Modulation of 11β-Hydroxysteroid Dehydrogenase Type 1 in Mature Human Subcutaneous Adipocytes by Hypothalamic Messengers

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Glucocorticoids are regulated at the receptor level by 11β-hydroxysteroid dehydrogenase (11β-HSD), which interconverts active cortisone and active cortisol. In a previous study, we noted that patients with hypothalamic obesity had an increased ratio of cortisol/cortisone metabolites, suggesting enhanced 11β-HSD-1 activity. In this in vitro study, we tested the hypothesis that adipose

11β-HSD-1 is regulated by the hypothalamus via circulating hormones, sympathetic nervous system innervation, and/or cytokines. Preadipocytes were retrieved from subcutaneous fat from healthy nonobese individuals and differentiated in vitro to mature adipocytes. Cells were incubated with several potential effectors, and the activity of 11β-HSD-1 was assayed by measuring conversion of added 500 nM cortisone to cortisol. Expression of 11β-HSD-1 mRNA was determined by real-time PCR, whereas lipolytic effects were determined by measuring glycerol concentration in the culture medium. CRH down-regulated 11β-HSD-1 activity with maximal effect at 10^{-9} M (65 ± 20%; P < 0.05) and reduced medium glycerol. Neither CRH nor ACTH affected 11β-HSD-1 mRNA expression. TNFα up-regulated 11β-HSD-1 activity maximally at 0.6 \times 10^{-9} M (140 ± 20%; P < 0.001); the same cytokine increased 11β-HSD-1 mRNA levels at 3-fold of control (P < 0.05) and increased medium glycerol levels to 165 ± 14% of control (P < 0.01). IL-1β also up-regulated 11β-HSD-1 activity maximally at 0.6 \times 10^{-9} M (160 ± 33%; P < 0.001) and caused an increase in glycerol levels (159 ± 11% of control; P < 0.001). Of the adrenergic agonists, salbutamol up-regulated 11β-HSD-1 activity maximally at 10^{-7} M (162 ± 46%; P < 0.02), and clonidine down-regulated it at 10^{-7} M (82 ± 15%; P < 0.005). We conclude that possible distinct hypothalamic mediators regulating adipose tissue 11β-HSD-1 might include down-regulation of 11β-HSD-1 activity by CRH, ACTH, and α2 sympathetic stimulation, and up-regulation of the enzyme by β2 sympathetic stimulation and by the cytokines TNFα and IL-1β. (J Clin Endocrinol Metab 88: 385–393, 2003)

A

BNORMAL METABOLISM OF glucocorticoids has been implicated in the pathophysiology of obesity for many years (1). Glucocorticoids are regulated at the receptor level by the microsomal enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD), which interconverts active cortisol and inactive cortisone. Human 11β-HSD type 1 (11β-HSD-1) is a reversible nicotinamide adenine dinucleotide phosphate (NADP)/reduced NADP-dependent, low-affinity enzyme whose in vivo action is predominantly o xo-reduction of inactive cortisone to active cortisol, functioning as a positive prreceptor signaling pathway modulator of glucocorticoid action (2). In humans, 11β-HSD-1 is expressed in glucocorticoid target tissues, such as the brain, liver, gonads, lung, and adipocyte tissue (3).

In the companion paper, we report that the obesity that developed in patients with hypothalamic obesity is associated with enhanced 11β-HSD-1 activity, as reflected by higher ratios of 11-hydroxy/11-oxo metabolites (4). We proposed that abnormal metabolism of exogenous glucocorticoids might be involved in the pathogenesis. Despite the recent increase in our understanding of adipose to hypothalamus signaling, mainly through research on leptin, neuropeptide Y, and α-melanocyte-stimulating hormone (5), our understanding of the reciprocal pathway remains lacking. The present study was designed to examine postulated hypothalamic messengers that might mediate hypothalamus-adipose signaling.

On the basis of the findings of the companion paper and on the aforementioned studies, the current in vitro study was based on the hypothesis that adipose tissue 11β-HSD-1 is regulated by the hypothalamus through hormones, the sympathetic nervous system, and/or cytokines. Previous studies have used sc or omental adipose stromal cells to study 11β-HSD-1 regulation (2, 6–8). These studies have provided valuable information on the regulation of steroid metabolism in preadipocytes. We now report on the regulation of 11β-HSD-1 in fully differentiated adipocytes.

Abbreviations: BMI, Body mass index; HPA, hypothalamic-pituitary-adrenal; 11β-HSD, 11β-hydroxysteroid dehydrogenase; NADP, nicotinamide adenine dinucleotide phosphate; PRL, prolactin.

Materials and Methods

Preadipocytes were retrieved by centrifugation after collagenase treatment from abdominal or thigh sc fat samples obtained by liposuction from otherwise nonobese, healthy individuals (Table 1). Preadipocytes were differentiated in vitro to mature adipocytes using 1 µM dexamethasone, 100 nM insulin, isobutylmethylxanthine 0.2 mM, and 10 µM PPARγ agonist (Zen-Bio, Inc., Research Triangle Park, NC). Differentiation to mature adipocytes was confirmed by microscopic appearance of intracellular lipid droplets, expression of the adipocyte specific genes aP2, PPARγ, and α(2) (leptin), and by the lipolytic response to isopro-
eroneol. Mature cells were maintained in media containing 1 \( \mu \)M dexamethasone and 100 nM human insulin.

After an overnight incubation with serum-free media and without dexamethasone or insulin, cells were incubated with and without the several effectors tested in this study. The supernatant was collected and centrifuged, and activity of 11\( \beta \)-HSD-1 was assayed in the supernatants by measuring conversion of added 500 nM cortisone (Sigma, St. Louis, MO) to cortisol with a RIA kit (Coat-a-Count cortisol, Diagnostic Products Corp., Los Angeles, CA), under conditions ensuring first-order kinetics (2-24 h; Fig. 1). Starvation of up to 48 h and also addition of reduced NADP to the incubation media did not influence 11\( \beta \)-HSD-1 activity in these cells (data not shown). All experiments were performed at least three times and in triplicate.

**Effectors tested**

GH, IGF-I, leptin, estradiol, dihydrotestosterone, T\(_4\), CRH, ACTH, TNF\(_\alpha\), phenylephrine, dobutamine, salbutamol, phenylpropanolamine, BRL 37344, prolactin (PRL), and acetyl choline were purchased from Sigma. IL-1\( \beta \) and IL-6 were purchased from R&D Systems, Inc. (Minneapolis, MN).

**Statistics**

As expected in a primary culture system originating from subjects with individual variations, individual results varied considerably. Replicate experiments were performed on each of the five subjects and with individual variations, individual results varied considerably. Replicated by RIA in the culture medium.

Concentration of cortisol was then measured at varying time intervals.

**Real-time RT-PCR**

After incubation with a known or putative effector as described above, total RNA was extracted from adipocytes using a single-step method (Tri Reagent, Molecular Research Center, Inc., Cincinnati, OH). RT-PCR experiments were performed according to the Thermoscript RT-PCR system kit instructions (Life Technologies, Inc., Gaithersburg, MD). Briefly, after treatment with dexamethasone and 1 (GenHunter Corp., Nashville, TN), total RNA (1 \( \mu \)g) was reverse transcribed to complementary DNA by a reaction containing 2 mM dithiothreitol, 40 U RNase inhibitor, 50 ng random primer, and 15 U Thermoscript reverse transcriptase. The reaction was run at 25 C for 10 min and 50 C for 50 min, heated to 85 C for 5 min, and then cooled to 4 C.

To quantitate expression of 11\( \beta \)-HSD-1 mRNA after incubation with an effector, we applied the TaqMan PCR method, using a 7700 Sequence Detector (PE Applied Biosystems, Foster City, CA). The reaction contained TaqMan Universal PCR Master Mix (900 nmol/liter) and the following forward and reverse primers: forward, 5'-TTGGAATTTTGCCCTAACCCTGTA-3'; reverse, 5'-CCTCTCTCAATT TTCTCTTCCTGAG-3. The dye utilized was a TaqMan probe 200 nmol/liter 5'-FAM-AGGTATTTATGTGCATGTGCCTCCCCAG-TAMRA-3'. 18S ribosomal RNA primers and probe were added at 50 nmol/liter. Thermal cycling proceeded with 40 cycles of 95 C for 15 sec and 60 C for 1 min. Input RNA amounts were calculated with a multiplex comparative method for mRNAs of 11\( \beta \)-HSD-1 and 18S ribosomal protein.

**Lipolysis**

Lipolysis was assessed by quantitative enzymatic determination of glycerol concentrations in the culture medium [Triglyceride (GPO-Trinder), Sigma].

**Results**

**CRH and ACTH**

CRH down-regulated 11\( \beta \)-HSD-1 activity (\( P < 0.1, \)) with maximal down-regulatory effect demonstrated at 10\(-9\) M (65 ± 10% of control; \( P < 0.01; \) Table 2). A representative dose-response demonstrating this effect is shown in Fig. 2A, presenting mean and distribution of five replicate wells. The CRH receptor-1 antagonist, antalarmin, at a concentration of 10\(-7\) M did not reverse CRH-suppressed 11\( \beta \)-HSD-1 activity.

| TABLE 1. Characteristics of subjects who donated liposuction tissue for this study |
|---------------------------------|-----|-----|-----|
| Subject no. | Sex | Age (yr) | BMI (kg/m\(^2\)) |
| 1 | F | 32 | 21.67 |
| 2 | F | 43 | 24.84 |
| 3 | F | 64 | 25.9 |
| 4 | M | 35 | 26.6 |
| 5 | F | 20 | 23.9 |

All tissues were abdominal sc fat except tissue from subject 2, which was sc fat from the thigh. F, female; M, male.
TABLE 2. Summary of effectors that modulated 11β-HSD-1 activity in a primary culture of mature human sc adipocytes

<table>
<thead>
<tr>
<th>Effector</th>
<th>Maximal effect on 11β-HSD-1 activity</th>
<th>Maximal effective concentration</th>
<th>Maximal lipolytic effect</th>
<th>Maximal effective concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRH</td>
<td>65 ± 10%</td>
<td>10^{-9} M</td>
<td>77 ± 21%</td>
<td>10^{-7} M</td>
</tr>
<tr>
<td>ACTH</td>
<td>65 ± 20%</td>
<td>10^{-9} M</td>
<td>77 ± 21%</td>
<td>10^{-7} M</td>
</tr>
<tr>
<td>TNFα</td>
<td>140 ± 20%</td>
<td>0.6 × 10^{-9} M</td>
<td>165 ± 14%</td>
<td>0.6 × 10^{-9} M</td>
</tr>
<tr>
<td>IL-1β</td>
<td>160 ± 33%</td>
<td>0.6 × 10^{-9} M</td>
<td>159 ± 11%</td>
<td>0.6 × 10^{-10} M</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>162 ± 46%</td>
<td>10^{-7} M</td>
<td>100%</td>
<td>None</td>
</tr>
<tr>
<td>Clonidine</td>
<td>82 ± 15%</td>
<td>10^{-7} M</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results represent the mean ± SD; n = 4–5 subjects.

(data not shown). CRH caused a dose-dependent reduction in glycerol concentration in the incubation media (ANOVA P < 0.05). Maximal effect was at a concentration of 10^{-7} M (77 ± 21% of control; Table 2). Likewise, ACTH down-regulated 11β-HSD-1 activity (P < 0.1) with maximal effect at a concentration of 10^{-9} M (65 ± 20%; P < 0.05; Table 2). A representative dose-response demonstrating the down-regulatory effect of ACTH is shown in Fig. 2B. A combination of CRH and ACTH was nonadditive (data not shown). ACTH caused a dose-dependent reduction in glycerol concentration (ANOVA P < 0.001), with maximal effect at a concentration of 10^{-7} M (72 ± 9% of control; P < 0.01; Fig. 2D). The individual response did not correlate with the subject’s age or body mass index (BMI; r = 0.323; P > 0.1; n = 5). Neither CRH nor ACTH affected 11β-HSD-1 mRNA levels (Fig. 2E).

**Cytokines**

TNFα up-regulated 11β-HSD-1 activity in mature adipocytes in a dose-dependent manner, exerting maximal effect at a concentration of 0.6 × 10^{-9} M (140 ± 20%; P < 0.01; Fig. 3A). TNFα at a concentration of 0.6 × 10^{-8} M increased 11β-HSD-1 mRNA levels to 300% of control (P < 0.05), whereas IL-6 did not have any effect (Fig. 3E). TNFα caused an increase in glycerol levels with maximal effect at 0.6 × 10^{-9} M (165 ± 14% of control; P < 0.01; Fig. 3C). IL-1β up-regulated 11β-HSD-1 activity in mature adipocytes, with maximal effect reached at 0.6 × 10^{-9} M (160 ± 33%; P < 0.001; Table 2). A representative dose-response of this effect is shown in Fig. 3B. IL-1β caused an increase in glycerol levels with maximal effect at 0.6 × 10^{-10} M (159 ± 11% of control; P < 0.001; Fig. 3D).

IL-6 demonstrated inconsistent effects on 11β-HSD-1 activity ranging from 50% to 150% of control. These differences did not correlate with patient characteristics (r = 0.297; P > 0.1). IL-6 did not influence glycerol levels in the incubation media.

**Adrenergic effectors**

To test possible effects of the sympathetic nervous system, mature adipocytes were incubated with agonists of each of the adrenergic receptors. The β2 agonist salbutamol up-regulated 11β-HSD-1 activity, demonstrating maximal effect at a concentration of 10^{-7} M (162 ± 46%; P < 0.02; Table 2). A representative dose response demonstrating the up-regulatory effect of salbutamol is shown in Fig. 4A. The α2 agonist, clonidine, demonstrated a slight but significant down-regulation of 11β-HSD-1 activity at 10^{-7} M (82 ± 15%; P < 0.005; Table 2). A representative dose-response demonstrating the down-regulatory effect of clonidine is shown in Fig. 4B. Salbutamol did not influence glycerol levels in the incubation media.

The α1 agonist phenylephrine, the β1 agonists dobutamine and phenylpropanolamine, and the β3 agonist BRL 37344 did not affect 11β-HSD-1 activity (Table 3). Likewise, acetylcholine did not affect 11β-HSD-1 activity (Table 3).

**Other effectors**

GH, IGF-I, leptin, estradiol, dihydrotestosterone, T₃, and PRL did not influence 11β-HSD-1 activity (Table 3).

**Discussion**

This study investigated potential messengers that might mediate hypothalamic signals to mature adipose cells, resulting in modulation of 11β-HSD-1 activities. The results of this study demonstrate a number of such potential mediators. We used a primary cell culture system of mature human abdominal sc adipocytes from nonobese subjects. Mature adipocytes might play an important role in adipose metabolism, and although 11β-HSD-1 activity in stromal cells has been shown to be influenced by various factors (2, 3, 6–8), the mature adipocyte has been studied far less in this respect than its precursors. Furthermore, study of modulation of 11β-HSD-1 activity in mature adipocytes is crucial in light of the recent report that 11β-HSD-1 activity switches direction from a predominant dehydrogenase activity in the preadipocyte to predominant oxo-reductase activity in the mature omental adipocyte (9). Despite the fact that this switch did not occur in sc adipocytes, this reversal of 11β-HSD-1 activity in mature omental adipocytes emphasizes the importance of cell differentiation stage in cellular function. Likewise, modulation of 11β-HSD-1 activity might be substantially different between the preadipocyte and the mature adipocyte in regard to hypothalamic regulation of the enzyme, with significance regarding hypothalamic control of adiposity. We encountered a number of problems with our study system. The major problem was variability of results, as expected in a primary culture system originating from subjects with individual variations. To combat these problems, we repeated experiments at least 3 times, and in most instances we repeated experiments between 8 and 11 times. Despite this and despite careful observation of patient characteristics and methods, considerable variability remained.

We postulated that CRH and ACTH, might play important roles as hypothalamic regulators of 11β-HSD-1 activity, be-
cause changes in adiposity during acute stress and during states of excess cortisol secretion or effect, such as in Cushing’s syndrome, can be striking. Rat adipocytes express high-affinity ACTH receptors (10), and ACTH induces lipolysis in rat adipocytes through these receptors (11). Melanocyte-stimulating hormone/ACTH (4–10), representing the core...
sequence of all melanocortins, reduced obesity when administered to human subjects (12). ACTH suppresses leptin levels in rats and has been proposed as the mediator of a hypothalamo-pituitary-adrenal (HPA) axis-leptin regulatory loop (13). Vicennati and Pasquali (14) demonstrated hyperactivity of the HPA axis in obese women and also proposed that the HPA axis influences obesity through two distinct mechanisms that lead to functional hypercortisolism. The first was suspected to be of central origin, and the second, a peripheral one, located in the liver and adipose tissue (14, 15).

**Fig. 3.** Effect of TNFα (A) and representative dose-response effects of IL-1β (B) on 11B-HSD-1 activity (A and B) and medium glycerol (C and D) in a primary culture of human adipocytes. E, Real-time RT-PCR. Cells were incubated for 3 h with TNFα or IL-1β and 500 nM cortisone. Mean ± sd. *, P < 0.05; ***, P < 0.001. ANOVA P < 0.001 (A), P < 0.05 (C and D).
The results of the present study demonstrate a significant decrease in 11β-HSD-1 activity when adipocytes were incubated with either CRH or ACTH. CRH and ACTH deficiency become important candidates to directly mediate, at the adipose tissue level, the high cortisol/cortisone ratio of patients with hypothalamic obesity (4) and would comply with the mechanisms detailed previously (14, 15).

Few studies have investigated the role of CRH and ACTH as modulators of the renal type 2 isoform of 11β-HSD, mostly in relation to the hypertension and electrolyte abnormalities found in hypercortisolism. These have mostly demonstrated down-regulation of 11β-HSD-2 activity, as we have shown for the type 1 isoform (16, 17). Interestingly, antalarmin, a specific CRH 1 receptor inhibitor (18), did not reverse CRH inhibition of 11β-HSD-1 activity, which might exert this effect through the CRH 2 receptor or possibly through a third, as yet unidentified, receptor. At the same time, ACTH and CRH down-regulated cellular lipolysis, as indicated by a decrease in medium glycerol. These results contrast those found in the rat adipocyte model described previously (11), an effect that seems to vary between species (10). Our results suggest that the down-regulatory effect of CRH and ACTH is exerted not at a transcriptional level, as evidenced by a lack of change in 11β-HSD-1 mRNA, but rather by direct non-additive modulation of enzymatic activity, possibly through posttranslational modification (phosphorylation) of the enzyme (19–22).

In the acute setting, stress-related CRH and ACTH would down-regulate 11β-HSD-1 activity, reducing cortisol conversion and reducing adiposity. This might contribute to the weight loss of stress, which is often in excess of that related to reduced caloric intake (23). In the acute and chronic setting, the down-regulatory effect of CRH and ACTH on 11β-HSD-1 activity might counteract the up-regulatory effect of inflammatory cytokines and adrenergic activity that we have shown on this enzyme.

Other hormones that we screened as putative hypothalamic modulators of adipose 11β-HSD-1 activity did not influence this activity in our system. These included GH, PRL, IGF-I, leptin, estradiol, dihydrotestosterone, and T3. GH has been shown to decrease 11β-HSD-1 activity in vivo (24–28), as an expression of total body 11β-HSD-1 activity. Activity of 11β-HSD-1 is tissue specific (29–31), so that decreased 11β-HSD-1 due to GH might reflect liver 11β-HSD-1 activity (25) and not hold true for mature sc adipocytes. Moore et al. (8) showed a down-regulation of 11β-HSD-1 activity due to GH in adipose stromal cells, through IGF-I. Like GH, IGF-I did not influence 11β-HSD-1 activity in mature sc adipocytes. The difference between these results might be explained by the distinct differentiation stage of the cells, because 11β-HSD-1 activity has been shown to depend on cell differentiation (9, 32).

T3 was tested with the thought that it would be the end effector of the TRH-TSH- T3 axis, possibly affecting adipose metabolism through modulation of 11β-HSD-1. In previous studies, albeit using different species and tissues, thyroid hormones have had up-regulating (30), down-regulating (31, 33–35), or no (30) effects. Sex steroids decreased 11β-HSD-1 activity in sheep, rat, and human liver (36–39) and in rat testes (40).

It was recently suggested that in bulimic and anorexic patients (41, 42), circulating TNFα is derived from the central nervous system, suggesting an endocrine mechanism of secretion and acting. TNFα is under neural control (43), and clonidine suppresses plasma concentrations of TNFα (44).
Mental stress delays increases in cytokine responses, suggesting modulation of TNFα by sympathetic activity (45). Recent research has revealed an important role for cytokines in the metabolism of adipose tissue (46–48). Cytokines are expressed at significant levels by adipose tissue and correlate in the metabolism of adipose tissue (46).

Fig. 5. A model of hypothalamic modulation of 11β-HSD-1 activity in adipose tissue.

TNFα up-regulates β receptors in adipocytes (62). We show that a β2 agonist up-regulates 11β-HSD-1 activity, making TNFα a potent recruiter of local glucocorticoids at times of inflammation, stress, and sympathetic hyperactivity.

Adrenergic stimulation of β-receptors has an important role in lipolysis (63) and constitutes an efferent brain-to-adipose signaling pathway. We sought to investigate whether this signaling might modulate adipose 11β-HSD-1 activity. Our results show up-regulation of 11β-HSD-1 activity by β2 stimulation, with the converse results seen with regard to α2 receptors. No effect on 11β-HSD-1 activity was demonstrated with a β1 agonist, strengthening the notion that modulation of 11β-HSD-1 activity by the sympathetic system is a separate pathway to that of the classic lipolytic one. β Adrenergic receptors have been shown to induce lipolysis in rodents (64), and because these receptors are found in human adipose tissue (65), we postulated that they might be the mechanism for hypothalamic modulation of adipose 11β-HSD-1. We found no effect of a β3 agonist, although one reason for this may be that the activity of this receptor is low in sc fat in comparison to omental fat (65, 66).

In summary, we have demonstrated modulation of 11β-HSD-1 activity and availability of cortisol for intracrine effect in mature human sc adipocytes in vitro by a number of possible hypothalamic mediators (Fig. 5). Possible mediators that are used by the hypothalamus to regulate adipose tissue cortisol might include down-regulation of 11β-HSD-1 activity by the HPA axis through a direct CRH and ACTH effect, up-regulation of the enzyme in these cells by the β2 adrenergic system, and stimulation of the enzyme activity by the cytokines TNFα and IL-1β.

Damage to the hypothalamus and hypothalamic obesity (4) might result from CRH and ACTH deficiency, increased sympathetic tone, and increased cortisol availability. The same system may also be involved in adipose tissue activity by stress, inflammation, and sympathetic hyperactivity.

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References

dehydrogenase isozymes by growth hormone and insulin-like growth factor: in vivo and in vitro studies. J Clin Endocrinol Metab 84:4172–4177
man subcutaneous adipocytes and preadipocytes. J Clin Endocrinol Metab 86:2817–2825
60. Petruschke T, Hauner H 1993 Tumor necrosis factor-α prevents the differentiation of human adipocyte precursor cells and causes delipidation of newly developed fat cells. J Clin Endocrinol Metab 76:742–747