Hormonal Regulation of 18S RNA, Leptin mRNA, and Leptin Release in Adipocytes From Hypothyroid Rats

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The present studies were designed to examine the regulation of leptin release in primary cultures of adipocytes from fed hypothyroid rats incubated with hormones for 24 hours. Leptin release was increased in the presence of dexamethasone, while the decrease in leptin mRNA content over a 24-hour incubation was reduced by dexamethasone. Dexamethasone did not affect the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA or 18S RNA content of adipocytes. Insulin increased leptin release by adipocytes in both the absence and presence of dexamethasone. Although insulin also prevented the loss of leptin mRNA, this effect was less than that observed for GAPDH mRNA or 18S RNA content. In isolated adipocytes, the loss of almost half the 18S RNA content over a 24-hour incubation was prevented in the presence of insulin but not oxytocin or epidermal growth factor (EGF). The specific β_3 catecholamine agonist CI 316,243 inhibited the effects of dexamethasone on leptin release and leptin mRNA accumulation, as did EGF, without affecting 18S RNA content. Oxytocin inhibited the increase in leptin release due to dexamethasone without affecting leptin mRNA levels. These data indicate that although dexamethasone and insulin are positive regulators of leptin release, only dexamethasone specifically prevented the loss of leptin mRNA in cultured rat adipocytes. In contrast, insulin, but not dexamethasone, prevented the marked loss in 18S RNA observed over a 24-hour incubation of rat adipocytes.

LEPTIN is produced by white adipocytes and is involved in the central regulation of appetite and metabolism.[1,2] The rat adipocyte in primary culture was used in our studies, since the level of leptin expression in established cell lines such as 3T3-L1 or 3T3-F422A preadipocytes is less than 1% of that in intact adipose tissue.[3] These cells represent a homogeneous preparation of a single cell type that can be incubated in primary culture for 24 hours. Adipocytes are responsive to most hormones that affect adipose tissue metabolism,[4,5] and release leptin in response to dexamethasone.[6,7] However, the present results demonstrate a marked loss of 18S RNA during the first 24 hours of primary culture in the absence of insulin. Furthermore, there is controversy with regard to insulin regulation of leptin formation and release by adipocytes, since an increase,[8] decrease,[9] and no change[6] due to insulin have been reported with respect to leptin mRNA accumulation.

The present studies were designed to determine whether insulin stimulation of leptin release in adipocytes is accompanied by similar changes in leptin mRNA content. We also examined whether oxytocin and epidermal growth factor (EGF) could mimic the effects of insulin on 18S RNA content and leptin release.

MATERIALS AND METHODS

Adipocytes were obtained from male Sprague-Dawley rats in which hypothyroidism was induced by a 3-week low-iodine diet with 6-*N*-propyl-2-thiouracil in the drinking water (62.5 mg/L).[10] The body weight of hypothyroid rats after 3 weeks on the diet was 335 ± 6 g (mean ± SEM of 20 rats). In the studies shown in Fig 1, adipocytes were also obtained from euthyroid rats age-matched to the hypothyroid rats, and the body weight was 330 ± 6 g (mean ± SEM of 20 rats). Adipocytes were prepared by digestion of the epididymal adipose tissue from each rat in 5.5 mL buffer containing 1 mg/mL bacterial collagenase (*Clostridium histolyticum* CLS1 238 U/mg; Worthington Biochemical, Freehold, NJ) as described by Gokmen-Polar et al.[5]

The buffer for digestion and incubation of the cells was Dulbecco's modified Eagle's medium/Ham's F12 (1:1, #2906; Sigma, St Louis, MO) containing 17.5 mmol/L glucose, 121 mmol/L NaCl, 4 mmol/L KCl, 1 mmol/L $CaCl_2$, 25 mmol/L HEPES, 2.4 mmol/L sodium bicarbonate, 40 mg/mL albumin, 5 μg/mL ethanolamine, 0.1 ng/mL sodium selenite, 90 μg/mL penicillin G, and 150 μg/mL streptomycin sulfate. For incubation of the cells, we added ascorbic acid (55 μmol/L), leupeptin (10 μg/mL), and aprotinin (10 μg/mL). The buffer with albumin and other additions was filtered through a 0.2-μm filter. Adipocytes were isolated and incubated under sterile conditions in 50-mL polypropylene tubes shaken at 11 rpm in a gyratory water bath. The adipocytes were filtered through 1,000-μm nylon mesh, and approximately 0.4 to 0.5 g packed cells (600,000 to 1,000,000) were incubated in 10 mL medium for 24 hours. Adipocytes obtained from two rats were divided among 13 to 14 tubes for a given experiment. After removal of the medium, the total RNA was extracted in each of 13 to 14 tubes by the procedure of Chomczynski and Sacchi.[11] The leptin content of 100-μL aliquots of incubation medium was measured using radioimmunoassay kits with antibody raised against rat leptin with rat leptin standards (Linco Research, St Charles, MO).

The analysis for RNA levels was made by Northern blotting. The leptin cDNA probe was a 209–base pair fragment of mouse leptin cDNA (nucleotides + 101 to + 309) that was cloned by reverse transcription and polymerase chain reaction.[10] The rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe was prepared from pTRI-GAPDH vector by religating the linearized plasmid (Ambion, Austin, TX).[10]

The total RNA from each tube was dissolved in 100 μL water. Twenty microliters of the extract was lyophilized and electrophoresed at 22V for 20 hours[12] in buffer containing ethidium bromide. The gels were photographed under UV transillumination (Alphaimager 2000; Alpha Innotech, San Leandros, CA). The digital images were stored as tagged-image format files and analyzed by the National Institutes of Health (NIH) Image program, which calculates the relative intensity of 18S RNA bands. RNA was transferred to Nytran membranes using a Turbo blotter (Schleicher and Schuell, Keene, NH), and Northern blot

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analyses for leptin and GAPDH mRNA were performed simultaneously.[10,12]

The blots were analyzed by electronic autoradiography using a Instantimager (Packard, Meriden, CT).[12] The total count under each peak was determined after subtraction of background counts as shown in Fig 2. The counts were corrected for recovery of 18S RNA as compared against the time 0 sample and are expressed as a percentage of the time 0 value. The data in Fig 2 demonstrate results from six lanes of a single experiment. The range of counts for GAPDH mRNA after correction for 18S RNA recovery (7,400 to 9,300) demonstrates the variability in counts in an individual experiment, since dexamethasone does not affect the GAPDH mRNA content. The direct quantitation of hybridized messages, the more consistent transfer of RNA to Nytran membranes using the Turbo blotter, and the fact that each experiment involving 13 to 14 experimental conditions was analyzed on a single gel allowed for a quantitative procedure. Each experiment was replicated at least three times.

RESULTS

Comparison of Adipocytes From Euthyroid Versus Hypothyroid Rats

The present studies used adipocytes from hypothyroid rats because initial experiments showed that the amount of leptin mRNA after a 24-hour incubation in the presence of dexamethasone was greater than the amount in adipocytes from euthyroid rats (Fig 1). It should be noted that the hypothyroid rats were large male rats maintained on the diet for only 3 weeks, and the body weight and epididymal fat pad weight was unaffected under these conditions.[10] Although the effect of dexamethasone on leptin mRNA content was greater in adipocytes from hypothyroid rats, the increase in leptin release and GAPDH mRNA content due to dexamethasone or insulin was comparable to the increase in adipocytes from euthyroid rats (Fig 1).

GAPDH is a housekeeping gene routinely used to normalize the relative intensity of RNA bands in Northern blots. The amount of GAPDH mRNA was similar in adipocytes from hypothyroid versus euthyroid rats and was unaffected by dexamethasone (Fig 1). However, insulin doubled GAPDH mRNA content at 24 hours, so it could not be used as a recovery standard. Our results confirm those of Alexander et al,[13] who found similar effects of insulin in 3T3 adipocytes.

18S RNA Content Is Influenced by Insulin

We used 18S RNA content as our housekeeping gene following the suggestion of Savonet et al.[14] However, we found a 35% loss of 18S RNA when adipocytes were incubated for 24 hours in the absence of insulin. This loss was abolished in the presence of 10 nmol/L insulin. In all subsequent studies, data for leptin and GAPDH mRNA were corrected for recovery of 18S RNA. Thereby, the effects of insulin on GAPDH mRNA were no longer statistically significant except in the presence of dexamethasone plus Cl 316,243, which is a specific β_3-adrenergic agonist.[15] There was no significant effect of dexamethasone on GAPDH mRNA (Table 1).

One factor that might account for the loss of 18S RNA in adipocytes over a 24-hour incubation is the use of hypothyroid rats. However, in adipocytes from euthyroid rats, the 18S RNA content at 24 hours was 54% ± 18% of the time 0 value in the absence of insulin and 146% ± 25% in the presence of insulin (mean ± SEM of five experiments), comparable to the values reported in Table 1 for adipocytes from hypothyroid rats.

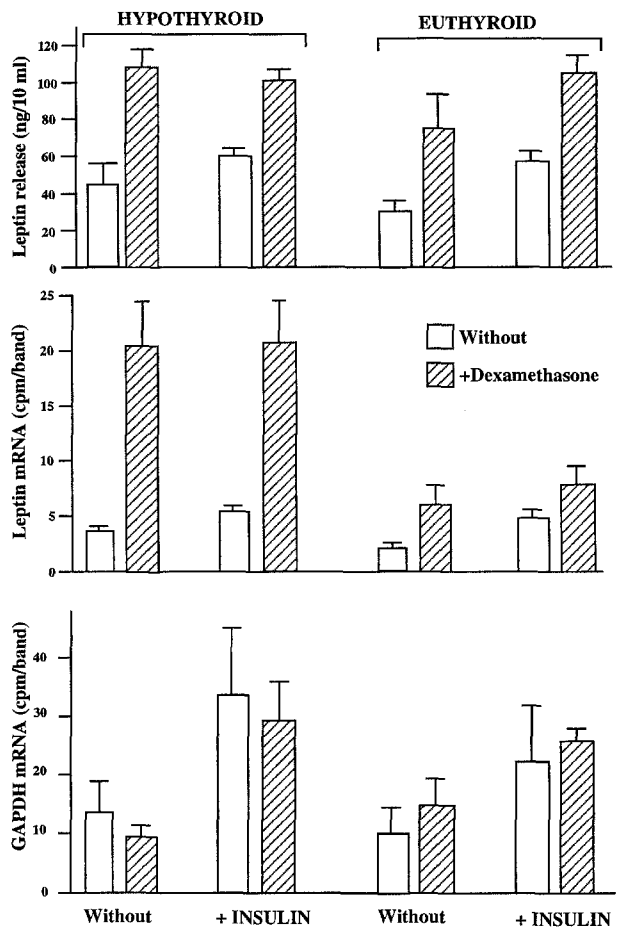

Fig 1. Comparison of dexamethasone and insulin effects on adipocytes from hypothyroid versus euthyroid rats. Adipocytes (1.2×10^6 from hypothyroid and 1.1×10^6 from euthyroid rats) were incubated for 24 hours either in the absence (□) or presence (▨) of 25 nmol/L dexamethasone. Values are the mean ± SEM of 5 experimental replications in the absence or presence of insulin (10 nmol/L).

We have found no agent that can mimic the ability of insulin to prevent the loss of 18S RNA observed over a 24-hour incubation of rat adipocytes. Neither dexamethasone, Cl 316,243, EGF, or oxytocin affected the 18S RNA content of adipocytes at 24 hours (Table 2). Similar negative effects were observed with phorbol myristate acetate (PMA) and forskolin (Table 3). However, the stimulatory effect of insulin on 18S RNA content in the presence of dexamethasone was reduced in the presence of Cl 316,243 (Table 2).

The concentration of insulin used in the studies shown in Table 2 was 10 nmol/L and resulted in a 103% increase in 18S RNA content at 24 hours. However, we found that 1 or 100 nmol/L insulin had similar effects (129% ± 16% and 111% ± 20%, respectively, mean ± SEM of three experiments).

Table 1. Effect of Insulin and CI 316,243 on Leptin Release, Leptin mRNA, GAPDH mRNA, and 18S RNA Accumulation by Adipocytes inthe Absence and Presence of Dexamethasone

Additions	Basal	+Dexamethasone (25 nmol/L)
Leptin in medium at 24 h (ng/10 mL)		
None	32 ± 2	91 ± 5
Insulin 10 nmol/L	56 ± 8*	120 ± 18
CI 316,243 10 nmol/L	31 ± 9	56 ± 4*
CI 316,243 + insulin	44 ± 8	108 ± 11*
Leptin mRNA at 24 h (% of time 0)		
None	16 ± 2	69 ± 8
Insulin 10 nmol/L	15 ± 3	44 ± 8
CI 316,243 10 nmol/L	13 ± 3	38 ± 5*
CI 316,243 + insulin	12 ± 4	49 ± 6
GAPDH mRNA at 24 h (% of time 0)		
None	173 ± 35	116 ± 19
Insulin 10 nmol/L	270 ± 55	162 ± 17
CI 316,243 10 nmol/L	110 ± 20	101 ± 15
CI 316,243 + insulin	185 ± 60	171 ± 16*
18S RNA mass at 24 h (% of time 0)		
None	64 ± 7	63 ± 8
Insulin 10 nmol?L	130 ± 28*	150 ± 15*
CI 316,243 10 nmol/L	79 ± 2	61 ± 9
CI 316,243 + insulin	101 ± 20	107 ± 12*

NOTE. Adipocytes (800,000) from hypothyroid rats were incubated in 10 mL medium for 24 hours, and all agents were added at the start of the incubation. Values are the mean ± SEM of 5 paired replications. The time 0 value for leptin mRNA was 19,000 and for GAPDH 8,000 counts. Leptin and GAPDH mRNA values are corrected for recovery of 18S RNA.

*$P < .05$, significant effects of CI 316,243 or insulin based on paired comparisons.

Dexamethasone Prevents the Loss of Leptin mRNA in Adipocytes During a 24-Hour Incubation

There was a loss of approximately 85% of leptin mRNA in adipocytes incubated for 24 hours in the absence of dexamethasone when the data were corrected for the loss of 18S RNA (Table 1). However, if no correction was made for the 35% decrease in 18S RNA over 24 hours, the total loss of leptin mRNA increased to about 90%. About half of this loss occurred during the first 6 hours of a 24-hour incubation (45% ± 5%, mean ± SEM of seven experiments). In contrast to the marked effect of dexamethasone on leptin mRNA content, it had no statistically significant effect on 18S RNA or GAPDH mRNA content (Tables 1 and 2).

Dexamethasone (25 nmol/L) markedly reduced the loss of leptin mRNA observed over a 24-hour incubation (Table 1). The effect of dexamethasone was equally dramatic at 6 hours, where there was no significant loss of leptin mRNA (−5% ± 8%, mean ± SEM of seven experiments) in the presence of dexamethasone versus a 45% ± 5% loss of leptin mRNA in the absence of dexamethasone. The dose-response relationship at 24 hours for prevention of leptin mRNA loss in the presence of dexamethasone is shown in Figs 2 and 3. Stimulatory effects could be observed with as little as 10 nmol/L dexamethasone, and maximal stimulation required only 50 nmol/L dexamethasone. Our standard concentration of dexamethasone was 25 nmol/L, which resulted in a response that was about 60% of maximal with respect to leptin release and about 70% of maximal with respect to leptin mRNA accumulation (Fig 3).

The data in Tables 2 and 3 demonstrate that dexamethasone was the only agent that prevented the marked loss of leptin mRNA over a 24-hour incubation. In the absence of dexamethasone, insulin had no significant specific effect on the loss of leptin mRNA (Tables 2 and 3), whereas PMA and forskolin enhanced the loss of leptin mRNA (Table 3). In the presence of dexamethasone, addition of insulin, EGF, or CI 316,243 partially blocked the ability of dexamethasone to prevent the loss of leptin mRNA (Table 2).

In Tables 1 to 3, the data on leptin mRNA are corrected for the recovery of 18S RNA, which was significantly affected by insulin and indicates that the effect of insulin to prevent the loss of leptin mRNA in the presence of dexamethasone was less than the effect on 18S RNA recovery. The data in Fig 1 are uncorrected for recovery of 18S RNA and show that insulin enhanced the recovery of leptin mRNA, but to a lesser extent than for GAPDH mRNA.

Regulation of Leptin Release by Hormones

Leptin release was elevated by 180% over a 24-hour incubation of adipocytes with 25 nmol/L dexamethasone (Table 2). More than 90% of the increase in leptin release occurred during the last 18 hours of a 24-hour incubation, since there was an increase in leptin release due to 10 nmol/L dexamethasone of only 18% ± 5% during the first 6 hours (mean ± SEM of 10 experiments), while the increase at 24 hours in the same

Table 2. Comparison of Hormone Effects on Leptin Release, Leptin mRNA, and 18S RNA

Added Agent	Leptin Release	Leptin mRNA	18S RNA
% change due to added agent			
Insulin 10 nmol/L (n = 14)	+56 ± 8*	−3 ± 12	+103 ± 28*
Dexamethasone 25 nmol/L (n = 9)	+180 ± 50*	+273 ± 40*	+26 ± 22
% change in the presence of dexamethasone			
Insulin 10 nmol/L (n = 9)	+49 ± 7*	−33 ± 10*	+145 ± 30*
EGF 100 nmol/L (n = 12)	−14 ± 5*	−18 ± 8*	+20 ± 14
Oxytocin 1 μmol/L (n = 10)	−32 ± 5*	−1 ± 11	−8 ± 15
CI 316,243 10 nmol/L (n = 8)	−34 ± 8*	−45 ± 8*	+2 ± 11
CI 316,243 + insulin (n = 6)	+24 ± 11	−25 ± 9*	+55 ± 10*

NOTE. Data are derived from studies using adipocytes from hypothyroid rats incubated for 24 hours in 10 mL medium. All agents were added at the start of the incubation period. Values are the mean ± SE of the paired differences for the number of comparisons (6 to 14) indicated for each agent expressed as % change either in the absence or presence of 25 nmol/L dexamthasone. Basal values were comparable to those shown in Table 1. Leptin mRNA values were corrected for recovery for 18S RNA.

*$P < .05$, significant effects of added agents.

Table 3. Neither Forskolin nor PMA Mimic the Effects of Insulin on Leptin Release, GAPDH mRNA, Leptin mRNA, or 18S RNA Content

Additions	Basal	PMA (10 nmol/L)	Forskolin (10 μmol/L)
Leptin in medium at 24 h (10 ng/10 mL)			
None	28 ± 2	23 ± 1	27 ± 2
Insulin 10 nmol/L	41 ± 3*	38 ± 4*	40 ± 4*
Leptin mRNA at 24 h (% time 0)			
None	16 ± 3	6 ± 2*	5 ± 2*
Insulin 10 nmol/L	15 ± 6	11 ± 2	8 ± 3
GAPDH mRNA at 24 h (% of time 0)			
None	132 ± 35	100 ± 20	105 ± 12
Insulin 10 nmol/L	231 ± 36	188 ± 34	185 ± 60
18S RNA mass at 24 h (% of time 0)			
None	52 ± 6	64 ± 11	62 ± 16
Insulin 10 nmol/L	128 ± 11†	94 ± 7	91 ± 12

NOTE. Rat adipocytes (840,000) from hypothryoid rats were incubated for 24 hours in 10 mL medium. All agents were added at the start of the incubation period. Values are the mean of 4 replications. Values for leptin mRNA and GAPDH mRNA are corrected for recovery of 18S RNA.

*$P < .05$, †$P < .001$: significant effects of insulin, PMA, or forskolin.

experiments was 174% ± 26%. However, over half of basal leptin release was observed during the first 6 hours of these incubations (22 ng at 6 hours *v* 39 ng at 24 hours, mean of 10 paired replications).

The effects of all hormones except insulin and oxytocin on leptin release were comparable to the effects on leptin mRNA (Table 2). Insulin significantly increased leptin release by about 50% in both the absence and presence of dexamethasone, but it had no specific effect on leptin mRNA except that it reduced the dexamethasone effect by 33% after correction for the effect of insulin on 18S RNA (Table 2). In contrast, oxytocin inhibited the stimulation of leptin release by dexamethasone by 32% without a significant effect on leptin mRNA. These data suggest that both the inhibition of leptin release by oxytocin and stimulation by insulin involve mechanisms other than the regulation of leptin mRNA stability.

The increase in leptin release and prevention of the loss of leptin mRNA in the presence of dexamethasone was inhibited by Cl 316,243 (Table 1), confirming prior reports that catecholamines inhibit leptin release.[7,16,17] When we added 10 μmol/L forskolin to adipocytes, it markedly decreased the basal leptin mRNA content. However, just as with Cl 316,243, no effect on basal or insulin-stimulated leptin release was found with forskolin (Table 3).

EGF is a potent activator of the mitogen-activated protein (MAP) kinase pathway in adipocytes but does not mimic insulin action on adipocyte metabolism.[18,19] EGF actually had a small but significant inhibitory effect on leptin release by adipocytes incubated in the presence of dexamethasone (Table 2).

Oxytocin is a potent stimulator of phosphoinositide breakdown in adipocytes that elevates intracellular Ca^{2+} and diacylglycerol.[18,20] Oxytocin has a small stimulatory effect on adipocyte glucose metabolism,[18] but unlike insulin, it inhibited leptin release in the presence of dexamethasone. Furthermore, oxytocin failed to mimic the effects of insulin on 18S RNA content or leptin mRNA (Table 2).

It is known that PMA is an activator of Glut 4 transporter movement to the surface of adipocytes but poorly mimics the effects of insulin on glucose metabolism.[21] Similar results are shown in Table 3, where PMA had no effect on GAPDH mRNA, leptin release, or 18S RNA content but drastically inhibited leptin mRNA accumulation.

DISCUSSION

We initially examined the 18S RNA content of adipocytes as a control for normalization of the amount of extracted RNA. Savonet et al,[14] reported that actin, albumin, and cyclophilin, as

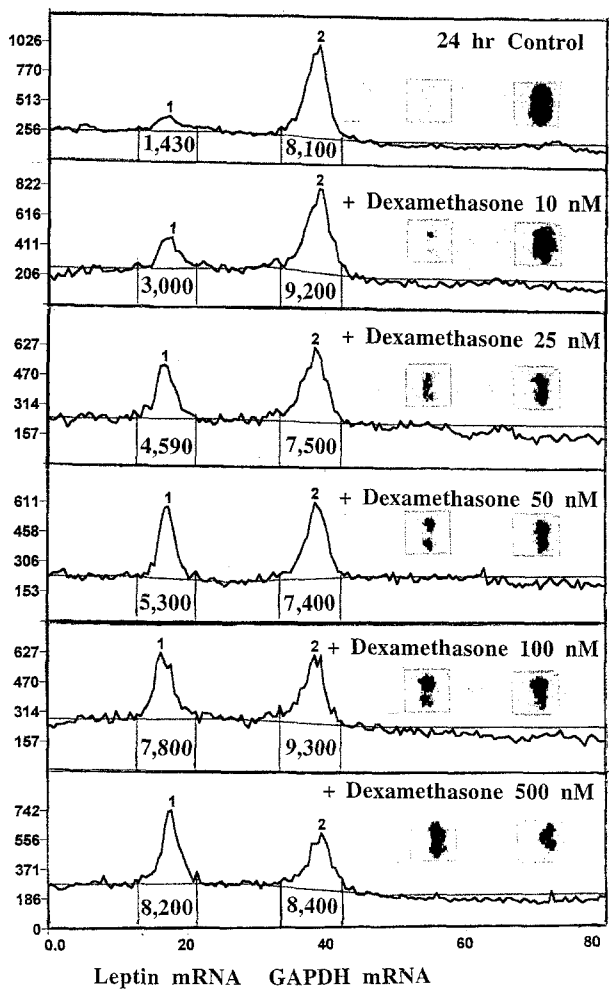

Fig 2. Electronic autoradiographic analysis of Northern blots for leptin mRNA and GAPDH mRNA. The data are from a single experiment comparable to those shown in Table 1, and all samples were first electrophoresed on the same gel and then transferred to Nytran membranes. The mRNA content was determined by simultaneous analysis with probes to leptin and GAPDH mRNA. The data are presented as cpm, and the numbers under the peaks are for the total count in the region between the vertical lines after subtraction of background counts and adjustment for recovery of 18S RNA. The digital image for each lane is also shown, and the boxed area represents the area for leptin mRNA (left) and GAPDH mRNA (right) analysis.

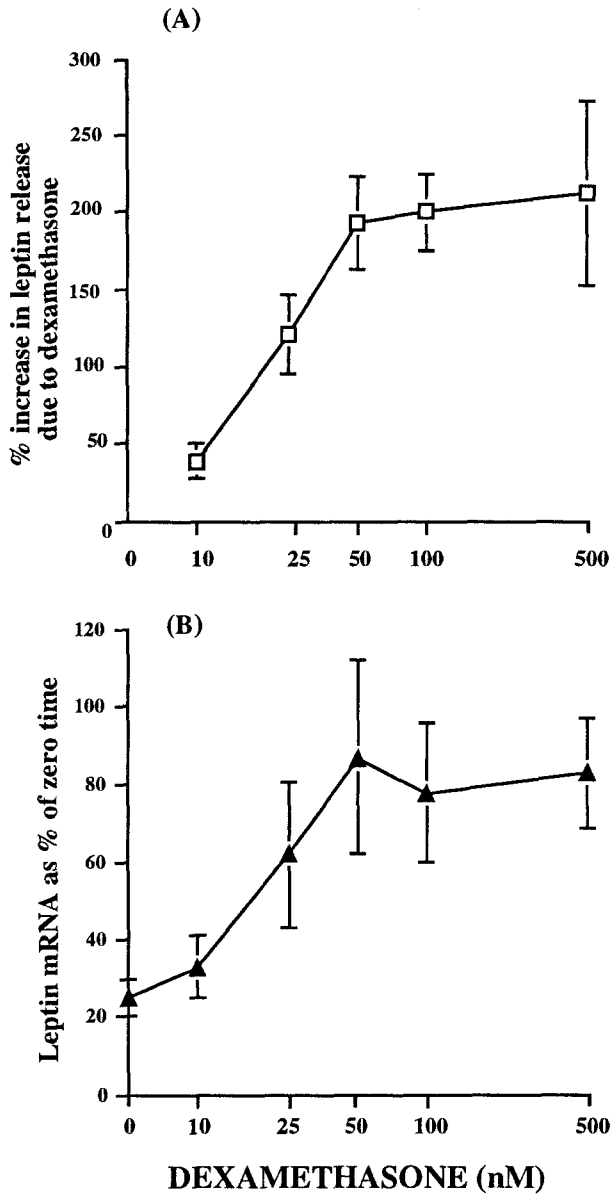

Fig 3. Dose-response curve for stimulation of leptin release and leptin mRNA accumulation by dexamethasone. Rat adipocytes (980,000) from hypothyroid rats were incubated for 24 hours in the absence or presence of the indicated concentration of dexamethasone. Values for leptin release (A) are shown as the mean ± SEM % increase of 3 paired replications in the presence of the indicated concentration of dexamethasone over the basal value of 50 ng. Values for leptin mRNA (B) are expressed as the % of the initial value corrected for recovery of 18S RNA.

well as GAPDH mRNA, four genes commonly used for total RNA normalization, were less suitable for quantitation of mRNA in thyrocytes than an analysis of 18S RNA. We were using GAPDH mRNA for the housekeeping gene until we realized the marked effect of insulin on GAPDH mRNA content in adipocytes (Fig 2). To our surprise, we found a marked loss of 18S RNA content over a 24-hour incubation of adipocytes that could be prevented by insulin (Table 1). Similar results of lesser magnitude were reported by Antonetti et al,[22] in primary cultures of rat hepatocytes. In their experiments, total RNA content was reduced by 26% after 4 days of culture in the absence of insulin, but was restored to control values within 48 hours. Ribosomal RNA constitutes 85% of total cellular RNA, and specific measurement of 18S RNA showed a 36% decrease in this RNA. The effect of insulin was attributed to both stimulation of ribosome biogenesis and inhibition of ribosomal RNA degradation in cultured hepatocytes.[22] The effects of insulin on the 18S RNA content of adipocytes were also found with respect to the 28S RNA content (unpublished data, Fain JN, Bahouth SW, July 1997), which suggests that, as in hepatocytes, insulin prevents the loss of total RNA in adipocytes incubated in suspension culture for 24 hours.

The phospholipase C pathway that is activated by oxytocin in adipocytes, the MAP kinase pathway activated by EGF, the protein kinase C pathway activated by phorbol esters, and the cyclic adenosine monophosphate (AMP)-dependent protein kinase pathway stimulated by β_3-adrenergic receptor agonists or forskolin are unable to mimic insulin's effects on 18S RNA or leptin release. We also failed to mimic the effects of insulin on the 18S RNA content or leptin release of adipocytes by addition of insulin-like growth factor 1 or tumor necrosis factor (unpublished data, Fain JN, Bahouth SW, August 1997). Hardie et al[17] similarly reported that these agents did not affect leptin release by rat adipocytes. Fetal calf serum (10%) is often included in the medium for cell culture studies, but it did not mimic the ability of insulin to affect 18S RNA content despite its ability to mimic the stimulation of leptin release by insulin (unpublished data, Fain JN, Bahouth SW, May 1997).

One study found that administration of insulin to rats that were fasted overnight restored the level of leptin mRNA to fed levels within 4 hours in the absence of food.[8] However, the effect may involve the indirect effects of insulin mediated through the release of other hormones, since Becker et al[23] reported that administration of insulin to diabetic rats resulted in an increase in the level of GLUT4 mRNA and fatty acid synthase mRNA in epididymal adipose tissue within 12 hours but no change in leptin mRNA even after 96 hours. Our cell culture studies using adipocytes from fed rats are in agreement with the data from Becker et al,[23] since there was no stimulatory effect of insulin on leptin mRNA at 24 hours (Table 2). Similar effects were reported by Slieker et al,[7] while Reul et al[9] found that insulin reduced the stabilization of leptin mRNA by dexamethasone. However, Barr et al[24] demonstrated that insulin increased leptin secretion from pieces of rat adipose tissue throughout a 4-hour incubation. They also concluded that insulin stimulates leptin secretion by a mechanism distinct from the release of stored secretory vesicles, since leptin appears to be secreted as it is produced.

We found approximately a 50% increase in leptin release due to 10 nmol/L insulin over a 24-hour incubation of adipocytes from hypothyroid rats both in the absence and presence of dexamethasone (Table 2). Dexamethasone also stimulated leptin release, but to a greater extent than insulin and by a distinctly different mechanism. The stimulatory effects of dexamethasone on leptin release were associated with similar increases in leptin mRNA content. In contrast, dexamethasone did not affect 18S RNA or GAPDH mRNA over the 24-hour incubation period. In the presence of insulin, the 18S RNA content of adipocytes was

twice the level observed after 24-hours in its absence and greater than the effects of insulin on leptin and GAPDH mRNA content. In effect, insulin prevents the loss of leptin mRNA, GAPDH mRNA, and 18S RNA to about the same extent, while dexamethasone specifically prevents the loss of leptin mRNA, over a 24-hour incubation of rat adipocytes. In contrast, catecholamines are negative regulators of leptin release. Trayhurn et al[25] found that cold-induced suppression of leptin gene expression involved catecholamines, and the same may be true during starvation.

The effects of the hormones examined in this study with respect to leptin release by adipocytes from hypothyroid rats over a 24-hour incubation are summarized in Fig 4. Insulin and glucocorticoids stimulate leptin release, while agents that stimulate protein kinase A, protein kinase C, or the MAP kinase pathway inhibit leptin release in the presence of glucocorticoids. Our data suggest that glucocorticoids are required for the maintenance of leptin release by rat adipocytes in the fed state, since no other hormone has been found to mimic their ability to maintain leptin release and leptin mRNA content.

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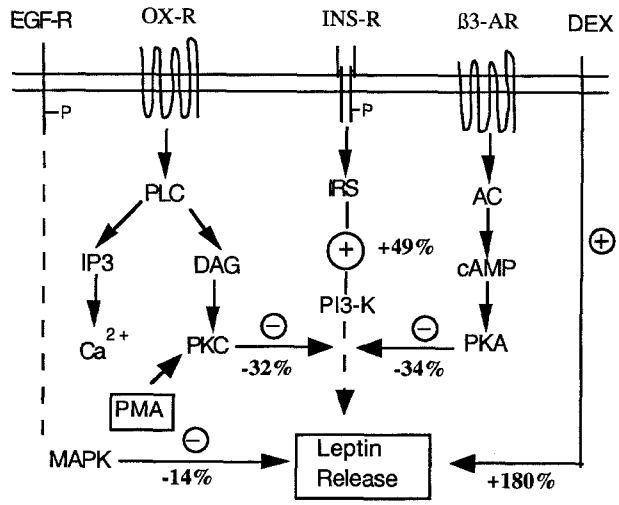

Fig 4. Regulation of dexamethasone-stimulated leptin release by insulin, Cl 316,243, oxytocin, and EGF. Activation of the insulin receptor causes recruitment of insulin-responsive substrates followed by stimulation of phosphatidylinositol 3-kinase (PI3-K) and upregulation of leptin release. Activation of β3-adrenergic receptors by Cl 316,243 promotes the production of cyclic AMP. Activation of oxytocin receptors elevates diacylglycerol and Ca^{2+} accumulation in adipocytes, while activation of the EGF receptor increases the activity of the MAP kinase pathway. The effects of added agents are shown as the % change.

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