Hormonal regulation of hormone-sensitive lipase activity and mRNA levels in isolated rat adipocytes

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Abstract Hormone-sensitive lipase (HSL) mediates the lipolysis of triacylglycerol from mammalian adipocytes, resulting in the release of non-esterified fatty acids and glycerol. Although numerous studies have examined the hormonal regulation of HSL, the measurement of HSL mRNA levels in response to hormonal regulators has not been studied. This study was designed to determine the effects of epinephrine, growth hormone, glucagon, and dexamethasone on HSL expression by measuring HSL mRNA levels and glycerol release in primary cultures of rat adipocytes. Exposure of adipocytes to epinephrine at 10^{-7} M and 10^{-5} M for 4 h resulted in an increase in medium glycerol (209 ± 46%, and 284 ± 58% of control, $P < 0.001$, respectively). However, no change in HSL mRNA levels occurred due to the epinephrine treatment. Similarly, the peptides glucagon (10^{-7} M and 10^{-5} M for 4 h) and growth hormone (100 ng/ml for 24 h) resulted in increased medium glycerol and had no effect on HSL mRNA levels in adipocytes. Dexamethasone was added to adipocyte cultures for 4 and 24 h, and resulted in a dose-dependent increase of medium glycerol (102 ± 8%, 138 ± 8% ($P < 0.001$), and 168 ± 24% ($P < 0.001$) for 10^{-8} M, 10^{-7} M, and 10^{-6} M, respectively). In contrast to the other hormones, however, dexamethasone yielded increases in HSL mRNA levels of 368 ± 87% and 452 ± 29% of control at 10^{-7} M and 10^{-6} M, respectively. Thus, epinephrine, glucagon, and growth hormone increased the activity of HSL when added to primary cultures of adipocytes through post-transcriptional mechanisms, and not through a change in HSL mRNA level. Dexamethasone, however, increased HSL mRNA levels by approximately 4-fold, and this study represents the first demonstration of HSL mRNA regulation in adipose tissue.—**Slavin, B. G., J. M. Ong, and P. A. Kern.** Hormonal regulation of hormone-sensitive lipase activity and mRNA levels in isolated rat adipocytes. *J. Lipid Res.* 1994. **35:** 1535–1541.

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Hormone-sensitive lipase (HSL) is the enzyme responsible for the hydrolysis of triacylglycerol from the lipid droplet of adipocytes into glycerol and non-esterified fatty acids (NEFA) (1). These NEFA are then released into the blood stream, where they provide energy for muscle tissue, and are taken up by the liver and oxidized, esterified, or converted into ketone bodies. The control of adipose tissue lipolysis by HSL is determined by a number of hormones (2–6). HSL activity is inhibited by insulin, adenosine, and prostaglandins, and stimulated by hormones that raise intracellular levels of cAMP, such as catecholamines and glucagon. In contrast to the HSL-mediated release of NEFA, the main enzyme involved in adipose tissue lipid accumulation is lipoprotein lipase (LPL). LPL is stimulated by insulin and inhibited by catecholamines (7). Thus, the regulation of adipose tissue triglyceride accumulation during normal cycles of fasting and feeding is determined mainly by the inversely regulated actions of LPL and HSL.

A number of studies have examined the cellular regulation of HSL. An important mechanism for HSL regulation occurs posttranslationally. The HSL protein contains 757 amino acids and is strongly activated by hormone-stimulated reversible phosphorylation of Ser^{563} by a cAMP-dependent protein kinase (3, 8, 9). A recent study suggested that activation of HSL was accompanied by translocation from the cytosol to the lipid storage droplet of adipocytes (10). Although much of the regulation of HSL likely occurs through posttranslational activation, it is possible that the synthesis of the HSL mRNA or protein may also be subject to regulation. Kraemer, Tavangar, and Hoffman (11) examined HSL mRNA levels in rats during different stages of development. In adipose tissue, there was no change in HSL mRNA level between 3 weeks and 2 years of age, although developmental changes were noted in heart, adrenal, and testes.

In this study, we examined the hormonal regulation of HSL using primary cultures of rat adipocytes. The hormones tested include epinephrine, glucagon, growth hormone, and dexamethasone, and HSL activity was measured, along with HSL mRNA levels.

Abbreviations: HSL, hormone-sensitive lipase; ATP, adenosine triphosphate; NEFA, nonesterified fatty acid; LPL, lipoprotein lipase.

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METHODS

Rat adipocyte isolation procedure

Primary cultures of rat adipocytes were prepared from the epididymal fat pads of male Sprague-Dawley rats (200–220 g), as previously described (12). After incubating the cells overnight in serum-free medium 199, hormones were added in the concentrations described below. After the indicated times, the medium was removed and glycerol was measured. RNA was then extracted from the cells, as described below.

Glycerol assay

HSL activity was assessed by measuring the amount of glycerol released into the medium. The assay for glycerol has been described previously (13). In this assay, glycerol was phosphorylated with [gamma-^{32}P]ATP and glycerokinase in a reagent mixture buffered with triethanolamine hydrochloride and bovine serum albumin. The residual [gamma-^{32}P]ATP underwent hydrolysis by exposure to phosphoric-perchloric acid at 95°C. After removal of the free [^{32}P]phosphate by ammonium molybdate/triethylamine precipitation, an aliquot of the supernatant was measured in a scintillation counter. Standard dose response curves for glycerol concentrations ranging from 375 to 3000 pmol were used in each experiment. Samples were assayed in duplicate and taken off the linear range of the standard curve.

RNA extraction and Northern blotting of HSL mRNA

RNA was isolated from adipocytes via the guanidinium–phenol–chloroform method of Chomczynski and Sacchi (14), as described by us previously (15), and subjected to Northern analysis. The extracted RNA samples were resolved by electrophoresis on a 2.2 M formaldehyde–1% agarose gel and transferred onto a nylon membrane (Hybond-N, Amersham Corp.). cDNA probes for HSL (8) and gamma-actin (16) were radiolabeled by the random-priming method (17) with [^{32}P]dCTP (3,000 Ci/mmol; ICN Biomedical, Irvine, CA). The nylon membranes were prehybridized in a solution containing sodium phosphate buffer (40 mM, pH 7.2), 5% SDS, 1% bovine serum albumin, 1 mM EDTA (pH 8.0), and 0.1% diethyl pyrocarbonate (DEPC)-treated water. The cDNA probe (1×10^6 cpm/ml) was hybridized to the blotted membrane in the same solution as above. The blots were washed four times with 2.5% SDS, 0.1% bovine serum albumin, and 40 mM sodium phosphate (pH 7.24) at 60°C. The blot was dried at room temperature and exposed to XAR-5 X-ray film (Eastman Kodak Co., Rochester, NY) at −70°C. The images from the Northern blots were quantitated by densitometry using a Soft Laser Scanning Densitometer SLR-2D/1D (Zeineh). Blots were scanned vertically through the bands, and the area under the curve generated by the scan was calculated. For all Northern blots, a mini-gel was also run with the same RNA and was stained with ethidium bromide to identify the rRNA bands. Except for cells exposed to dexamethasone, the ratio of actin mRNA to total cellular RNA remained constant, and actin cDNA hybridization permitted accurate comparisons between samples (see text).

Statistics

All data are expressed as the mean ± SEM and were analyzed using ANOVA.

RESULTS

A number of hormones are known to affect HSL activity. To determine their effects on HSL mRNA levels, several hormones were added to the medium of primary cultures of adipocytes. Medium glycerol was measured to assess HSL activity, and RNA was extracted for Northern blotting.

Epinephrine

To assess the effects of epinephrine on HSL, adipocytes were treated with epinephrine (10^{-9} M to 10^{-5} M) for 4 and 24 h. The medium was then collected and assayed for HSL activity as described under Methods. As shown in **Fig. 1A,** epinephrine treatment of isolated adipocytes led to a dose-dependent increase in medium glycerol, up to a maximum of 284 ± 58% of control at 10^{-5} M at 4 h ($P < 0.001$). This effect was sustained when the cells were exposed to epinephrine for 24 h. To determine whether the increase in HSL activity by epinephrine was reflected by an increase in HSL mRNA level, Northern blots were performed. As shown by the representative Northern blots in Fig. 1B, and the summarized data in Fig. 1C, treatment of adipocytes with epinephrine had no effect on HSL mRNA levels (Fig. 1B).

Glucagon and growth hormone

Glucagon was added to the rat adipocyte cultures for 4 h, and HSL expression was assessed. As with epinephrine, a 4-h treatment with glucagon produced a dose-dependent increase of HSL activity, with a maximum increase at a concentration of 10^{-5} M of 163 ± 10% of control ($P < 0.001$) (**Fig. 2A**). A similar effect on glycerol release was observed at 24 h. When HSL mRNA levels were measured in these cells, there were no differences between control and hormone-exposed adipocytes (Figs. 2B and 2C). Northern blots of a 24-h exposure to glucagon also showed no effect on HSL mRNA levels (data not shown).

In a similar manner, the effects of growth hormone were assessed. Growth hormone was added to the cultures for 4 and 24 h at a concentration of 100 ng/ml. As shown

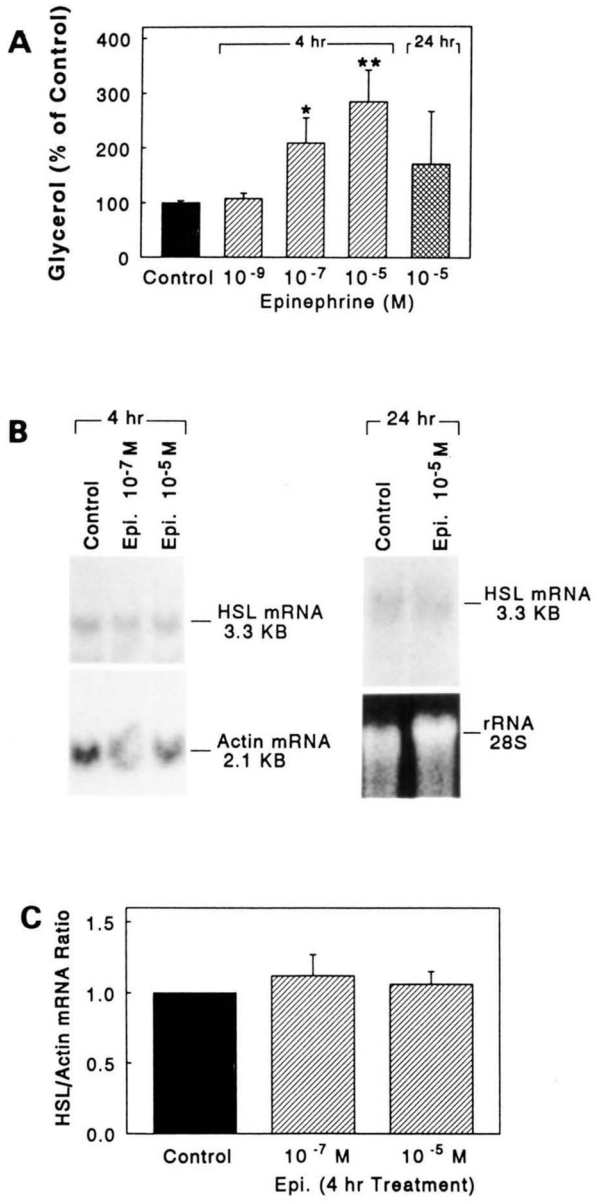

Fig. 1. Effect of epinephrine on HSL expression in isolated rat adipocytes. (A) Primary cultures of rat adipocytes were exposed for 4 or 24 h at the indicated concentrations of epinephrine, and medium glycerol levels were measured. Medium glycerol in the control cultures was 0.161 ± 0.01 μmol/min per 10^6 cells. Data are expressed as the percentage of control (adipocytes not exposed to epinephrine); mean ± SE. $^*P < 0.001$ versus control. (n = 4–5). (B) Total RNA was extracted from the cultured cells and analyzed by Northern blotting with ^{32}P-labeled cDNA probes for HSL and gamma-actin. For the 24-h experiments, equal loading of the gels was confirmed by ethidium bromide staining of the rRNA bands. (C) HSL/actin ratio of the Northern blots from the 4-h time points (n = 3–4).

in **Fig. 3**, growth hormone produced a small but significant increase in medium glycerol levels, which was 127 ± 1.2% ($P < 0.05$) of untreated control cells at 24 h (Fig. 3A). No effect of growth hormone was observed at 4 h. A higher concentration of growth hormone (500 ng/ml) did not yield a greater increase in media glycerol level (data not shown). Northern blot analysis revealed no changes in HSL mRNA levels at either 4 or 24 h of treatment, as shown in Figs. 3B and 3C.

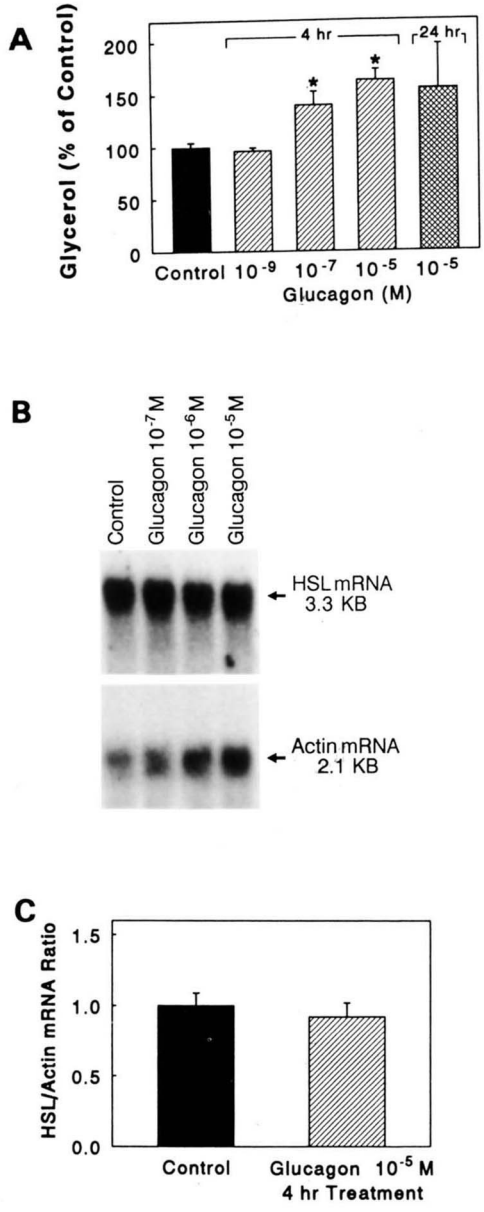

Fig. 2. Effect of glucagon on HSL expression. (A) Cultured rat adipocytes were treated with glucagon at the indicated concentrations for 4–24 h followed by measurement of medium glycerol levels. Data are expressed as the percentage of control (mean ± SE). Medium glycerol level in control cultures was 0.112 ± 0.01 μmol/min per 10^6 cells. $^*P < 0.001$ versus control; n = 4–6. (B) Representative Northern blot of HSL mRNA levels as described in the Methods section. (C) HSL/actin ratio of all the Northern blots at the 4-h time point.

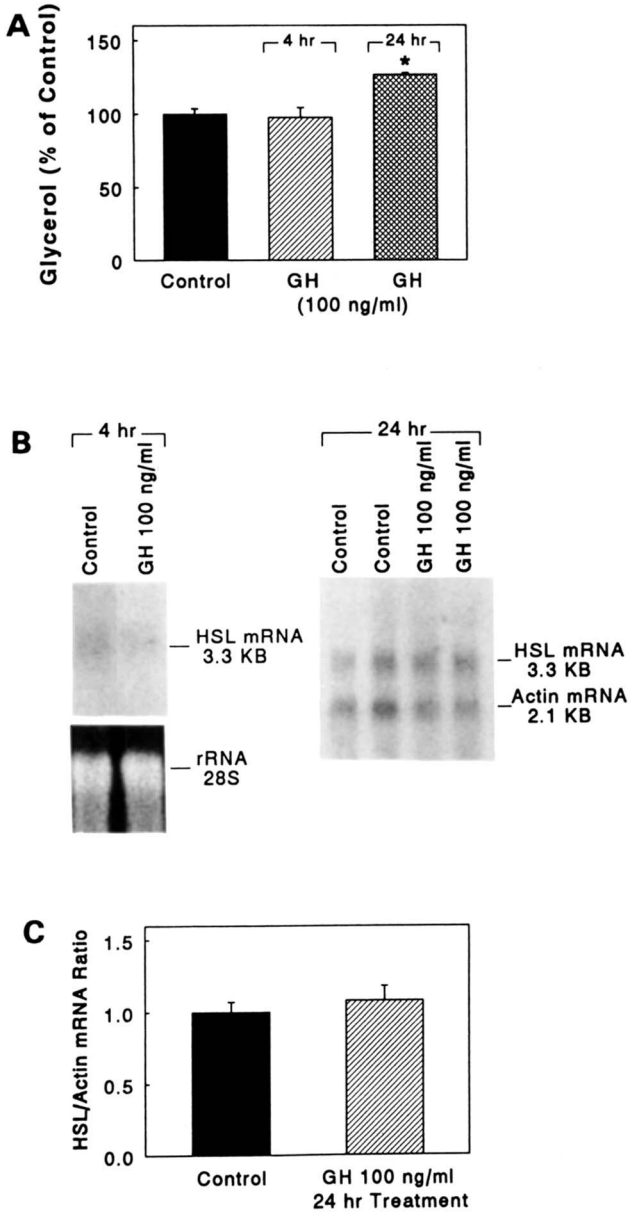

Fig. 3. HSL expression in growth hormone-treated rat adipocytes. (A) After rat adipocytes were treated for 4 or 24 h with human growth hormone (100 ng/ml), the medium of both treated and control cells was removed for measurement of glycerol. Medium glycerol levels in control cultures were 0.115 ± 0.01 μmol/min per 10^6 cells. Data are expressed as the percentage of control (mean ± SE, n = 4–5). *$P < 0.05$ versus control. (B) Representative Northern blots at the indicated times and concentrations of growth hormone. For the 4-h experiments, equal loading of the gels was confirmed by ethidium bromide staining of the rRNA bands. (C) HSL/actin ratio of the Northern blots from the 24-h time point.

Effect of dexamethasone on HSL expression

To assess the effect of dexamethasone on HSL expression, cells were exposed to various concentrations of the steroid hormone for a 4- or 24-h period. At 24 h, treatment of adipocytes with dexamethasone resulted in a dose-dependent increase in medium glycerol, with a maximum increase at a concentration of 10^{-6} M of 168 ± 24% of control ($P < 0.001$) (**Fig. 4A**). This increase in medium glycerol was not observed in cells treated with dexamethasone for 4 h. HSL mRNA levels of these cells were assessed and representative Northern blots are shown in Fig. 4B. Dexamethasone resulted in a significant increase in HSL mRNA levels. As demonstrated by us previously (18), dexamethasone also resulted in a decrease in the level of the gamma-actin mRNA. This is demonstrated in Fig. 4B using the 4-h and 24-h data. At both time points, the gels were loaded with equal amounts of total RNA. The identification of the rRNA bands by ethidium bromide staining is shown for the 4-h time point. Therefore, the HSL/actin ratio may exaggerate the increase in HSL expression because of the fall in actin expression. To quantitate the level of induction of the HSL mRNA, the 24-h data was expressed using both gamma-actin, and total RNA. When the data were expressed as HSL/gamma-actin mRNA ratio (Fig. 4C, insert), HSL mRNA increases to 482 ± 166 ($P < 0.01$) and 1325 ± 96% of control ($P < 0.001$) for 10^{-7} M and 10^{-6} M, respectively. When the effects of dexamethasone were expressed relative to total RNA, HSL mRNA levels increased to 368 ± 87 and 452 ± 29% of control at 10^{-7} M and 10^{-6} M, respectively.

DISCUSSION

During adipose tissue lipolysis, free fatty acids and glycerol are mobilized from adipocytes through the action of HSL. Much of the regulation of HSL is due to the reversible phosphorylation of Ser^{563}, which results in a large increase in HSL specific activity (3). Hormones that stimulate adenylate cyclase, such as catecholamines, glucagon, and ACTH, increase HSL phosphorylation by stimulation of a cAMP-dependent protein kinase (3, 8, 9). Whether or not reversible phosphorylation/dephosphorylation is the only mechanism by which HSL is regulated is not known. With the recent development of the cDNA for HSL (8, 19), the mechanism of HSL regulation can be examined at the mRNA level.

In this study, primary cultures of isolated rat adipocytes were used, such that only direct hormonal effects on adipocytes would be detected without any contribution from possible paracrine effects from other cells in adipose tissue. Epinephrine and glucagon, which stimulate adenylate cyclase, resulting in HSL phosphorylation and increased HSL activity (3), predictably increased HSL activity, but had no effect on HSL mRNA levels. Growth hormone increases plasma NEFA levels when injected into animals or humans (20), although the effects of growth hormone in vitro are more modest (21–24). In this

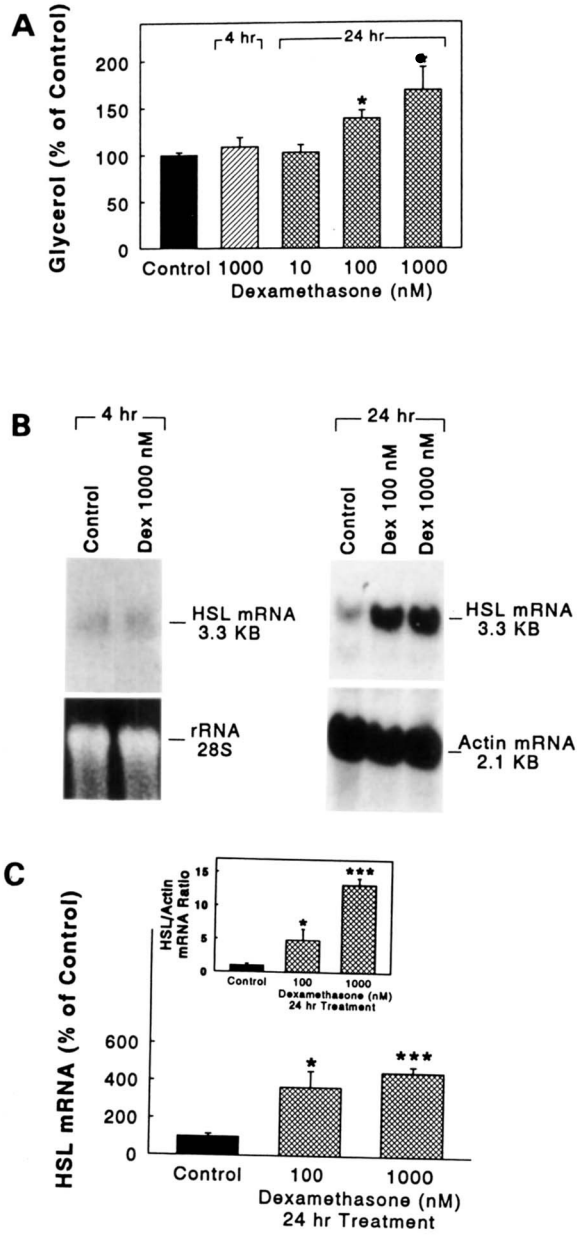

Fig. 4. Effect of dexamethasone on rat adipocyte HSL expression. Isolated rat adipocytes were exposed to dexamethasone for 4 and 24 h at the indicated concentrations. HSL activity was then assessed by measuring medium glycerol (A), and HSL mRNA levels were determined by Northern blotting (B and C). For (A), data are expressed as the percentage of control (mean ± SE, n = 4–6), and control medium glycerol levels were 0.130 ± 0.01 μmol/min per 10^6 cells. In (B), equal loading of the gels was confirmed by ethidium bromide staining of the rRNA bands for the 4-h time point. In (C), the HSL mRNA levels are expressed in relation to total RNA, and dexamethasone-treated cells are expressed as the percent of control cells. As demonstrated previously (18), dexamethasone treatment of adipocytes results in a decrease in actin expression. (C) inset: HSL/gamma-actin mRNA ratio (see text). Mean ± SE, n = 4–5. $^*P < 0.05$ and $^{**}P < 0.01$, $^{***}P < 0.001$ versus control.

study, growth hormone was added to primary cultures of adipocytes, and yielded a small but consistent increase in HSL activity, and no significant effect on HSL mRNA levels.

Thus, our studies suggest that the changes in HSL activity due to epinephrine, glucagon, and growth hormone are presumably due to posttranscriptional changes. Because of the well-described activation of HSL by adenylate cyclase-mediated reversible phosphorylation, glucagon and epinephrine likely operate through this mechanism. Other studies, however, have suggested other mechanisms for the epinephrine-mediated increase in lipolysis (25). In a recent study, the activation of lipolysis in adipocytes was controlled by the translocation of the lipase protein to the surface of the lipid storage droplet (10). The mechanism of the increase in lipolysis by growth hormone is not clear. Whereas catecholamines increase lipolysis very quickly, the effect of growth hormone takes several hours (26, 27). Gorin et al. (28) have provided evidence for a role for protein kinase C in the stimulation of lipolysis by growth hormone. Lipolysis was stimulated by a phorbol ester, and the lipolytic response to growth hormone was decreased by inhibitors of phorbol esters. Therefore, these authors suggest that growth hormone may trigger a cellular increase in cAMP, leading to a stimulation of HSL, through a mechanism independent of adenylate cyclase.

In agreement with previous studies (23, 28–32), dexamethasone increased HSL activity; however, this increase in HSL activity was accompanied by an increase in HSL mRNA levels. Indeed, the increase in HSL mRNA level was greater than the increase in medium glycerol, suggesting that the adipocytes may have partially compensated for the increased HSL mRNA through other mechanisms. The increase in HSL mRNA in response to dexamethasone may explain the potentiation of other hormonal responses by dexamethasone (21, 23, 24, 28, 29, 32). Whereas other hormones increase HSL through posttranscriptional mechanisms, dexamethasone increases HSL mRNA levels, and therefore amplifies posttranscriptional effects. Although this study used primary cultures of adipocytes, preadipocytes could have been adherent to the cells. Thus, the stimulation of preadipocyte development by dexamethasone (33) could have contributed to these observations. This study contains the first demonstration of HSL mRNA regulation in adipose tissue in vitro. A previous study of HSL ontogeny demonstrated essentially no change in adipose tissue HSL mRNA levels in rats during development, although other tissues demonstrated some changes with sexual maturation (11). In a recent study, there was a small increase in HSL mRNA in testes of rats that were injected with hCG (human chorionic gonadotropin) (34).

The increase in HSL mRNA in response to the glucocorticoid dexamethasone has potential clinical significance. Excessive glucocorticoids cause the development of central (intraabdominal) adiposity, along with clinical sequellae such as insulin resistance, hypertension, and hyperlipidemia (35, 36). Many of the sequellae of

common forms of intraabdominal obesity may be due to the high rate of lipolysis of omental adipose tissue (37, 38). Thus, the increased expression of HSL in certain adipose depots, perhaps in response to glucocorticoids, may be a mechanism for the elevated lipolysis of abdominal adipose tissue.

This study used primary cultures of rat adipocytes. Other cells in adipose tissue, as well as adipocytes themselves, are known to produce hormones that affect adipocyte lipolysis, and these local effects may be as important as the direct effects of the hormones studied herein. For example, adenosine, prostaglandins, and tumor necrosis factor (TNF) are all locally produced substances in adipose tissue that have been demonstrated to affect lipolysis in adipocytes (39–43). Thus, this in vitro study with adipocytes may not necessarily be reflective of the effects of the same hormones in vivo.

In summary, primary cultures of rat adipocytes were treated with epinephrine, glucagon, growth hormone, and dexamethasone, and HSL activity and mRNA levels were measured. Whereas each of these hormones resulted in increases in HSL activity (manifested by increased medium glycerol), only dexamethasone resulted in an increase in HSL mRNA. Therefore, much regulation of HSL is likely due to reversible phosphorylation/dephosphorylation, although some regulation at the mRNA level is possible in response to dexamethasone.

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