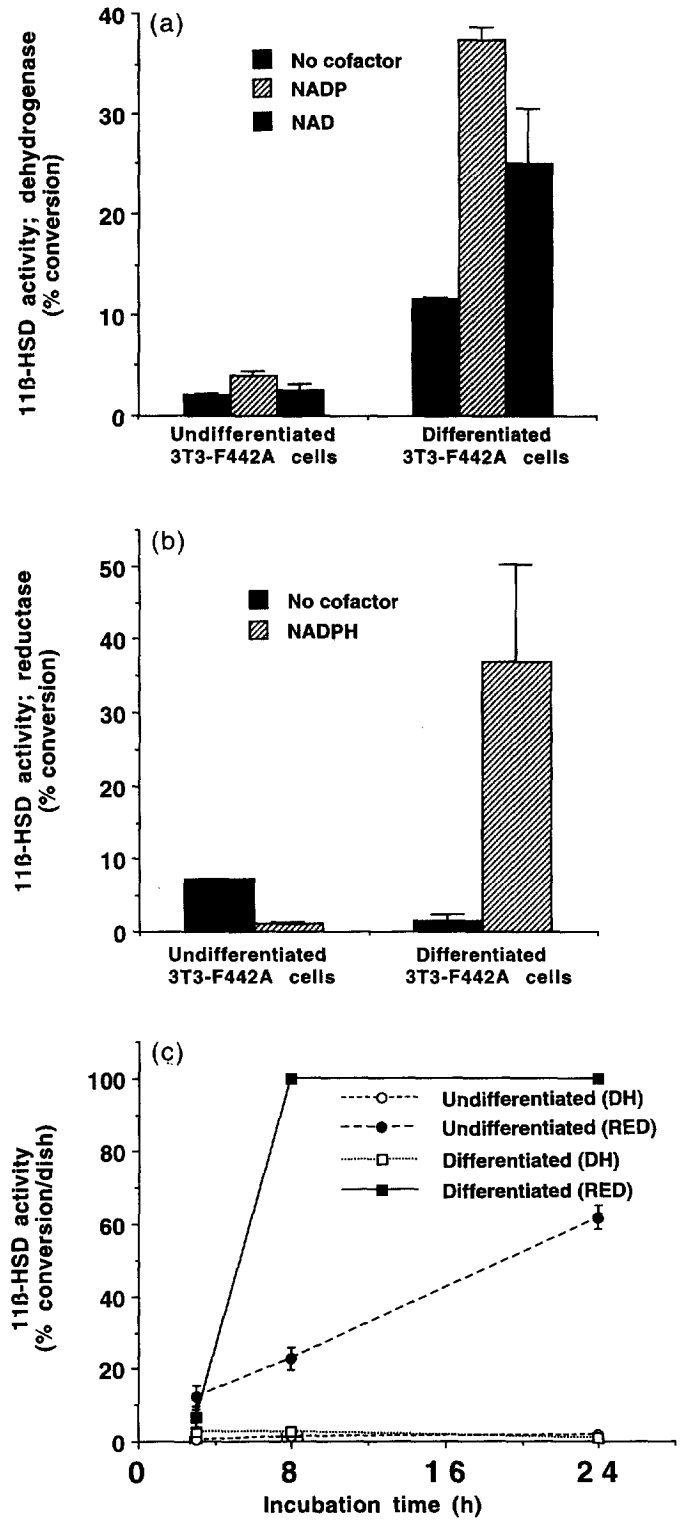

Fig. 2. 11β-HSD enzyme activity is induced upon differentiation of 3T3-F442A cells. (A and B) Homogenates of differentiated 3T3-F442A cells contain 11β-HSD enzyme activity. (A) 11β-HSD activity in the dehydrogenase direction and (B) 11β-HSD activity in the reductase direction was measured in homogenates made from undifferentiated 3T3-F442A cells (day 0 post-confluence) and differentiated 3T3-F442A cells (day 10 post-confluence) in the absence of added cofactor, or in the presence of NADP(H) or NAD, as indicated. Activity is expressed as % conversion of [^{3}H]-corticosterone to [^{3}H]11-dehydrocorticosterone (A) or % conversion of [^{3}H]11-dehydrocorticosterone to [^{3}H]-corticosterone (B). Values are means of duplicate samples ± SEM from 2 independent experiments. (C) 11β-HSD-1 in intact 3T3-F442A cells encodes an enzyme with reductase activity. 11β-reductase activity (expressed as % conversion [^{3}H]11-dehydrocorticosterone to corticosterone) and 11β-dehydrogenase activity (expressed as % conversion [^{3}H]-corticosterone to 11-dehydrocorticosterone) were measured in the medium overlying undifferentiated 3T3-F442A cells (day 0 post-confluence) or differentiated 3T3-F442A cells (day 10 post-confluence) at 3, 8 and 24 h after addition of steroid. Values are means obtained from samples taken from triplicate or quadruplicate dishes ± SEM.

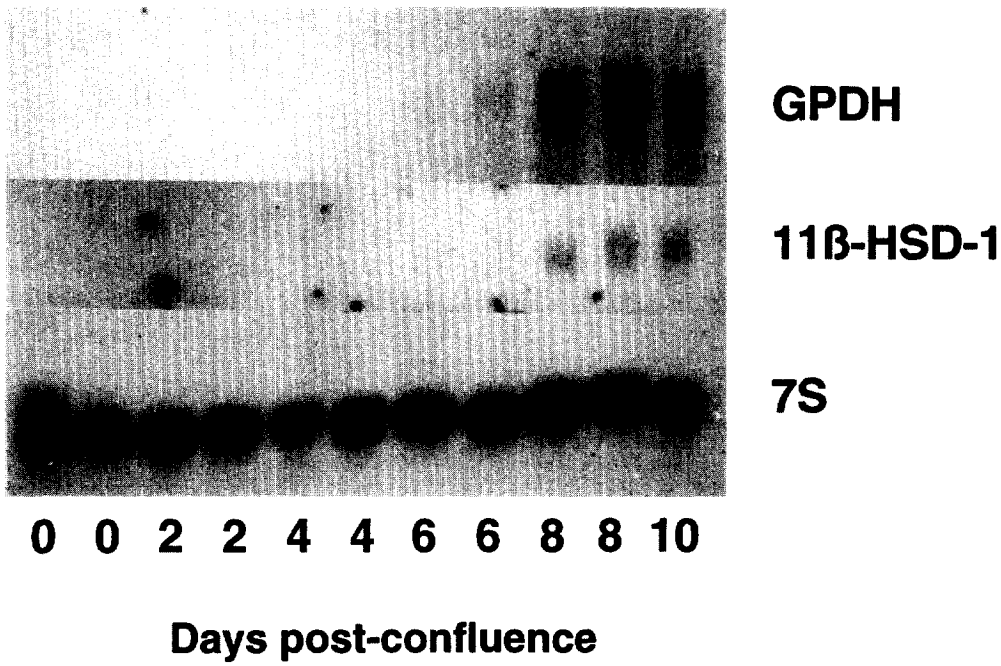

Fig. 3. Time course of 11β-HSD-1 gene expression during differentiation of 3T3-F442A cells. Autoradiograph of a representative Northern blot of 30 μg total RNA isolated from confluent 3T3-F442A cells (0 days post-confluence) and from post-confluent cells (2, 4, 6, 8 and 10 days post-confluence), hybridized to [^{32}P]-labelled cDNAs encoding rat 11β-HSD-1, mouse GPDH and 7S RNA. Replicates represent RNA isolated from cells from independent culture dishes. The experiment is representative of 3 independent experiments.

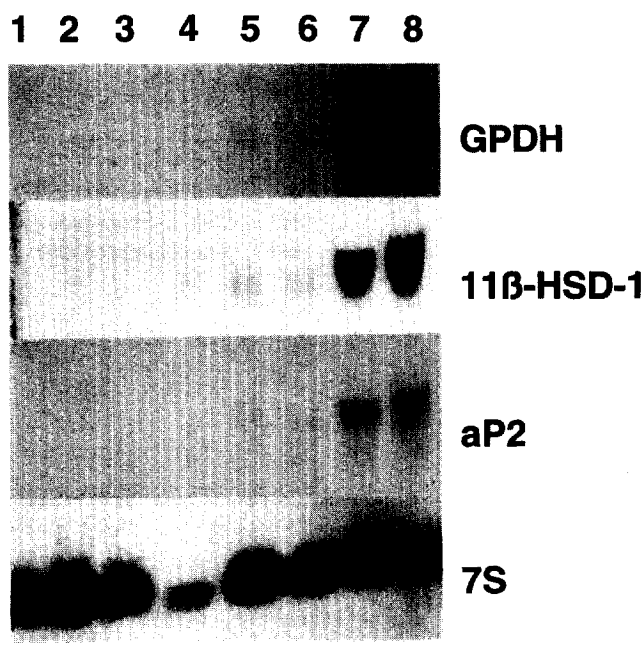

Fig. 4. 11β-HSD1 is expressed in differentiated 3T3-L1 cells. Autoradiograph of a representative Northern blot of 25 μg total RNA extracted from 3T3-L1 cells on the day of confluence (lanes 1 and 2) and 7 days post-confluence (lanes 3–8), hybridized to [^{32}P]-labelled cDNAs encoding rat 11β-HSD-1, mouse GPDH, mouse aP2 and 7S RNA. Confluent cells were treated for the 7 day post-confluent period with complete 'differentiation medium' (lanes 7 and 8) (containing insulin, dexamethasone and methylxanthine), 'differentiation medium' from which the insulin had been omitted (lanes 3 and 4), or complete 'differentiation medium' (containing insulin, dexamethasone and methylxanthine) on the first day of confluence only (the insulin was omitted for the remainder of the incubation period) (lanes 5 and 6). Each lane represents pooled RNA from 2 replicate dishes.

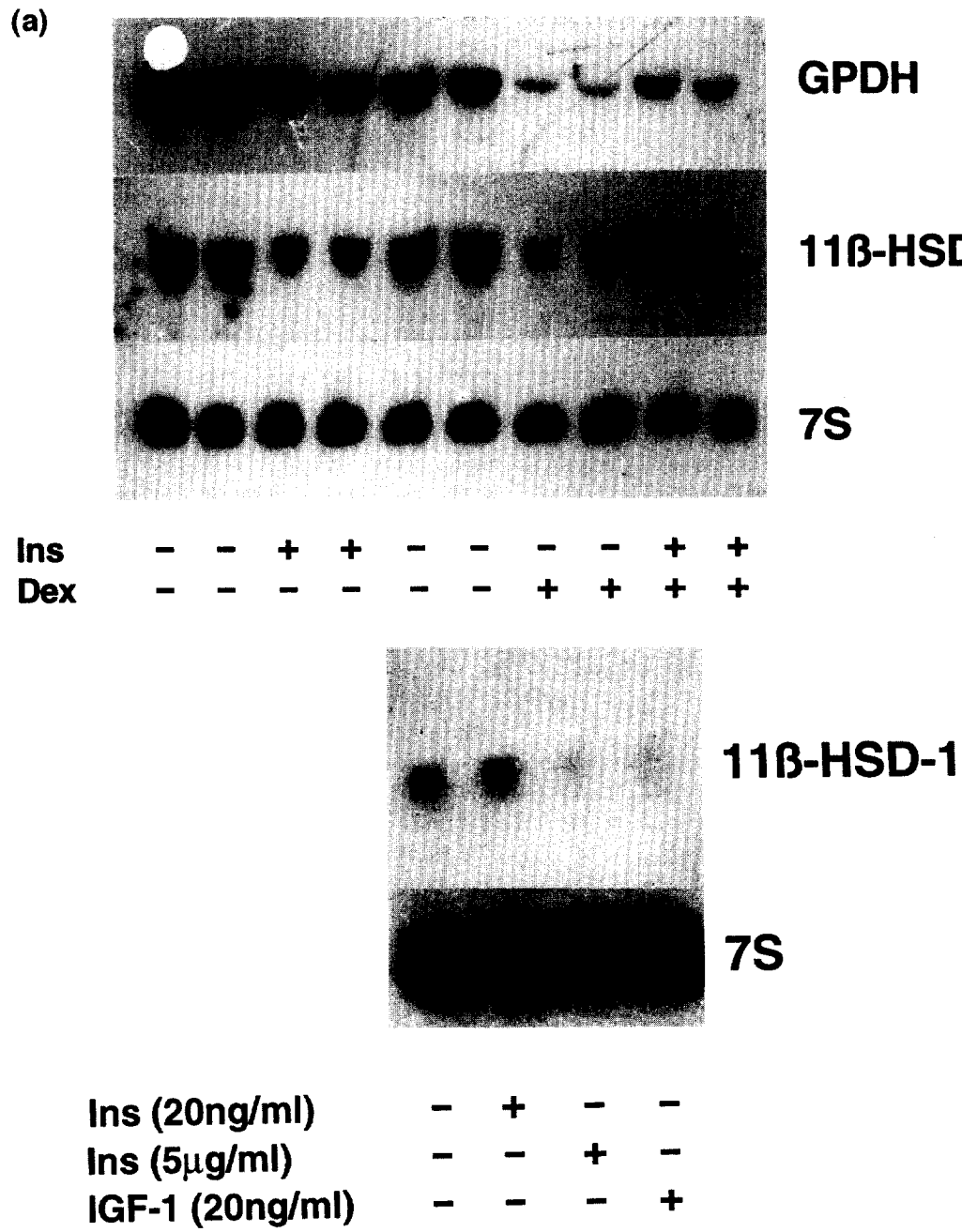

Fig. 5. Effect of hormonal treatments on 11β-HSD-1 gene expression in 3T3-F442A cells. (A) Autoradiograph of a representative Northern blot of 30 μg total RNA isolated from untreated and from hormone-treated differentiated 3T3-F442A cells, hybridized to [^{32}P]-labelled cDNAs encoding rat 11β-HSD-1, mouse GPDH and 7S RNA. Fully differentiated 3T3-F442A cells (day 10 post confluence) were transferred to 'basal medium' for 24 h prior to hormone treatment. Cells were then incubated in 'basal medium' alone (lanes 1, 2, 5 and 6) or 'basal medium' containing insulin (5 μg/ml) (lanes 3 and 4), dexamethasone (10^{-7} M) (lanes 7 and 8) or a combination of both (lanes 9 and 10), for a further period of 48 h before harvesting cells for RNA isolation. Replicates represent RNA isolated from cells from independent culture dishes. The autoradiograph is representative of 2 independent experiments. (B) Autoradiograph of a representative Northern blot of 30 μg total RNA isolated from untreated and from hormone-treated differentiated 3T3-F442A cells, hybridized to [^{32}P]-labelled cDNAs encoding rat 11β-HSD-1 and 7S RNA. Cell conditions were as for (A) except that the hormonal treatments used were 20 ng/ml insulin (lane 2), 5 μg/ml insulin (lane 3) or 20 ng/ml IGF-1 (lane 4). Lane 1 contains RNA from untreated cells. The autoradiograph is representative of 2 similar experiments.

lipolysis in adipose tissue. Although the actions of glucocorticoids upon adipose tissue have been extensively studied, few studies have addressed adipocyte glucocorticoid metabolism, although this crucially determines steroid access to nuclear receptors in other tissues [2–4]. In this study we have demonstrated 11β-HSD-1 gene expression in rat adipose tissue and isolated rat adipocytes, as well as the stromal/

vascular preadipocyte fraction, extending previous data demonstrating 11β-HSD activity in the adipose component of the rat mammary gland [30] and in the stromal cells of omental fat in humans [31]. To investigate further the tissue-specific nature of 11β-HSD-1 in adipose tissue we examined enzyme expression in 3T3-F442A and 3T3-L1 cells, which represent well-defined clonal cell models of adipocyte development and function. We have demonstrated that 11β-HSD-1 mRNA is expressed in a differentiation-dependent manner in 3T3-F442A cells, with significant expression only in fully differentiated adipocytes. The presence of low but detectable reductase activity in intact undifferentiated 3T3-F442A cells is consistent with the presence of low levels of 11β-HSD-1 mRNA, detectable by RT-PCR (Lyons *et al.*, unpublished data) but not by Northern analysis and may reflect either the presence of a small number of differentiated adipocytes in the population of confluent undifferentiated 3T3-F442A cells, or may be due to low levels of 11β-HSD-1 expression in all undifferentiated cells. Support for the latter contention comes from the finding that intact 3T3-C2 cells, a clone of mouse 3T3 fibroblasts which undergo adipose conversion at a low frequency [37], showed 11β-reductase, but not 11β-dehydrogenase activity and contained low levels of 11β-HSD-1 mRNA, detectable only by RT-PCR (A. Napolitano *et al.*, unpublished data).

Homogenates of fully-differentiated 3T3-F442A cells possessed both 11β-dehydrogenase and 11β-reductase activities. However, intact differentiated 3T3-F442A cells showed only 11β-reductase activity, with no dehydrogenase activity, suggesting that the predominant 11β-reductase activity in 3T3-F442A adipocytes may reactivate inert glucocorticoids, potentiating glucocorticoid action. These results are in agreement with findings in intact hepatocytes and 2S FAZA cells (a liver-derived cell line) [7, 15]. By contrast, intact human adipose stromal cells cultured in the presence of insulin and cortisol showed bidirectional 11β-HSD-1 activity (although reductase activity predominated in cells derived from omental fat) [31], suggesting that cell-specific factors play a key role in the determination of enzyme direction, even within different cell types from a single tissue.

In fully-differentiated 3T3-F442A adipocytes, 11β-HSD-1 gene expression is regulated by insulin/IGF-1 and glucocorticoids. Dexamethasone decreased 11β-HSD-1 expression. This contrasts with reports that dexamethasone increases 11β-HSD-1 mRNA expression in rat liver and hippocampus *in vivo* [40] and in rat hepatocytes, 2S FAZA cells and human skin fibroblasts *in vitro* [7, 12, 15]. However, a similar situation has been reported for PEPCK, where dexamethasone increases expression in liver, but decreases expression of PEPCK in adipose tissue *in vivo* and 3T3-L1 adipocytes *in vitro* [25]. Clearly the glucocorticoid control of 11β-HSD-1 expression is highly tissue-specific and may also exhibit temporal specificity [41]. Insulin at supraphysiological (μM) concentrations reduced the expression of 11β-HSD-1 mRNA in 3T3-F442A cells; this is likely to reflect activation of the type 1 insulin-like growth factor (IGF) receptor, as IGF-1 treatment reduced 11β-HSD-1 mRNA to a similar extent. In contrast, more physiological doses of insulin (20 ng/ml, ~3.5 nM), which would not be expected to activate the IGF-1 receptor, had little effect on the expression of 11β-HSD-1 mRNA and even slightly increased 11β-HSD-1 expression in some experiments (A. Napolitano, unpublished data). The regulation of 11β-HSD-1 by insulin also appears to be cell type specific. Thus, in primary hepatocytes, insulin reduced 11β-HSD1 mRNA levels, but had no effect on enzyme activity [7]. In 2S FAZA cells, physiological concentrations of insulin or IGF-1 reduced 11β-HSD-1 activity, to a similar extent, but only insulin significantly reduced 11β-HSD-1 mRNA in these cells [15]. In fibroblasts insulin also inhibited 11β-HSD-1 activity in a dose-dependent manner [12]. These discrepancies are likely to reflect in part, cell and tissue-specific differences in insulin regulation of 11β-HSD-1, but may also reflect differences in experimental conditions (cell culture conditions, length of hormone treatment for example). However, the hormonal regulation of 11β-HSD-1 mRNA in 3T3-F442A cells paralleled that of GPDH mRNA, encoding a key enzyme in triglyceride synthesis, and a well-characterised marker of adipocyte differentiation for which glucocorticoid and insulin regulation is well established [24]. The closely related regulation of 11β-HSD-1 and GPDH suggest that 11β-HSD-1 plays a similar central role in adipocyte function. Clearly, further investigation is necessary to establish the mechanisms by which glucocorticoids, insulin and IGF-1 regulate 11β-HSD-1 mRNA in 3T3-F442A adipocytes. Nevertheless, these data suggest that 3T3-F442A cells represent a novel and useful cell culture model for studying the hormonal and developmental regulation of 11β-HSD-1 gene expression.

3T3-F442A and 3T3-L1 cells have proved to be of crucial importance in the investigation of the factors which regulate metabolically important genes in adipocytes. Specifically a number of *trans*-acting factors have been identified that regulate the co-ordinate transcription of adipose-specific genes during adipocyte differentiation [22, 23]. Notable among these factors is C/EBPα, expressed at high levels in liver and adipose tissue [42] which plays an important role in the commitment of preadipocytes and the regulation of genes central to the adipocyte differentiation programme [22, 23]. The genes encoding members of the C/EBP transcription factor family are differentially regulated by glucocorticoids in adipose tissue *in vivo* [38] and by glucocorticoids and insulin in 3T3

adipocytes *in vitro* [38, 43]. Interestingly, co-transfection of C/EBPα into the human hepatoma cell line, HepG2, increases 11β-HSD-1 promoter activity and bacterially expressed C/EBPα binds to a number of sites within the 11β-HSD-1 promoter [44]. Electrophorectic mobility shift assays using nuclear extracts from undifferentiated and differentiated 3T3-F442A cells suggest that C/EBPα may play a similar role in regulating 11β-HSD-1 expression in adipocytes [45], raising the possibility that the regulation of 11β-HSD-1 by insulin and glucocorticoids may be indirectly mediated through changes in the complement of C/EBP-related proteins in the adipocyte.

The biological importance of 11β-HSD-1 mRNA expression and enzyme activity in differentiated 3T3-F442A and 3T3-L1 adipocytes is undetermined, but points to a role in regulating glucocorticoid access to glucocorticoid and/or mineralocorticoid receptors and hence, perhaps, the control of energy balance in fat cells. The involvement of mineralocorticoid receptors in adipocyte differentiation was suggested by the unexpected finding that aldosterone can elicit terminal differentiation of 3T3-L1 cells [46]. However, we have been unable to detect NAD-dependent 11β-HSD-2 activity (which confers aldosterone-specificity on mineralocorticoid receptors in the distal nephron) in these cells, in agreement with the findings of Bujalska *et al.* who reported the absence of 11β-HSD-2 mRNA in human adipocytes [31]. Furthermore the predominant reductase activity of 11β-HSD-1 in intact 3T3 cells should increase intracellular glucocorticoid levels in differentiated adipocytes, rather than inactivating intracellular glucocorticoids. The precise role of 11β-HSD-1 in modulating glucocorticoid action remains unclear, however, the 3T3 cells now present a unique system in which to investigate the function of 11β-HSD-1 in glucocorticoid sensitive metabolic pathways in adipocytes.

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