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Growth Hormone (GH) or Insulin-like Growth Factor (IGF)-I Represses 11β-Hydroxysteroid Dehydrogenase Type 1 (HSD1) mRNA Expression in 3T3-L1 Cells and Its Activity in Their Homogenates

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Abstract. Patients with growth hormone (GH) deficiency (GHD) have a clinical feature of visceral adiposity and it has been reported that these patients have an increased active cortisol (F)/inactive cortisone (E) metabolite ratio. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is an enzyme expressed in liver and adipose tissue that acts principally as a reductase converting E to F. In the present study, we investigated the effects of GH or IGF-I on the activity of 11β-HSD1 in 3T3-L1 cell homogenates and its mRNA expression. First, we showed that 11β-HSD1 activity and mRNA levels were low in preadipocytes and increased throughout the process of adipogenesis. When fully differentiated adipocytes were treated with GH for various times, the activity of 11β-HSD1 was significantly decreased after 4 h and 8 h but was restored to basal levels after 24 h. After 8 h of GH stimulation, 11β-HSD1 mRNA levels were decreased compared with basal levels. IGF-I treatment of adipocytes resulted in rapid decreases in 11β-HSD1 activity as well as mRNA levels; however, IGF-I treatment for 24 h increased 11β-HSD1 activity. In long-term cultured adipocytes, GH or IGF-I showed only inhibitory effects on 11β-HSD1 activity. In conclusion, 11β-HSD1 activity was suppressed by GH or IGF-I in differentiated adipocytes, probably due to a reduction of 11β-HSD1 mRNA levels. These data suggest that under the conditions of low GH or IGF-I concentrations, 11β-HSD1 activity in adipose tissue is maintained at high levels, leading to an increase in active cortisol that induces adipogenesis and/or lipogenesis. Thus, visceral adiposity in patients with GHD might be related to increased 11β-HSD1 activity.

Key words: Growth hormone (GH), Insulin-like growth factor-I (IGF-I), 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1), Adipocytes

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GROWTH hormone (GH) is well known to induce animal growth mediated by insulin-like growth factor (IGF)-I production. In addition, GH possesses direct anabolic activity and anti-insulin activity as metabolic regulatory actions, such as increasing protein synthesis in various tissues, enhancing lipolysis in adipose tissue, and reducing glucose uptake in liver [1]. Clinically, a state of GH excess, such as acromegaly, results in increased lean body mass, abnormal glucose metabolism and hypertension, and disproportional acral growth. In contrast, patients with GH deficiency (GHD) are characterized by visceral obesity,

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Abbreviations: Growth hormone, GH; insulin-like growth factor-I, IGF-I; Growth hormone deficiency, GHD; 11β-hydroxysteroid dehydrogenase type 1, 11β-HSD1; Cortisol, F; Cortisone, E; Dulbecco's modified Eagle's medium, DMEM; Hanks' balanced salt solution, HBSS; fetal bovine serum, FBS; calf serum, CS; bovine serum albumin, BSA; nicotinamide adenine dinucleotide phosphate, NADP; CCAAT enhancer-binding protein, C/EBP; peroxisome proliferator-activated receptor, PPAR; free fatty acid, FFA.

decreased lean body mass, dyslipidemia and osteoporosis. As stated above, patients with GHD present metabolic syndrome-like features; however, the causes of this syndrome remain largely unknown.

Recent studies have revealed that many of the clinical features observed in patients with either GH excess or deficiency may be mediated by changes in the tissue action of cortisol [2, 3]. In peripheral tissues, corticosteroid action is partly regulated by 11β-hydroxysteroid dehydrogenase (11β-HSD) which interconverts hormonally active cortisol (F) and inactive cortisone (E). Among the two isoforms, the type 1 isoform (11β-HSD1) is an NADPH-dependent, bidirectional enzyme that predominantly acts as a reductase converting cortisone to cortisol [4]. It is expressed in adipose tissue, liver, gonads and the central nervous system and acts as a tissue-specific pre-receptor amplifier of glucocorticoid action [5]. Type 2 isoform (11β-HSD2), by contrast, is a high affinity NADP-dependent enzyme that inactivates cortisol to cortisone in mineralocorticoid target tissues, such as kidney and colon, thereby protecting the mineralocorticoid receptor from cortisol [6]. It has been reported that transgenic mice overexpressing 11β-HSD1 in white adipose tissue develop visceral adiposity and exhibit features of the metabolic syndrome [7]. This study suggests that glucocorticoid converted by 11β-HSD1 in adipose tissue may play a role in adipocyte differentiation and may be involved in the development of metabolic syndrome, which includes insulin resistance, glucose intolerance, and dyslipidemia.

Studies in adult GHD patients treated with GH revealed a reduction in the urinary tetra-metabolite ratio of cortisol and cortisone (THF+allo-THF/THE) [2]. Converse results were obtained in patients with acromegaly, showing that urinary THF/THE ratios rise when GH secretion is adequately suppressed to normal values by trans-sphenoidal surgery [3]. The changes in THF+allo-THF/THE by GH treatment are presumed to reflect suppression of 11β-HSD1 reductase activity; however, this ratio is determined wholly by production and inactivation of glucocorticoids in the whole body that might reflect the activity of 11β-HSD1 and 11β-HSD2. This ratio is in fact higher in patients with apparent mineralocorticoid excess due to inactivating mutations in the gene for 11β-HSD2. Whether GH or IGF-I directly regulates expression and activity of 11β-HSD1 in adipose tissue is still unclear.

This study was undertaken to investigate the effect of GH or IGF-I on 11β-HSD1 mRNA levels and its activity in homogenates of cells in the process of adipogenesis and in mature adipocytes. For this purpose, we utilized the mouse fibroblast 3T3-L1 cell line as a model in which expression of 11β-HSD1 has been reported [8].

Materials and Methods

Materials

3T3-L1fibroblasts were obtained from the American Type Tissue Culture Collection and 293T cells were a kind gift from Dr. Kunio Shiota (University of Tokyo, Tokyo, Japan). GH was obtained from Wako (Osaka, Japan) and IGF-I was from Sigma (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), Hanks' balanced salt solution (HBSS) and fetal bovine serum (FBS) were obtained from Nissui Pharmaceutical Co., Ltd. (Ibaraki, Japan). Calf serum (CS) was from Invitrogen (Carlsbad, CA, USA). Penicillin was obtained from Banyu Pharmaceutical Co., Ltd. (Ibaraki, Japan) and bovine serum albumin (BSA) was obtained from Sigma (St. Louis, MO, USA). Unless otherwise noted, all other chemicals and reagents were obtained from Nacalai Tesque, Inc. (Kyoto, Japan).

Cell culture

3T3-L1 fibroblasts were maintained in DMEM containing 10% CS and an antibiotic mixture (50 μg/ml streptomycin, 50 μg/ml penicillin, and 100 μg/ml kanamycin) in an atmosphere of 5% CO_2 at 37°C. Two days after the fibroblasts had reached confluence, differentiation was induced by treating the cells with DMEM containing 25 mM glucose, 10% FBS, 1 μg/ml insulin, 1 mM dexamethasone, and 0.5 mM 3-isobutyl-1-methylxanthine. After 4 days, the medium was replaced with DMEM containing 10% FBS, plus 1 μg/ml insulin for an additional 4 days. The medium was then changed to DMEM containing 10% FBS every 2 days for 10 days. More than 90% of the cells morphologically differentiated into adipocytes. Prior to all experimental treatments, the cells were serum starved in DMEM containing 0.1% BSA for 24 h at 37°C. The undifferentiated and differentiated 3T3-L1 cells were treated with various concentrations of

GH or IGF-I for various times.

Preparation of recombinant 11β-HSD1

Human 11β-HSD1 cDNA was a kind gift from Dr. Edmund Maser (Institute of Toxicology and Pharmacology for Natural Scientists, University Medical School Schleswig-Holstein, Kiel, Germany). The human 11β-HSD1 cDNA was subcloned into the pcDNA3.1 vector and this expression plasmid was transfected into 293T cells by the calcium phosphate precipitation method. The recombinant protein was used as a positive control for 11β-HSD1 activity.

Measurement of 11β-HSD1 and 11β-HSD2 mRNA

Total RNA was extracted and purified using the RNeasy Plus Mini Kit (Qiagen, Hilden, Germany). RNA was quantified by measuring absorbency at 260nm with reference wavelength 280 nm. A total of 0.2 μg RNA was transcribed into cDNA using a High Capacity cDNA Reverse Transcription Kit and random primers (Applied Biosystems, Foster City, CA). The expression of 11β-HSD1 mRNA was measured by real-time PCR analysis. A total of 2.5 μl cDNA was amplified with 1x Taqman Universal PCR Master Mix, 250 nM Taqman probe and 450 nM of each primer (Applied Biosystems, Foster City, CA) in real-time quantitative PCR using a 7500 Real-Time PCR System (Applied Biosystems). Briefly, after incubation at 50°C for 2 min and then 95°C for 10 min, 40 cycles of PCR (95°C for 15 sec and 60°C for 1 min) were performed. The TaqMan probe and primer for 11β-HSD1 (assay identification number Mm00476182 1.5 μ1) and 11β-HSD2 (assay identification number Mm01251104_m1 1.5 μ1) were assay-on-demand gene expression products (Applied Biosystems). The standard curve was generated by amplifying serial dilutions of cDNA from cell samples studied. 18S rRNA was used as the internal reference to normalize specific mRNA levels.

Measurement of 11β-HSD1 activity in cell homogenates

11β-HSD1 activity was determined according to previously described methods [8]. 11β-HSD1 activity in homogenized cells was evaluated by measuring the rate of conversion of [^{3}H]corticosterone to [^{3}H]dehydrocorticosterone. This bidirectional enzyme exists in microsomes. The presence of NADP in these assays drives the activity in the direction of dehydrogenation and no reductase activity is detected under these *in vitro* assay conditions [9]. The cells were harvested and homogenized 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 were measured using a Bio-Rad protein assay solution (Bio-Rad, Hemel Hempstead, Hertfordshire, UK). 11β-HSD1 activity was assayed by incubating 250 μg protein with 200 μM NADP (Sigma), and 12 nM [1, 2, 6, 7] [^{3}H]corticosterone (specific activity 88 Ci/mmol, Amersham International, Hertfordshire, UK) in a total volume of 250 μl Krebs-Ringer buffer for 15 min at 37°C. Steroids were extracted with 3 volumes of ethyl acetate, and the organic layer was separated and evaporated to dryness. The residues were dissolved in 40 μl methanol. Duplicate aliquots were spotted onto TLC plates (LK6D, Whatman, UK). The thin layer was developed in chloroform-ethanol-butanol (9: 1: 0.5) and dried. The fractional conversion of corticosterone to 11-dehydrocorticosterone was calculated after scanning the TLC plate on a Bio-Imaging Analyzer BAS-5000 system (FUJI Photo Film, Japan). Activity levels are presented as the percent change from the control value.

Statistical analysis

Results are expressed as means±SEM. For comparisons, data were analyzed utilizing a two-way or three-way ANOVA followed by a Fisher PSLD post-hoc test. Differences were considered to be statistically significant at $P<0.05$. Calculations were performed using Stat-View ver. 5.0 (SAS Institute Inc., Cary, NC).

Results

Effects of adipogenesis on mRNA levels and activity of 11β-HSD1and 11β-HSD2 in cell homogenates of 3T3-L1 cells

11β-HSD1 activity (Fig. 1A) and mRNA levels (Fig. 1B) were low in preadipocytes and increased throughout the process of adipogenesis (7.3±0.3 to 43.3±1.1%, $P<0.05$ for activity, and 1.2±0.1 to 7.8±0.6,

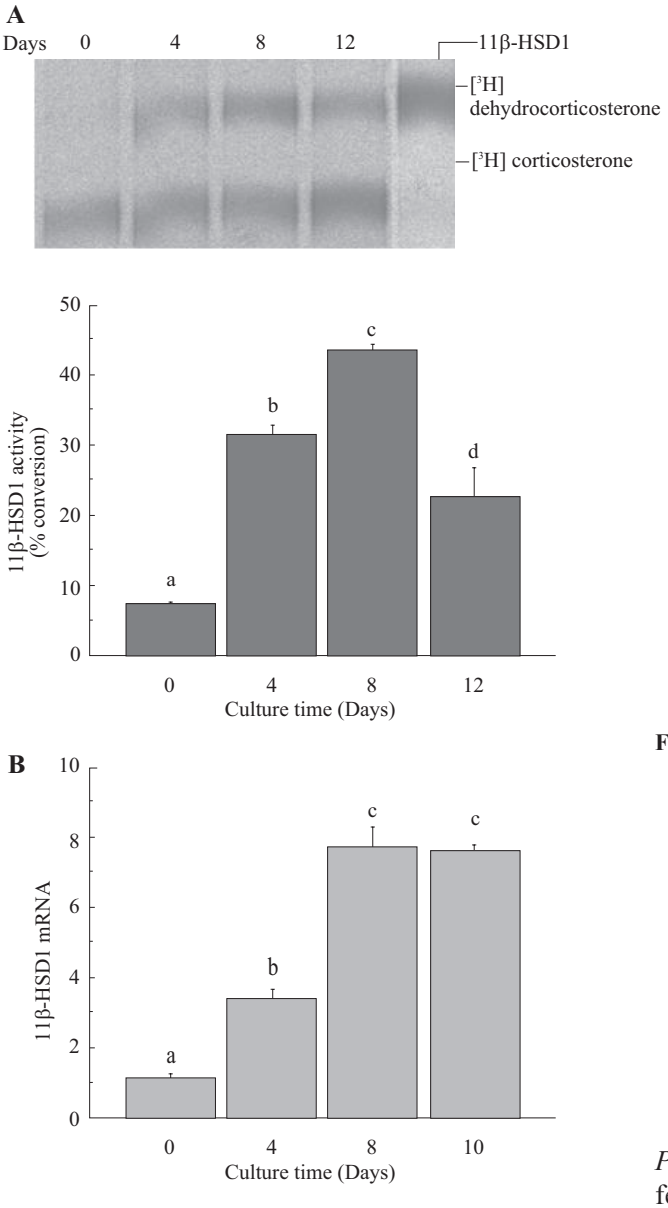

Fig. 1. Effects of adipogenesis on 11β-HSD1 activity in cell homogenates and its mRNA levels in 3T3-L1 cells. Cells were differentiated as described in "Materials and Methods." On the 4th, 8th, and 12th days, cell lysates were prepared. (A) 11β-HSD1 activity in homogenized 3T3-L1 cells was determined using corticosterone as a substrate at a final concentration of 12 nM and 250 μM of NADP as a co-factor. A representative autoradiograph image of TLC is shown (the inserted blot). Recombinant 11β-HSD1 converted 100% of corticosterone into dehydrocorticosterone. (B) Quantitative real-time PCR was performed and levels of 11β-HSD1 mRNA were normalized to 18S rRNA. Results are shown as the relative amounts, taking the mean of the group "Day 0" as 100. Values are means±SEM. n=3. Bars in the figures with different superscript letters are significantly different at $P < 0.05$.

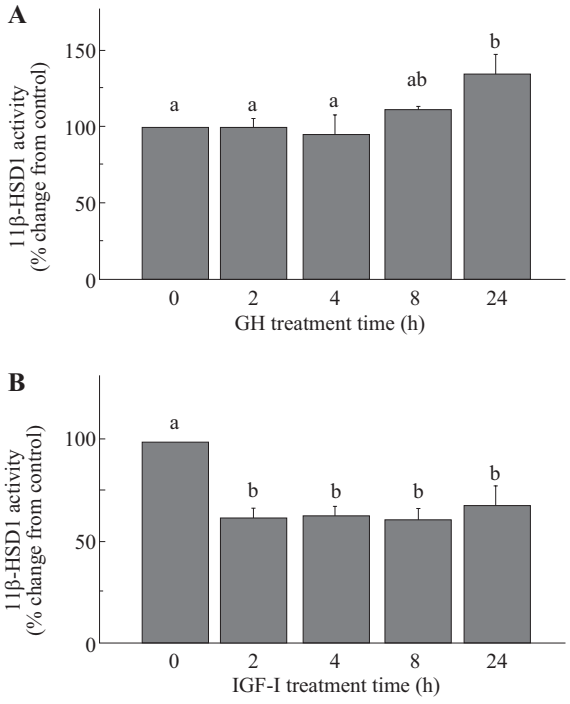

Fig. 2. Effect of GH or IGF-I on 11β-HSD1 activity in cell homogenates during differentiation of 3T3-L1 cells. On day 4 after induction of differentiation, cells were in the process of adipogenesis. Cells were cultured with 100 nM GH (A) or 10 nM IGF-I (B) in DMEM containing 0.1% BSA for the indicated times. 11β-HSD1 activity in homogenized 3T3-L1 cells was determined as described in "Materials and Methods." The results are shown as the relative activity, setting the mean of the time 0 group at 100. Values are means±SEM. n=3. Bars in the figures with different superscript letters are significantly different at $P < 0.05$.

$P<0.05$ for mRNA on the 8th day after induction of differentiation). On the other hand, 11β-HSD2 mRNA was not detected before adipogenesis or throughout the process of adipogenesis (data not shown), indicating that activity to convert corticosterone to dehydrocorticosterone reflects only 11β-HSD1 activity in cell homogenates.

Effects of GH or IGF-I on 11β-HSD1 activity in cell homogenates during differentiation of 3T3-L1 cells

On the 4th day after induction of differentiation, cells were treated with GH (100 nM) or IGF-I (10 nM) for various times and 11β-HSD1 activity was measured (Fig. 2). GH treatment did not repress but enhanced 11β-HSD1 activity after 24 h of stimulation.

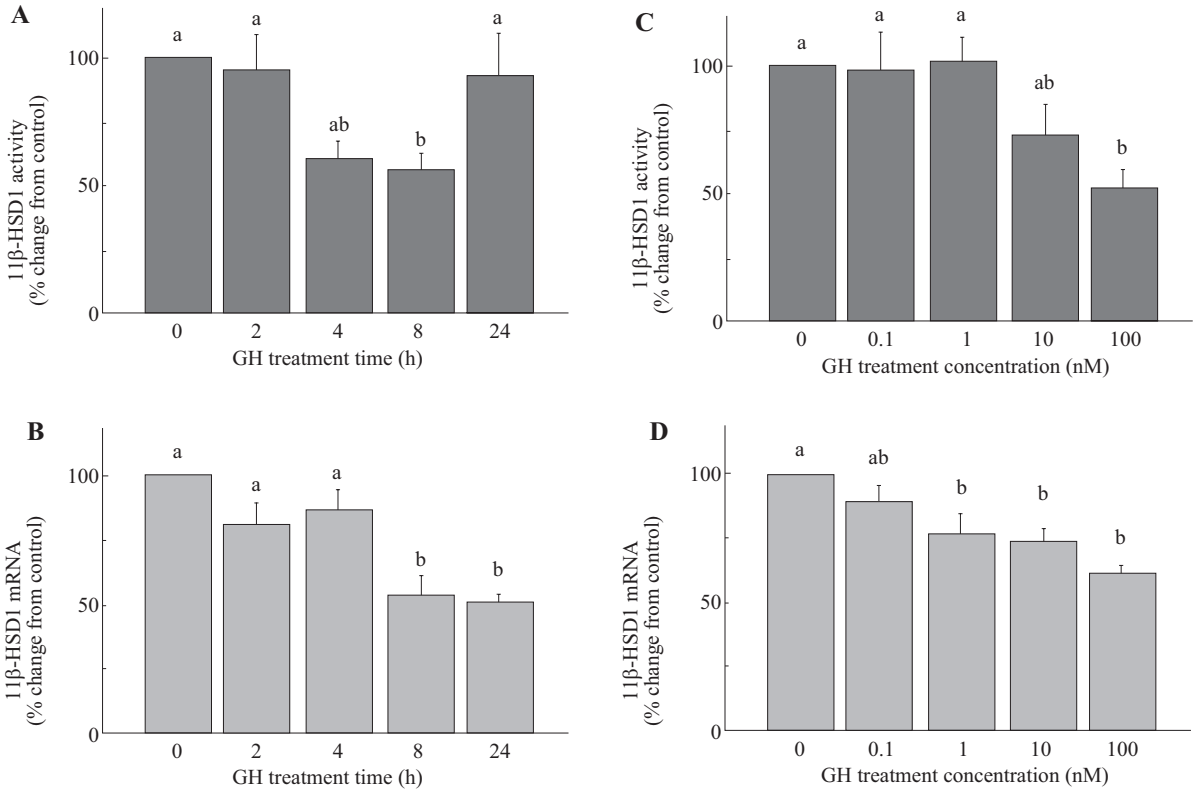

Fig. 3. Effects of GH on 11β-HSD1 activity in cell homogenates and its mRNA levels in 3T3-L1 adipocytes. (A and B) 3T3-L1 adipocytes which had undergone induction of adipogenesis for 8 days were treated with 100 nM GH for the indicated periods of time. 11β-HSD1 activity in homogenized 3T3-L1 cells was determined (A) and the mRNA levels of 11β-HSD1 were measured (B) as described in "Materials and Methods." The results shown are the relative activity, setting the mean of the time 0 group at 100. Values are means±SEM. n=3. (C and D) 3T3-L1 adipocytes were treated with various concentrations of GH for 8 h for measurement of activity (C) and measurement of mRNA (D). 11β-HSD1 activity in homogenized 3T3-L1 cells was determined (C) and the mRNA levels of 11β-HSD1 were measured (D) as described in "Materials and Methods". The results are shown as the relative activity, setting the mean of the 0 nM group at 100. Values are means±SEM. n=3. Bars in the figures with different superscript letters are significantly different at $P < 0.05$.

In contrast, after 2 h of IGF-I treatment, 11β-HSD1 activity was repressed.

Effects of GH on 11β-HSD1 activity in cell homogenates and its mRNA levels in 3T3-L1 adipocytes

Fully differentiated adipocytes (on the 8th day after induction of differentiation) were treated with GH for various times and the activity and mRNA levels of 11β-HSD1 were measured. 11β-HSD1 activity after 4 and 8 h of stimulation decreased to 61.0±5.3% of control and 56.7±5.2% of control (P<0.05), respectively, but gradually increased after 24 h (to 93.3±3.8% of control) of stimulation with 100 nM GH (Fig. 3A). mRNA levels of 11β-HSD1 after 2, 4, 8 and 24 h of stimulation of GH (100 nM) decreased to 81.6±8.5%, 86.5±7.8%, 54.0±7.1% and 51.3±2.8%, respectively (Fig. 3B), compared with the non-treated 3T3-L1 adipocytes defined as 100%, and a significant reduction was observed in the cases of treatment for 8 and 24 h (P<0.05).

At 8 h after treatment, the inhibition of 11β-HSD1 activity was observed with concentrations of 10 nM (71.8±11.0%) and 100 nM GH (51.7±7.18%, P<0.05) (Fig. 3C). At this time point, GH caused a concentration-dependent decrease in 11β-HSD1 mRNA (Fig 3D). Significant inhibition was observed with 1 nM, 10 nM, and 100 nM of GH with the levels decreasing to 76.3±8.5%, 73.8±5.0% and 61.1±2.3% of control (P<0.01 vs. control (100%), respectively.

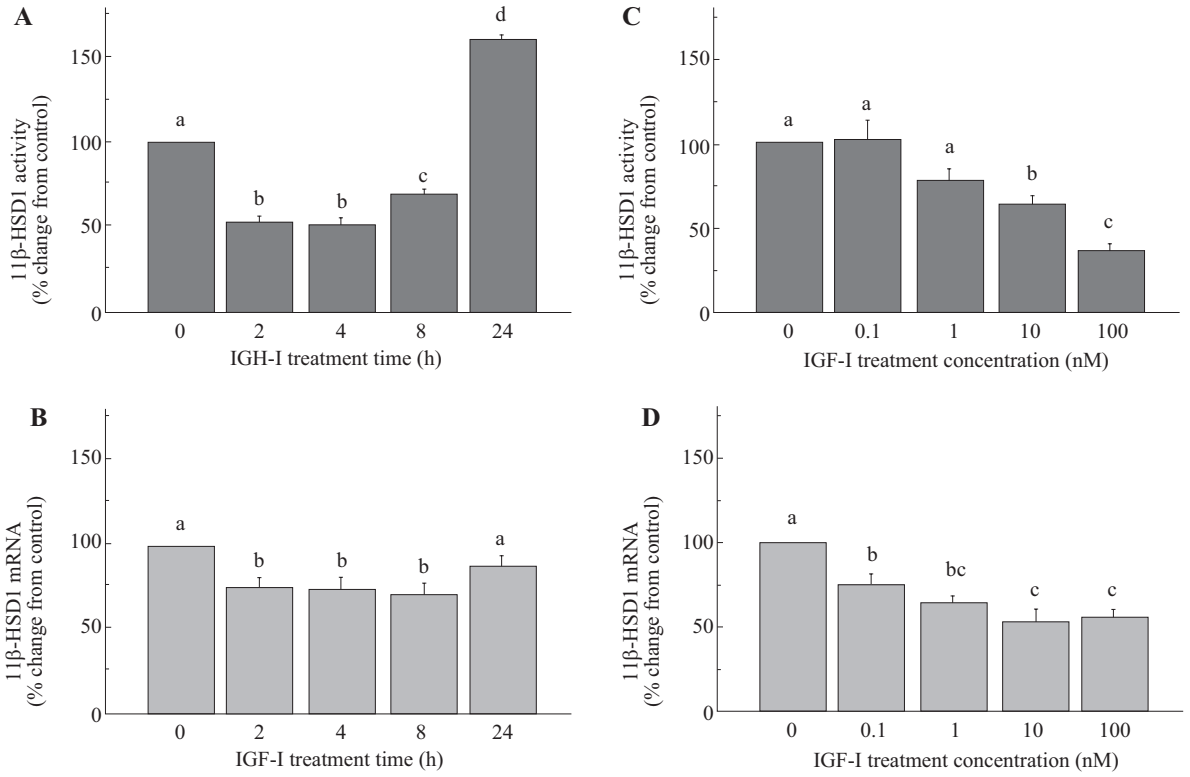

Fig. 4. Effects of IGF-I on 11β-HSD1 activity in cell homogenates and its mRNA levels in 3T3-L1 adipocytes. (A and B) 3T3-L1 adipocytes which had undergone induction of adipogenesis for 8 days, were treated with 10 nM IGF-I for the indicated periods of time. 11β-HSD1 activity in homogenized 3T3-L1 cells was determined (A) and the mRNA levels of 11β-HSD1 were measured (B) as described in "Materials and Methods." The results are shown as the relative activity, setting the mean of the time 0 group at 100. Values are means±SEM; n=3. (C and D) 3T3-L1 adipocytes were treated with various concentrations of IGF-I for 4 h for measurement of activity (C) and 8 h for measurement of mRNA (D) as described in Materials and Methods. Results are shown as the relative activity, setting the mean of the 0 nM group at 100. Values are means±SEM. n=3. Bars in the figures with different superscript letters are significantly different at $P < 0.05$.

Effects of IGF-I on 11β-HSD1 activity in cell homogenates and its mRNA levels in 3T3-L1 adipocytes

IGF-I (10 nM) treatment of fully differentiated 3T3-L1 adipocytes for 2 h and 4 h caused a significant decrease in 11β-HSD1 activity compared to the basal median levels (100%) (52.4±3.1% and 50.4±3.7%, $P<0.05$). After 8 and 24 h of treatment with IGF-I, 11β-HSD1 activity was elevated gradually to 68.1±2.9% and 159.0±2.0%, respectively, indicating that IGF-I has a biphasic effect on 11β-HSD1 activity (Fig. 4A). In contrast, IGF-I (10 nM) treatment of adipocytes for 2, 4, 8, and 24 h caused decreases in 11β-HSD1 mRNA levels to 74.1±5.5%, 72.8±6.6%, 70.3±6.1%, and 86.2±5.9%, respectively, of the non-treated adipocyte level defined as 100%, and a significant decrease was observed at 2, 4 and 8 h after stimulation ($P<0.05$, Fig. 4 B).

Following the time course of IGF-I stimulation, we studied the effects of IGF-I concentration at the time point when strongest reduction of 11β-HSD1 activity and mRNA levels was observed. At 4 h after IGF-I treatment when the greatest inhibition of activity was observed, significant inhibition was observed with 10 nM (64.2±5.1%; $P<0.05$) and 100 nM (37.5±2.4%; $P<0.01$ vs. control) IGF-I (Fig. 4C). At 8 h after IGF-I treatment, IGF-I caused a concentration-dependent inhibition of mRNA levels. Significant inhibition was observed with 0.1 nM, 1 nM, 10 nM, and 100 nM IGF-I treatment, with 11β-HSD1 mRNA levels decreased to 75.1±6.4% (IGF-I 0.1 nM), 64.2±3.9% (IGF-I 1 nM), 53.6±6.4% (IGF-I 10 nM) and 56.2±3.6% of control (IGF-I 100 nM, $P<0.05$ vs. control as 100%) (Fig. 4D).

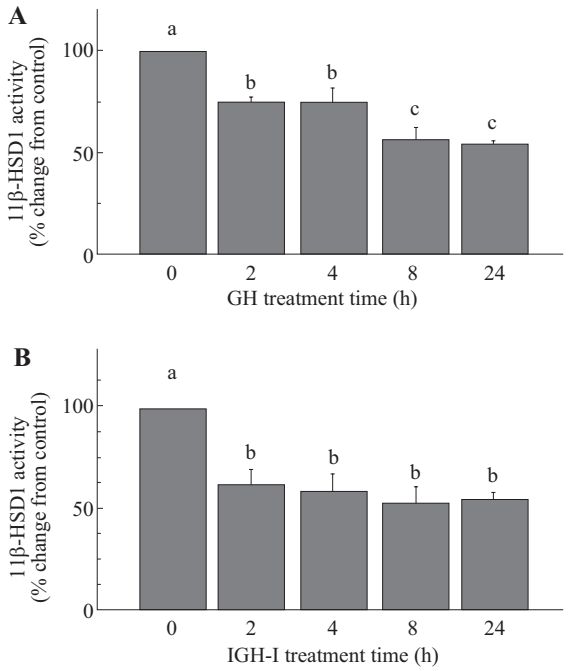

Fig. 5. Effect of GH or IGF-I on 11β-HSD1 activity in cell homogenates from long-term cultured 3T3-L1 adipocytes. On the 12th day after induction of differentiation, large droplets had accumulated in cells. These cells were cultured with 100 nM GH (A) or 10 nM IGF-I (B) in DMEM containing 0.1% BSA for the indicated periods of time. 11β-HSD1 activity in homogenized 3T3-L1 cells was determined as described in Materials and Methods. The results are shown as the relative activity, setting the mean of the time 0 group at 100. Values are means±SEM; n=3. Bars in the figures with different superscript letters are significantly different at $P < 0.05$.

Effect of GH or IGF-I on 11β-HSD1 activity in cell homogenates in long-term cultured 3T3-L1 adipocytes

On the 12th day after induction of differentiation, large oil droplets had accumulated in cells (data not shown). These cells were treated with GH (100 nM) or IGF-I (10 nM) for various times followed by measurement of 11β-HSD1 activity (Fig. 5). GH treatment for 2, 4, 8, and 24 h caused gradual decreases in 11β-HSD1 activity to 73.9±3.1%, 74.6±6.7%, 56.1±6.0%, and 54.1±1.4% compared to the basal median levels defined as 100%. IGF-I treatment reduced 11β-HSD1 to 61.7±6.0% ($P<0.05$) of basal at 2 h of treatment and maintained this low level of activity.

Effect of GH or IGF-I on 11β-HSD2 mRNA levels in 3T3-L1 adipocytes

11β-HSD2 mRNA was not detected in fully differentiated 3T3-L1 adipocytes, and GH (100 nM) or IGF-I (10 nM) treatment did not affect the mRNA levels.

Discussion

It has been demonstrated that 11β-HSD1 is one of the gene markers expressed during the late period of adipocyte differentiation [8]. Our study confirmed that 11β-HSD1 mRNA expression and activity in preadipocyte murine 3T3-L1 cells were both low and both increased throughout the process of adipogenesis. These results support our hypothesis that the increased 11β-HSD1 activity is due to an increase in its mRNA expression. Transcription of 11β-HSD1 in preadipocytes has been shown to be enhanced by glucocorticoids [10, 11] and CCAAT enhancer-binding proteins (C/EBP) α and β, transcription factors which play crucial roles in directing the differentiation process [12]. When 11β-HSD1 levels in 3T3-L1 fibroblasts were reduced by RNA interference, differentiation by inactive glucocorticoids was prevented and the expression of adipocyte-specific proteins such as peroxisome proliferator-activated receptor (PPAR) γ and C/EBP α was eventually reduced [13]. Taken together, these data suggest that the expression and activity of 11β-HSD1 may increase in an autocrine and/or paracrine manner during the process of adipogenesis, and that the biological importance of an increase in 11β-HSD1 activity during adipogenesis may be to bring about the differentiation of preadipocytes and initiate adipogenesis in the preadipocytes around them.

The effects of GH or IGF-I on 11β-HSD1 during adipogenesis were investigated. We found that IGF-I itself, but not GH, represses 11β-HSD1 activity in cells treated to be differentiated for 4 days. IGF-I is an important factor in the proliferation of many cell types including preadipocytes [14]. Inhibition of 11β-HSD1 activity by IGF-I reduces the levels of active glucocorticoid in adipose tissues, leading to an induction of proliferation and impairment of adipogenesis.

On day 8 after induction of differentiation by the conventional method, more than 90% of cells had morphologically differentiated into adipocytes and on day 12 mature adipocytes were observed to have

prominently accumulated lipids. High levels of 11β-HSD1 activity may contribute to the ability to produce active glucocorticoids. At the same time, active glucocorticoids are reported to repress mRNA levels and activity of 11β-HSD1 in 3T3-L1 adipocytes [15], suggesting that the amounts of active glucocorticoid in adipose tissues are strictly regulated by this feedback mechanism in the short term.

In adipose tissues, glucocorticoids increase lipolysis by up-regulating the expression of hormone-sensitive lipase [16] and fatty acid synthesis in the presence of insulin, leading to a release of free fatty acids (FFA) into the circulation. High levels of glucocorticoid by itself or with other catabolic hormones such as catecholamines, adipokines, and FFAs induce insulin resistance followed by hyperglycemia. The increase in 11β-HSD1 activity after adipocyte differentiation may be of significance for adipogenesis in nearby cells and the maintenance of adipocyte function. Chronic excess 11β-HSD1 activity may contribute to the enhanced insulin resistance that is typically observed in Cushing's syndrome.

On day 8 after induction of differentiation, GH treatment gradually inhibited 11β-HSD1 activity which then recovered after 24 h; however, GH treatment gradually repressed 11β-HSD1 mRNA levels up to 24 h, suggesting that the restoration of activity in the late phase is independent of changes in mRNA levels. On the other hand, IGF-I treatment rapidly reduced 11β-HSD1 activity, but surprisingly enhanced it later, indicating that IGF-I exhibits biphasic effects on 11β-HSD1 activity. As in the case of GH treatment, IGF-I-induced repression of 11β-HSD1 mRNA can not explain the enhancement of 11β-HSD1 activity after 24 h. In addition, the repression by GH and IGF-I are both observed at physiological concentrations, suggesting that the regulation is biologically relevant. In 3T3-L1 cell lines, GH stimulates increases in IGF-I mRNA in differentiating cells [17]. Based on these results, we postulate that the modification of 11β-HSD1 mRNA and activity in response to GH is mediated by IGF-I production, and that in the short term, IGF-I represses 11β-HSD1 mRNA levels, leading to decreases in its activity and that in the long term, it enhances 11β-HSD1 activity via a post-translational mechanism.

We then treated cells with GH or IGF-I on day 12 after the induction of differentiation and 4 days after the cells' maturation. GH, which may act through IGF-I production, and IGF-I itself both decreased 11β-HSD1 activity, clearly indicating that the response of 11β-HSD1 to IGF-I is quite different up to the point of differentiation or maturation of adipocytes. Inhibition of 11β-HSD1 activity by IGF-I can be explained by one of the underlying mechanisms: that IGF-I antagonizes the catabolic effects of glucocorticoids. Other *in vitro* studies have reported that GH had no effect on 11β-HSD1 activity in omental adipose stromal cells or 293T cells [3, 18]. However, in these studies, cells were treated with GH for a longer period than in our study. Alternatively, the status of these adipose cells may be different from our study, suggesting that the 3T3-L1 cell is a sensitive model to analyze the effects of various factors on 11β-HSD1 at different developmental stages of adipocytes. Recently, insulin has been reported to increase 11β-HSD1 activity due to enhancement of its mRNA stability in 3T3-L1 adipocytes [15]. It is interesting how insulin and IGF-I signals differ in this respect, because these hormones share common signaling pathways.

As mentioned in "Materials and Methods", the 11β-HSD1 activity we measured in cell homogenates was evaluated as the rate conversion of corticosterone to dehydrocorticosterone, because this bidirectional enzyme acts mainly as a dehydrogenase under *in vitro* assay condition adding NADP as a cofactor. We could not detect any mRNA expression of 11β-HSD2 in 3T3-L1 cells, either before adipogenesis or throughout the process of adipogenesis. These data indicate that this dehydrogenase activity reflects 11β-HSD1 activity. On the other hand, in intact cells (not in the cell-free assay) this enzyme activity is known to be modulated by other factors such as NADPH/NADP ratio or the existence of hexose-6-phosphate dehydrogenase (H6PDH) [19]. H6PDH is an ER-lumenal enzyme which catalyzes the conversion of glucose-6-phosphate (G6P) to 6-phosphogluconolactone of the pentose-phosphate pathway within this compartment, thereby generating NADPH from NADP [20]. Since NADPH and NADP cannot penetrate the ER-membrane, H6PDH exerts a key role in determining the ratio of NADPH/NADP in the ER lumen and regulates the reaction direction of 11β-HSD1 [21]. It might be expected that the inhibition of H6PDH in intact cells would demonstrate similar effect as 11β-HSD1 inhibition. However, the relative contribution of changes in H6PDH expression or activity to changes in 11β-HSD1 reductase activity in intact cells and *in vivo* has yet to be clearly understood. Indeed, a re-

cent study indicated a discordant regulation of expression of H6PDH mRNA and 11β-HSD1 activity and mRNA by dexamethasone and insulin [15]. Whether GH or IGF-I regulate 11β-HSD1 reductase activity by regulating expression of H6PDH or 11β-HSD1, and furthermore, whether GH or IGF-I stimulates 11β-HSD2 dehydrogenase activity in intact cells and *in vivo* needs to be investigated.

Our study has demonstrated that IGF-I represses the activity of 11β-HSD1 in cell homogenates from adipocytes during adipogenesis as well as in mature adipocytes. 11β-HSD1 is highly expressed in adipose tissues and its activity and expression are significantly higher in omental preadipocytes than subcutaneous ones [10]. Therefore, the increase in visceral obesity observed in patients with GHD could be partly explained by increased 11β-HSD1 activity due to low concentration of plasma IGF-I. Other symptoms, including dyslipidemia and insulin resistance seen in patients with GHD, might also be caused by excess local production of active glucocorticoids in preadipocytes and adipose tissues.

In contrast, the abnormal glucose metabolism and insulin resistance observed in patients with acromegaly may not be an effect of 11β-HSD1 activity since the urinary THF/THE ratio is low in these patients [3]. A reduction in serum IGF-I resulting from a GH receptor blockade by pegvisomant caused a increase in 11β-HSD1 activity, and was independent of changes in body composition and insulin sensitivity, suggesting that long-term GH excess may be a cause of insulin resistance [22]. GH replacement therapy for patients with GHD resulted in a significant reduction in abdominal adipocyte size, a reduced rate of adipogenesis, and an improvement in insulin sensitivity [23]. GH itself has strong lipolytic action, but by mediating IGF-I to suppress the expression and activity of 11β-HSD1 in adipose tissue, it may contribute to changes in body composition and fat distribution.

In summary, we have shown that GH or IGF-I inhibits 11β-HSD1 activity in cell homogenates partially due to a reduction of its mRNA levels in 3T3-L1 cells. These results may provide a clue to explain the phenotypes of acromegaly and adult GHD and the effects of treatment of these conditions on body composition and fat distribution.

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