0013-7227/03/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 88(1):385–393

doi: 10.1210/jc.2002-020510

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

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Glucocorticoids are regulated at the prereceptor level by 11β-hydroxysteroid dehydrogenase (11β-HSD), which interconverts inactive 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 sc 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 ± 10% of control; $P < 0.001$) and caused a reduction in lipolysis. Likewise, ACTH 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 0.6×10^{-9} M (140 ± 20%; $P < 0.001$); the same cytokine increased 11β-HSD-1 mRNA levels to 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 0.6×10^{-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)

ABNORMAL METABOLISM OF glucocorticoids has been implicated in the pathophysiology of obesity for many years (1). Glucocorticoids are regulated at the prereceptor 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 oxo-reduction of inactive cortisone to active cortisol, functioning as a positive prereceptor 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-to-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.

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 *ob* (leptin), and by the lipolytic response to isoprot-

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

eronol. Mature cells were maintained in media containing 1 μM 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 11β-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 11β-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_3, CRH, ACTH, TNF α, phenylephrine, dobutamine, salbutamol, phenylpropanolamine, BRL 37344, prolactin (PRL), and acetyl choline were purchased from Sigma. IL-1β 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 averaged. ANOVA were performed on the mean values of the subjects ($n = 5$) to compare the expression across concentrations, applying Student's t test when it was significant, to compare treated cells to control untreated cells using Sigmastat 32 (SPSS, Inc., Chicago, IL), Origin 5 (Microcal Software Inc., Northampton, MA) and Excel (Microsoft Corp., Redmond, WA) computer software. In the case of ACTH and CRH, when in two subjects most results indicated inhibition but some unexpected results showed stimulation, the binomial test was used to test the hypothesis that the negative response is the dominant one (*i.e.* the probability to get a negative response is higher than 0.5). The probability of type I error was set at 0.1, and a P value less than 0.1 was considered significant. Correlations were calculated by the Pearson's coefficient.

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.

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 deoxyribonuclease I (GenHunter, Corp., Nashville, TN), total RNA (1 μg) was reverse transcribed to complementary DNA by a reaction containing 2 mM deoxynucleotide mix, 100 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 11β-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′-TTGGAATATTTGGGCTAACAGTGA-3′; reverse, 5′-CCTCCTCTAATTTTCCTTCCTTGAG-3. The dye utilized was a TaqMan probe 200 nmol/liter 5′-FAM-AGGATTAAAATGCTGATTCTGCCCCCAG-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 11β-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 11β-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 11β-HSD-1 activity

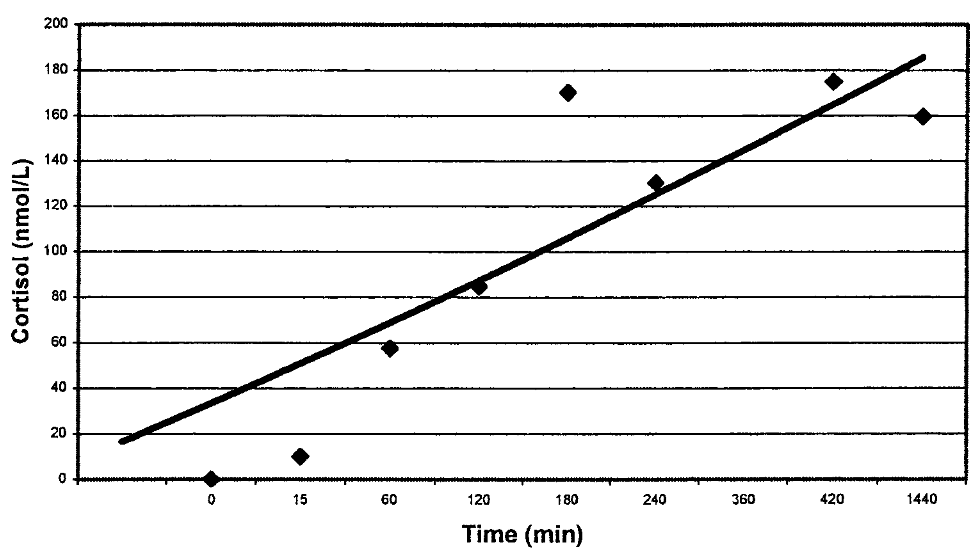

FIG. 1. Time course of adipocyte 11β-HSD-1 activity. Subcutaneous mature adipocytes were incubated with 500 nM cortisone for varying time intervals. Concentration of cortisol was then measured by RIA in the culture medium.

TABLE 2. Summary of effectors that modulated 11β-HSD-1 activity in a primary culture of mature human sc adipocytes

Effector	Maximal effect on 11β-HSD-1 activity	Maximal effective concentration	Maximal lipolytic effect	Maximal effective concentration
CRH	65 ± 10%	10^{-9} M	77 ± 21%	10^{-7} M
ACTH	65 ± 20%	10^{-9} M	72 ± 9%	10^{-7} M
TNFα	140 ± 20%	0.6×10^{-9} M	165 ± 14%	0.6×10^{-9} M
IL-1β	160 ± 33%	0.6×10^{-9} M	159 ± 11%	0.6×10^{-10} M
Salbutamol	162 ± 46%	10^{-7} M	100%	None
Clonidine	82 ± 15%	10^{-7} M		

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; $P = 0.05$; 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; $P < 0.001$; 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%; $P < 0.001$; 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^{-9} 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 adipocyte 11β-HSD-1 activity (Table 3). Likewise, acetylcholine did not affect adipocyte 11β-HSD-1 activity (Table 3).

Other effectors

GH, IGF-I, leptin, estradiol, dihydrotestosterone, T_3, 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-

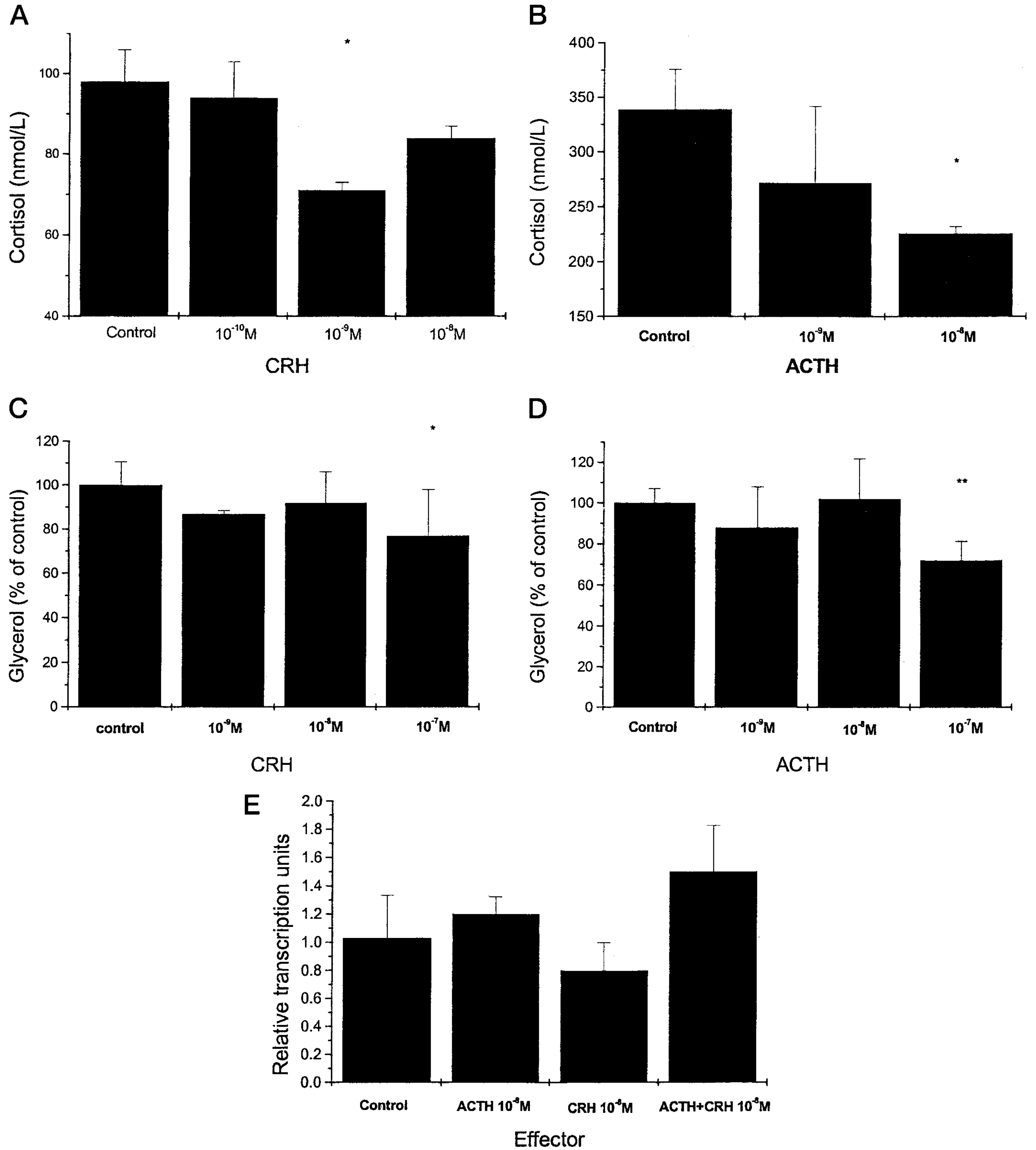

FIG. 2. Representative dose-response effects of CRH (A and C) and ACTH (B and D) on 11β-HSD-1 activity (A and B) and medium glycerol (C and D) in a primary culture of human adipocytes. E, Real-time RT-PCR of CRH and ACTH. Cells were incubated for 3 h with CRH or ACTH and 500 nM cortisone. Mean ± SD of five replicate wells. *, $P < 0.05$; **, $P < 0.01$. ANOVA $P < 0.05$ (A and C); $P < 0.001$ (D).

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

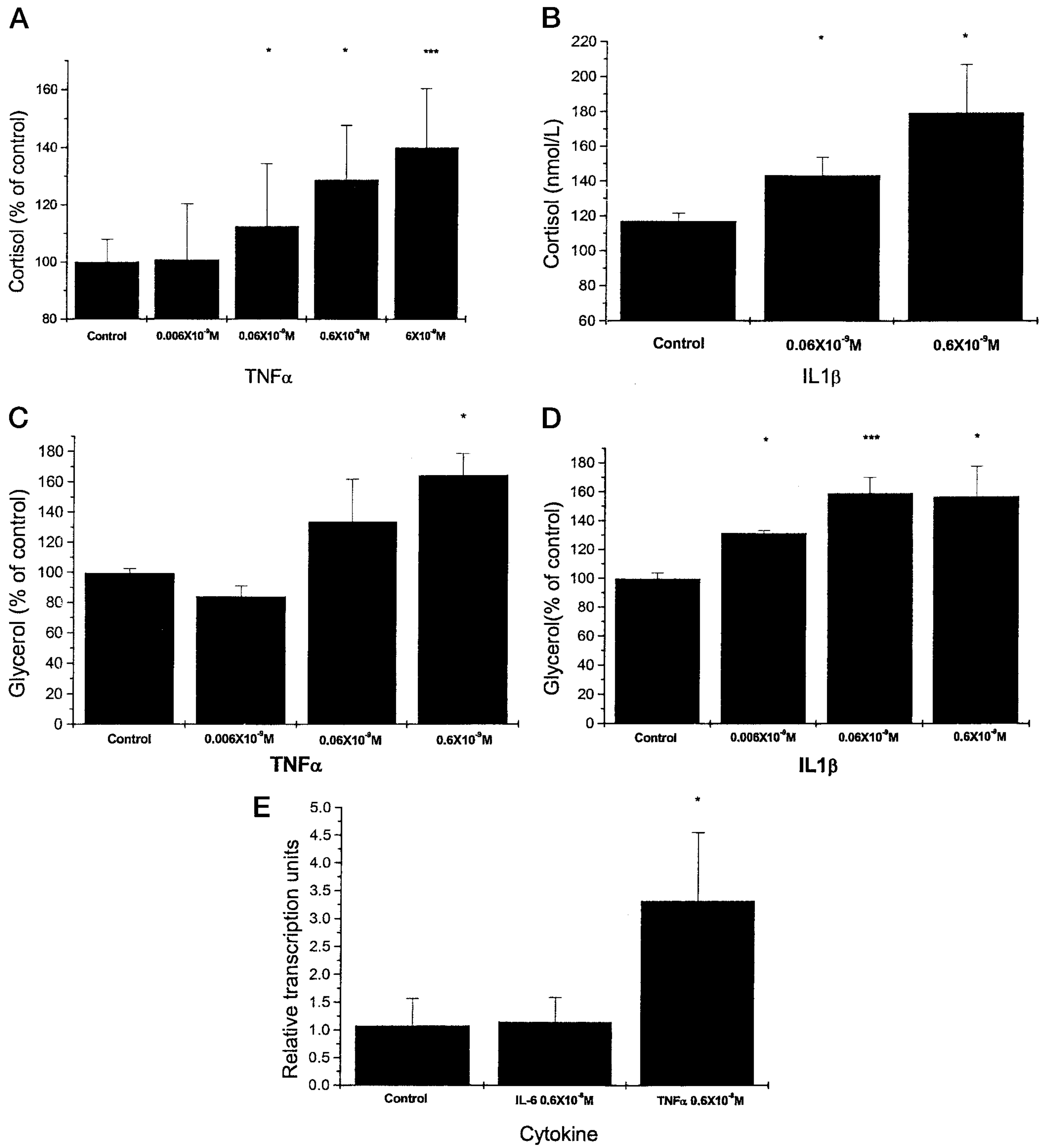

FIG. 3. Effect of TNFα (A) and representative dose-response effects of IL-1β (B) on 11B-HSD-1 activity (A and B) and medium glycerol (C and D) in a primary culture of human adipocytes. E, Real-time RT-PCR. Cells were incubated for 3 h with TNFα or IL-1β and 500 nM cortisone. Mean ± SD. *, $P < 0.05$; ***, $P < 0.001$. ANOVA $P < 0.001$ (A), $P < 0.05$ (C and D).

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).

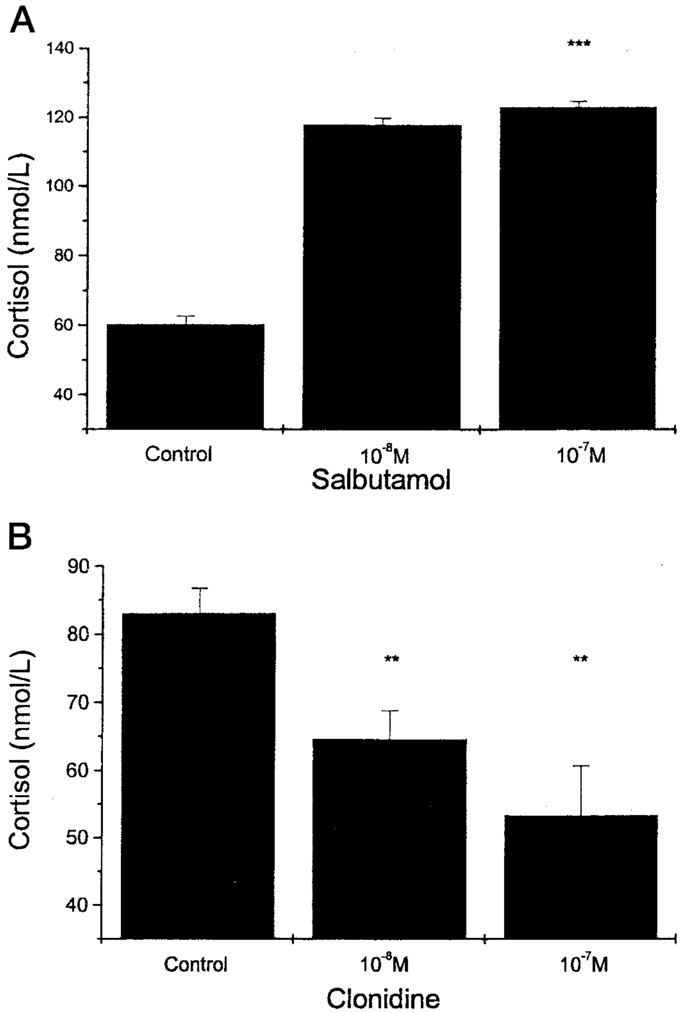

FIG. 4. Representative dose-response effects of salbutamol (A) and clonidine (B) on 11B-HSD-1 activity in a primary culture of human adipocytes. Cells were incubated for 3 h with salbutamol or clonidine and 500 nM cortisone. Mean ± SD. **, $P < 0.01$; ***, $P < 0.001$. ANOVA $P < 0.001$(A), $P < 0.01$(B).

TABLE 3. Effectors tested that did not influence 11-HSD-1 activity in a primary culture of human adipocytes

- GH
- IGF-I
- Leptin
- PRL
- Estradiol
- Dihydrotestosterone
- T_3
- IL-6
- Phenylephrine (α1 agonist)
- Dobutamine (β1 agonist)
- Phenylpropanolamine (β1 agonist)
- BRL 37344 (β3 agonist)
- Acetyl choline

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 nonadditive 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 cortisone to 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 T_3. 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).

T_3 was tested with the thought that it would be the end effector of the TRH-TSH- T_4-T_3 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 testis (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).

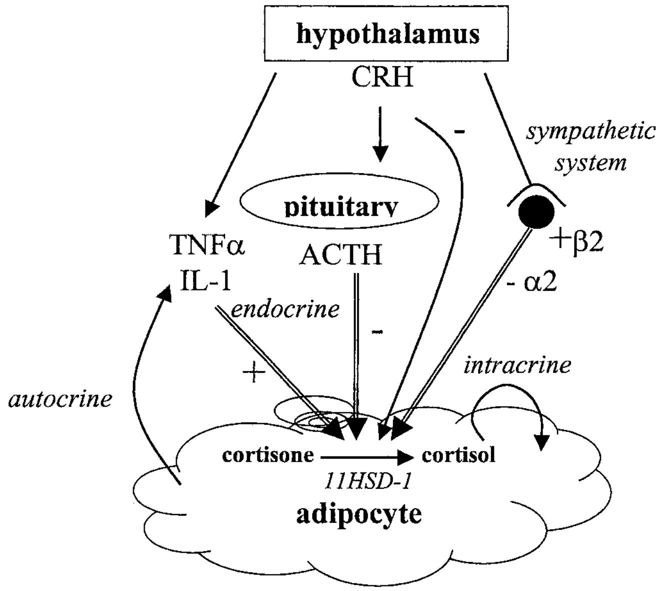

FIG. 5. A model of hypothalamic modulation of 11B-HSD-1 activity in adipose tissue.

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 with BMI (49–52). Also, cytokines have been shown to affect 11β-HSD-1 activity in various tissues (7, 53–57), but to the best of our knowledge, their effect on 11β-HSD-1 activity has not been studied in mature, fully differentiated adipocytes. On this basis, and in the context of the study of putative mechanisms of hypothalamic regulation of mature adipocytes, we investigated possible modulation of mature adipocytic 11β-HSD-1 activity by cytokines. Both TNFα and IL-1β up-regulated 11β-HSD-1 activity in sc adipocytes. These results are in accord with previous results obtained from a model using 11β-HSD-2 expression by rat glomerular mesangial cells (53), and they expand on the recent observation of a similar up-regulatory effect of cytokines in human sc and omental stromal cells (54). Moreover, we show for the first time that in the mature adipocyte, TNFα markedly up-regulates this activity through a gene transcription effect. IL-6, also produced at high levels by adipose tissue itself, had no effect on 11β-HSD-1 activity.

There is conflicting data about whether glucocorticoids regulate TNFα release by adipocytes, while they inhibit its effects in adipose tissue (56–58). Therefore, locally generated, intracrine cortisol might counter the effects of adipose paracrine/autocrine TNFα. In hypothalamic obesity, a lack of CRH and ACTH might allow for unopposed adipose paracrine/autocrine TNFα, thus leading to an increase in 11β-HSD-1 activity and an increase in local cortisol effect and obesity. During an acute stress or inflammatory process, TNFα and IL-1β could recruit cortisol via an autocrine/paracrine mechanism for a local intracrine effect. In a longer time frame, TNFα (cachexin) and IL-1β reduce adiposity through arrest of adipocyte differentiation, increase of lipolysis, and an increase in apoptosis (59–61). Interestingly, TNFα up-regulates β2 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. β3 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 cytokine availability. The same system may also be involved in adipose tissue activity by stress, inflammation, and sympathetic hyperactivity.

Acknowledgments

Received March 31, 2002. Accepted October 15, 2002.

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