Regulation of *ob* Gene Expression and Leptin Secretion by Insulin and Dexamethasone in Rat Adipocytes

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Leptin, the *ob* gene product, is produced by adipocytes, and it acts to decrease caloric intake and increase energy expenditure. To better understand the molecular mechanisms of hormone-regulated leptin synthesis and secretion, we assessed the ability of insulin and dexamethasone to acutely modulate *ob* gene expression and leptin secretion in rat adipocytes. Incubation of rat adipocytes with 100 nmol/l insulin for 2 h had no effect on *ob* mRNA levels, but it stimulated a twofold increase in leptin secretion. Dexamethasone (100 nmol/l) stimulated both a two- to fourfold increase in *ob* mRNA and a twofold increase in leptin secretion. Consonant with a posttranscriptional and transcriptional regulatory mechanism for insulin- and dexamethasone-stimulated leptin secretion, respectively, actinomycin D blocked dexamethasone-stimulated leptin secretion but did not affect insulin-stimulated leptin secretion. Cycloheximide treatment did not significantly affect *ob* mRNA accumulation, but it reduced total secreted leptin. Interestingly, however, insulin was still able to stimulate a twofold increase in leptin secretion. These data suggest that insulin, but not dexamethasone, is able to stimulate leptin secretion from a preexisting intracellular pool, although de novo protein synthesis is required for the full insulin-stimulated effect. Signaling pathways involved in leptin synthesis/secretion were also evaluated. The phosphatidylinositol 3-kinase inhibitor LY294002, the Map/Erk kinase inhibitor PD98059, and the immunosuppressant rapamycin had no effect on basal levels of leptin secretion. However, all three inhibitors markedly decreased both insulin- and dexamethasone-stimulated leptin secretion. These findings suggest a complex set of signaling pathways involved in mediating insulin- and dexamethasone-stimulated leptin synthesis and secretion. *Diabetes* 48:272–278, 1999

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KRPB, Krebs-Ringer phosphate buffer; PI, phosphatidylinositol; RIA, radioimmunoassay; SSC, sodium chloride–sodium citrate.

Obesity is a common disorder in industrialized societies that is associated with a myriad of cardiovascular and metabolic anomalies, including hypertension, hyperlipidemia, hyperglucocorticoidemia, and type 2 diabetes (1,2). The recently identified *ob* gene (3) encodes a 16-kDa protein called leptin that is secreted primarily from adipocytes. Leptin is a plasma protein hormone that acts as a key signaling factor for regulating body weight and energy expenditure (4). These effects of leptin are believed to be mediated through a closed-loop feedback system involving the hypothalamus, within which leptin receptors have been identified (5). Leptin receptors have also been detected at lower amounts in several peripheral tissues (6–8), which suggests that leptin signals to a variety of tissues and organ systems.

The underlying biochemical and molecular mechanisms that regulate leptin synthesis and secretion are unclear, as several contradictory reports persist. Most systematic studies have focused on two principal candidates, glucocorticoids and insulin. Glucocorticoids have more or less consistently been shown to increase *ob* gene expression and leptin secretion in vivo in both normal rats and humans (9–11), as well as in vitro in primary cultures of adipocytes (12–14), but not in the 3T3-L1 cell line (15). In addition, it was shown that in humans, there exists an inverse relationship between changes in plasma leptin levels and cortisol levels (16). With regard to the effects of insulin, there is currently substantial controversy over the ability of insulin to modulate *ob* gene expression and leptin protein synthesis and secretion. Some studies have shown a stimulatory effect of insulin on *ob* gene expression and/or leptin secretion (13,17,18), whereas others have reported no effect of insulin on these parameters (12,14,19,20). These disparate reports have been suggested to result from the use of different culture models (21). In support of an in vivo stimulatory effect, one study indicated that *ob* mRNA and leptin protein levels in rats are tightly regulated by diet and insulin. Under fasting conditions, *ob* mRNA and leptin protein levels decreased to barely detectable levels, but they were restored to normal within 4 h after refeeding or administration of insulin (18). In addition, rats rendered hyperinsulinemic in clamp studies showed a marked increase in *ob* mRNA compared with controls (18). However, insulin's effects on *ob* expression and leptin secretion in vitro remain unresolved. There is some evidence that insulin may acutely increase leptin secretion from isolated adipocytes (13,17,22). For the most part, though, numerous in vitro studies have reported

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conflicting results (13–15,18,22–25). The studies reported here were undertaken to better characterize the molecular and biochemical regulation of leptin synthesis and secretion in vitro by both insulin and dexamethasone. In the current study, we used freshly isolated rat adipocytes, which are one of the best, physiologically significant, insulin-sensitive tissues available, as opposed to cell lines differentiated using adipogenic reagents. One objective of the present study was to evaluate the acute effects of insulin and dexamethasone on *ob* gene expression and leptin protein secretion from freshly isolated rat adipocytes. In addition, experiments were performed to identify intracellular signaling pathways that may be involved in regulating leptin synthesis and secretion.

RESEARCH DESIGN AND METHODS

Materials. Type I collagenase was obtained from Worthington Biochemical (Lakewood, NJ), and fatty acid–free bovine serum albumin was obtained from Intergen (Purchase, NY). Insulin was from Boehringer Mannheim (Indianapolis, IN). Dexamethasone, actinomycin D, and cycloheximide were purchased from Sigma (St. Louis, MO). Rapamycin, LY294002, and PD98059 were acquired from CalBiochem (La Jolla, CA). The mouse *ob* cDNA probe was kindly provided by Dr. Gokhan Hotamisligil, Harvard School of Public Health, and the C/EBPδ probe was a generous gift from Dr. Ulupi Jhala, Joslin Diabetes Center. The human β-actin cDNA probe was from Clontech (Palo Alto, CA). A RadPrime DNA-labeling kit was purchased from GIBCO-BRL (Gaithersburg, MD), and [α-^{32}P]dCTP was from Du Pont-NEN (Boston, MA).

Animals. Male Sprague-Dawley rats from Taconic Farms (Germantown, NY) weighing 150–200 g were used for these studies. Rats were housed in a temperature-controlled room (24°C) under alternating 12 h light/dark periods and allowed access to food and water ad libitum. Animals were allowed to acclimate for 5 days after their arrival. Rats were killed between 10:00 and 11:00 A.M. to prevent circadian variations.

Adipocyte isolation and incubations. Epididymal fat pads were harvested, and adipocytes were prepared essentially as described (26). Briefly, the fat pads were immersed in Krebs-Ringer phosphate buffer (KRPB) containing 20 mmol/l HEPES pH 7.4, 120 mmol/l NaCl, 6 mol/l KCL, 1.2 mmol/l $MgSO_4$, 1 mmol/l $CaCl_2$, 0.6 mmol/l Na_2HPO_4, 0.4 mol/l NaH_2PO_4, and 1% bovine serum albumin. Cells were minced and subjected to collagenase digestion (2 mg/g fat) for 1 h at 37°C with constant shaking at 100 cycles/min. Adipocytes were filtered through 400-μm nylon mesh (Tetko, Kansas City, MO) and washed three times in KRPB. Cells were adjusted to a 20% concentration with KRPB and equilibrated at 37°C for 30 min with constant shaking at 80 cycles/min. At the end of this period, the conditioned medium was changed, and the cells were incubated in the absence or presence of 100 nmol/l insulin, 100 nmol/l dexamethasone, or both concurrently for 2 h at 37°C with constant shaking at 80 cycles/min. For studies to characterize transcriptional/translational regulatory mechanisms and potential signaling pathways mediating leptin synthesis and secretion, adipocytes were isolated exactly as described above; pre-incubated for 30 min with or without either 500 nmol/l actinomycin D, 20 μg/ml cycloheximide, 50 μmol/l LY294002, 10 μmol/l PD98059, or 20 nmol/l rapamycin; and then incubated for 2 h in the absence or presence of insulin or dexamethasone. At the end of the incubation period, the cells were collected and immediately processed for RNA isolation. In addition, conditioned medium for each incubation condition was collected, frozen at -80°C, and later assayed for leptin secretion by radioimmunoassay (RIA).

RNA isolation and analysis. Total RNA was isolated from rat adipocytes by the RNAzol method (Biotecx, Houston, TX). Ten-microgram samples of total RNA per lane were run in 1.2% formaldehyde-agarose denaturing gels and transferred to Hybond-N membranes (Amersham, Arlington Heights, IL) in 20× sodium chloride–sodium citrate (SSC) (3 mol/l NaCl plus 0.3 mol/l sodium citrate · $2H_2O$). Denaturing gels were run at 75 V for 3.5 h and were washed twice with 10× SSC before setting up transfer in 20× SSC. A mouse *ob* cDNA labeled to high specific activity with [α-^{32}P]dCTP was used to probe all blots. Hybridizations were carried out for 2 h at 65°C using Quick Hyb (Stratagene, La Jolla, CA). Membranes were washed twice with 2× SSC + 0.1% SDS for 15 min at room temperature and once with 0.1× SSC + 0.1% SDS for 15 min at 55°C. Membranes were exposed to Kodak BioMax MR film for 2–6 h at –80°C with dual intensifying screens. Transcript intensity was determined by densitometric scanning. In addition, blots were stripped and reprobed with a human β-actin cDNA probe as a control for RNA integrity and loading, and a C/EBPδ probe was used to demonstrate the effectiveness of actinomycin D at the concentration used for these studies.

Leptin RIA and data analysis. Leptin secreted into the conditioned medium was measured by RIA using a commercially available rat leptin RIA kit (Linco Research, St. Charles, MO) as described by the manufacturer. All samples were assayed in duplicate. Data are presented as the mean ± SE from multiple experiments. Statistical analyses were performed using StatView (Abacus Concepts, Berkley, CA). Differences among groups of animals were determined by analysis of variance and Scheffe's test. A probability value of <0.05 was considered significant.

RESULTS

Effects of insulin and dexamethasone on *ob* mRNA and leptin secretion. To gain some fundamental insight into hormonal regulation of *ob* gene expression in freshly isolated rat adipocytes, the acute effects of insulin and dexamethasone on *ob* gene expression in these cells were studied by Northern analysis. As shown in Fig. 1*A*, insulin treatment had no effect on *ob* mRNA levels during a 2-h incubation. In contrast, dexamethasone consistently stimulated a two- to fourfold increase in *ob* mRNA accumulation (Fig. 1*A*; $P < 0.05$), indicating that dexamethasone acutely regulates *ob* gene transcription in isolated rat adipocytes. To determine if concurrent treatment with both hormones would have an acute additive, synergistic, or inhibitory effect on *ob* expression, we examined the effects of insulin and dexamethasone combined on *ob* gene expression. Adipocytes were incubated for 2 h in the presence of both hormones simultaneously. As shown in Fig. 1*A*, the addition of insulin had no effect on dexamethasone-stimulated *ob* gene expression. Analysis of hormone-stimulated leptin protein secretion is shown in Fig. 1*B*. In contrast to its lack of effect on *ob* mRNA, insulin stimulated a twofold increase in leptin protein secretion ($P < 0.05$). Similar to the effect of insulin, incubation of the adipocyte preparations with 100 nmol/l dexamethasone also showed a twofold increase in leptin secretion (Fig. 1*B*; $P < 0.02$). Taken together, these data suggest a potential transcriptional regulatory mechanism for dexamethasone's effect on leptin synthesis and secretion, whereas insulin's ability to stimulate leptin secretion is through a posttranscriptional event. We also examined the effect of concurrent treatment with both hormones on leptin secretion. As depicted in Fig. 1*B*, stimulation of leptin secretion in the presence of both insulin and dexamethasone was the same as in the presence of the individual agents (Fig. 1*B*; $P < 0.05$).

Transcriptional regulation of insulin- and dexamethasone-mediated *ob* gene expression and leptin secretion. The above data suggest a transcriptional regulatory mechanism for dexamethasone-stimulated leptin synthesis/secretion, whereas insulin regulates leptin synthesis/secretion at a posttranscriptional step. To confirm this hypothesis, we utilized the transcriptional inhibitor actinomycin D. As depicted in Fig. 2*A*, insulin had no effect on *ob* mRNA, whereas dexamethasone elicited a threefold increase ($P < 0.02$), consistent with our earlier observations (Fig. 1*A*). Actinomycin D treatment alone caused upregulation of *ob* mRNA to similar levels in control ($P < 0.02$), insulin-treated ($P < 0.05$), and dexamethasone-treated ($P < 0.05$) cells (Fig. 2*A*). This suggests the presence of either a short-lived repressor or destabilizer protein for *ob* mRNA gene expression. To confirm that actinomycin D was indeed inhibiting gene transcription, the blot was stripped and reprobed with a C/EBPδ DNA probe. This probe was chosen because members of the C/EBP family of transcription factors have short half-lives. In the absence of actinomycin D, this transcript was expressed at similar levels in control, insulin-treated, and dexamethasone-treated cells. However, in the presence of actinomycin D, C/EBPδ gene expression was

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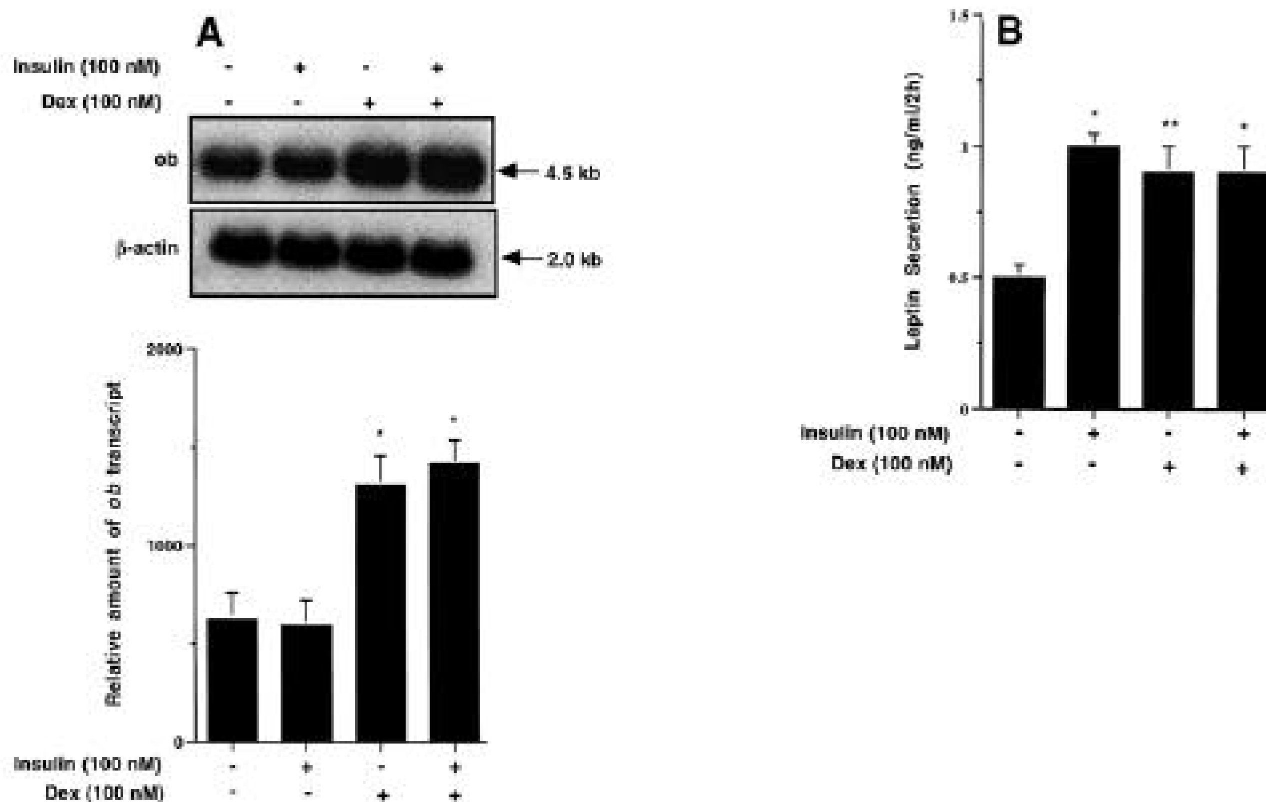

FIG. 1. ***A:*** **Northern blot showing the individual and combined effects of insulin and dexamethasone on *ob* gene expression in freshly isolated rat adipocytes. Adipocytes were incubated with or without 100 nmol/l insulin, 100 nmol/l dexamethasone, or both simultaneously for 2 h. Each lane was loaded with 10 µg total RNA and hybridized with a mouse *ob* cDNA probe. Densitometric scanning was used to determine the relative amount of *ob* transcript, and blots were stripped and reprobed with a human β-actin cDNA probe as a control for RNA integrity and loading.** ***B:*** **Individual and combined effects of insulin and dexamethasone on leptin secretion from freshly isolated rat adipocytes. Incubation conditions were those described above. Leptin secreted into the conditioned medium was measured using a commercially available rat leptin RIA kit. All results are expressed as means ± SE and are representative of three independent experiments (n = 8 per assay). *P < 0.05 vs. control (–/–); **P < 0.02 vs. control (–/–).**

completely abolished in all conditions (Fig. 2*A*), demonstrating the efficacy of actinomycin D in this study. In this experimental model, pretreatment of adipocytes with actinomycin D for 30 min before incubation with dexamethasone caused a complete inhibition of the dexamethasone-stimulated *ob* mRNA accumulation (Fig. 2*A*). Consistent with an actinomycin D–induced increase in basal *ob* mRNA levels, there was an elevated level of secreted leptin in the control samples (Fig. 2*B*; P < 0.02). However, pretreatment of adipocytes with actinomycin D blocked dexamethasone-stimulated leptin secretion (Fig. 2*B*; P < 0.05). In contrast, insulin stimulated leptin secretion to levels similar to those seen in the absence of actinomycin D (Fig. 2*B*; P < 0.05). These data are consistent with transcriptional and posttranscriptional regulatory mechanisms for dexamethasone and insulin, respectively, in the stimulation of leptin synthesis and secretion.

Effects of cycloheximide on insulin- and dexamethasone-stimulated leptin synthesis/secretion. To further analyze the regulatory mechanisms underlying stimulation of leptin synthesis/secretion by dexamethasone and insulin, we utilized the protein synthesis inhibitor cycloheximide. Treatment with cycloheximide did not have any significant effect on *ob* mRNA accumulation (Fig. 3*A*), but it significantly decreased total secreted leptin (Fig. 3*B*; P < 0.05). However, despite an overall reduction in leptin secretion after cycloheximide treatment, insulin nonetheless stimulated a twofold increase in leptin secretion relative to control cells in the presence of cycloheximide (Fig. 3*B*; P < 0.02). In contrast, dexamethasone had no effect on leptin secretion in the presence of cycloheximide. These data suggest the presence of preformed, insulin-regulated pools of leptin in rat adipocytes.

Signaling pathways mediating leptin synthesis/secretion. Lastly, we examined potential signaling pathways mediating leptin synthesis and secretion in freshly isolated rat adipocytes. Specifically, the effects of the phosphatidylinositol (PI) 3-kinase inhibitor LY294002, the mitogen-activated protein kinase (MEK) inhibitor PD98059, and the immunosuppressant rapamycin on insulin- and dexamethasone-mediated *ob* gene expression and on leptin secretion were determined. As shown in Fig. 4*A*, during a 2-h incubation, LY294002 increased basal (P < 0.05) and decreased dexamethasone-stimulated *ob* mRNA accumulation (P < 0.05). No other effect of any of these inhibitors on *ob* mRNA expression in basal, insulin-treated, or dexamethasone-treated cells was observed. In addition, basal levels of secreted leptin were not affected by any of the inhibitors (Fig. 4*B*). However, all three inhibitors markedly decreased (P < 0.05) both insulin- and dexamethasone-stimulated leptin secretion (50 ± 3 and 41 ± 4%, respectively, for LY294002; 67 ± 3 and 53 ± 4% for PD98059; 61 ± 3 and 42 ± 4% for rapamycin). These findings suggest that the signaling pathways mediating insulin- or

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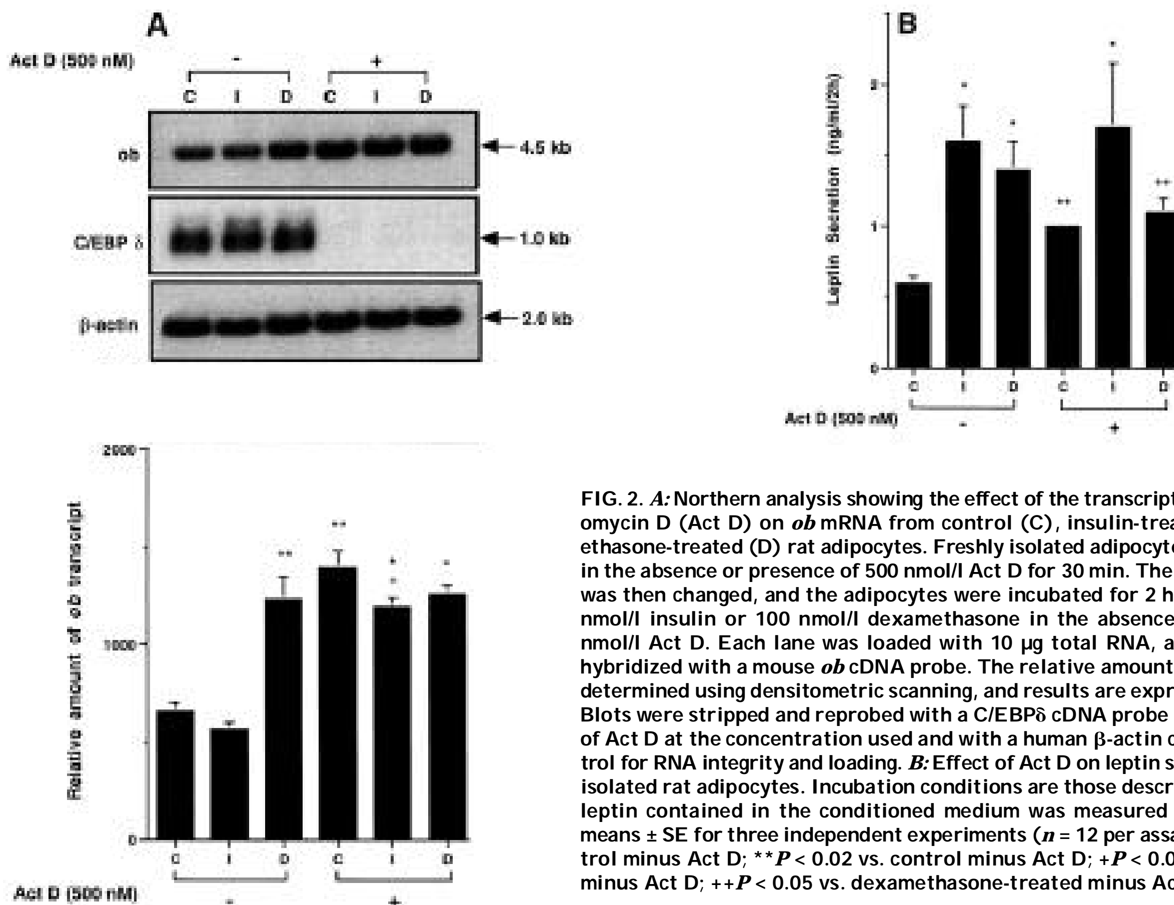

FIG. 2. ***A:*** **Northern analysis showing the effect of the transcriptional inhibitor actinomycin D (Act D) on *ob* mRNA from control (C), insulin-treated (I), and dexamethasone-treated (D) rat adipocytes. Freshly isolated adipocytes were preincubated in the absence or presence of 500 nmol/l Act D for 30 min. The conditioned medium was then changed, and the adipocytes were incubated for 2 h with or without 100 nmol/l insulin or 100 nmol/l dexamethasone in the absence or presence of 500 nmol/l Act D. Each lane was loaded with 10 µg total RNA, and membranes were hybridized with a mouse *ob* cDNA probe. The relative amount of *ob* transcript was determined using densitometric scanning, and results are expressed as means ± SE. Blots were stripped and reprobed with a C/EBPδ cDNA probe to verify the efficacy of Act D at the concentration used and with a human β-actin cDNA probe as a control for RNA integrity and loading. *B:* Effect of Act D on leptin secretion from freshly isolated rat adipocytes. Incubation conditions are those described above. Secreted leptin contained in the conditioned medium was measured by RIA. Results are means ± SE for three independent experiments (n = 12 per assay). *P < 0.05 vs. control minus Act D; **P < 0.02 vs. control minus Act D; +P < 0.05 vs. insulin-treated minus Act D; ++P < 0.05 vs. dexamethasone-treated minus Act D.**

dexamethasone-stimulated leptin synthesis and/or secretion are complex, involving steps that are PI 3-kinase dependent and steps that are sensitive to both PD98059 and rapamycin.

DISCUSSION

In the present study, we have shown that transcription of the *ob* gene in rat adipocytes is unaffected by insulin but strongly stimulated by dexamethasone. A number of studies using a variety of different culture systems have yielded conflicting results regarding the effects of these hormones on *ob* expression. Both direct regulatory effects (18) and no effect (14,20) of insulin on rat adipocyte *ob* gene expression have been reported. Our results document no acute effect of insulin on *ob* gene expression in freshly isolated rat adipocytes. In addition, with one exception (15), there is a growing body of evidence showing potent stimulation of *ob* gene expression by dexamethasone in cultured adipocytes (14,20), as well as in cultured explants of rat and human adipose tissue (12,21). Our observations using freshly isolated adipocytes confirm the potent effect of dexamethasone on *ob* gene transcription. There are reports of an inhibitory (21,27), as well as an additive, effect of insulin on glucocorticoid-stimulated *ob* gene expression (28) and leptin protein production (13,28). However, these effects were noted at time points well beyond the 2-h incubation period used in our study. We show no acute effect of insulin on dexamethasone-stimulated *ob* gene expression, which is consistent with our observations of the individual effects of these hormones over a 2-h period. With regard to their acute effects on leptin protein secretion, stimulation with either insulin or dexamethasone consistently elicited a twofold increase in leptin secretion. Interestingly, leptin secretion in the presence of both insulin and dexamethasone concurrently was the same as in the presence of the individual agents. Given their independent mechanisms of action, it seemed likely to expect an additive effect of both hormones, but this was not the case. A possible explanation for this may be that leptin secretion was already maximally stimulated in the presence of insulin or dexamethasone individually, given the relatively high concentrations of these agents used in this study. Therefore, coincubation with both hormones would not elicit an additional increase in leptin secretion if this were indeed the case. Alternatively, it is also possible that such an additive effect does not occur acutely and may manifest itself during a more prolonged incubation, as has been reported elsewhere (13,28).

The transcriptional inhibitor actinomycin D has been shown to prevent the stimulatory effects of dexamethasone on *ob* gene expression in cultured explants of both rat and human adipose tissue (10,21). In the present study, treatment with the transcriptional inhibitor actinomycin D inhibited dexamethasone-stimulated *ob* mRNA accumulation and leptin secretion in freshly isolated rat adipocytes. In contrast, actinomycin D had no effect on insulin-stimulated leptin secretion. These results are consistent with a posttranscriptional regulatory mechanism mediating insulin-stimulated leptin secretion and the involvement of transcriptional mechanisms in dexamethasone-stimulated leptin secretion. Unexpectedly, although it repressed dexamethasone-induced *ob* mRNA accumulation, actinomycin D treatment upregulated *ob* mRNA in control, insulin-treated, and dexamethasone-treated

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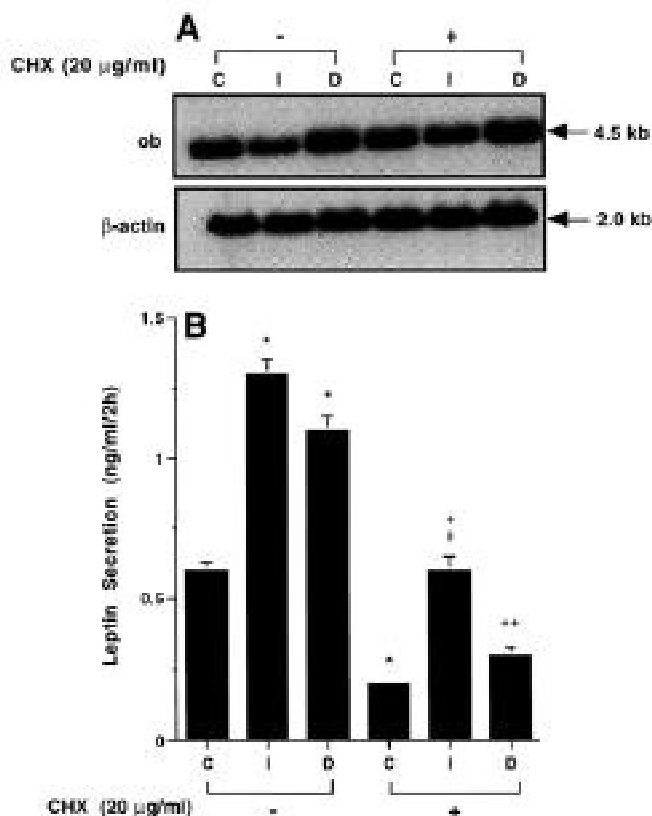

FIG. 3. ***A:*** **Northern blot showing the effect of the protein synthesis inhibitor cycloheximide (CHX) on *ob* gene expression in control (C), insulin-treated (I), and dexamethasone-treated (D) rat adipocytes. Freshly isolated adipocytes were preincubated in the absence and presence of 20 μg/ml CHX. The conditioned medium was changed. The adipocytes were then incubated with or without 100 nmol/l insulin or 100 nmol/l dexamethasone in the absence or presence of CHX (20 μg/ml). Each lane was loaded with 10 μg total RNA, and membranes were hybridized with a mouse *ob* cDNA probe. Membranes were stripped and reprobed with a human β-actin cDNA probe as a control for RNA integrity and loading.** ***B:*** **Effect of CHX (20 μg/ml) on leptin secretion. Secreted leptin contained in the conditioned medium from the 2-h incubation described above was measured by RIA. Results are means ± SE for 12 rats. ****P*** **< 0.05 vs. control minus CHX; +*P* < 0.05 vs. insulin-treated minus CHX; #*P* < 0.02 vs. control plus CHX; ++*P* < 0.05 vs. dexamethasone-treated minus CHX.**

cells to similar levels. This was not a general effect of actinomycin D on cellular mRNAs, as C/EBPδ mRNA was completely abolished under the same conditions. Based on these results, we propose that actinomycin D may inhibit a short-lived regulatory protein that either represses transcription of the *ob* gene or destabilizes the message. In support of our observations, a very recently published study reported that actinomycin D treatment stabilized leptin mRNA and increased leptin release from rat adipocytes (29). In addition, labile mRNA destabilizers controlling the turnover of different mRNAs have been identified in several cell types (30). However, at the present time, very little is known about the mechanisms by which these destabilizers operate.

Inhibition of protein synthesis with cycloheximide had no significant effect on *ob* mRNA levels in control, insulin-treated, or dexamethasone-treated cells. This finding is in agreement with a recent study showing no effect of cycloheximide on dexamethasone-induced *ob* mRNA accumulation in the rat (21) and extends these data by showing no effect of cycloheximide and insulin combined on *ob* gene expression. We further show that cycloheximide treatment dramatically decreases total secreted leptin from freshly isolated rat adipocytes. Interestingly, despite this decrease, insulin (but not dexamethasone) was still able to stimulate a twofold increase in leptin secretion in the presence of cycloheximide relative to control cells. These data suggest the existence of preformed insulin-regulated pools of leptin in rat adipocytes. Therefore, we propose that insulin (but not dexamethasone) may stimulate leptin secretion in the presence of cycloheximide via mobilization of these preformed leptin pools. By immunofluorescence, Barr et al. (22) showed the colocalization of intracellular leptin with markers of the endoplasmic reticulum and not in secretory-like compartments in adipocytes. Pilot studies in our own laboratory show accumulated leptin in subcellular internal membrane fractions prepared from freshly isolated rat adipocytes and a dramatic decrease in the leptin content of these internal membrane fractions from insulin-treated cells relative to controls (R.L.B., B.C., unpublished observations). Thus, it seems that insulin acts in part to stimulate the movement of preformed leptin stored in the endoplasmic reticulum. However, de novo protein synthesis is also required for the full insulin-stimulated response.

To gain a better understanding of the signaling pathways mediating hormone-stimulated leptin synthesis and secretion, we investigated the effects of inhibitors on various signaling molecules in adipocytes. Treatment with the PI 3-kinase inhibitor LY294002, the MEK inhibitor PD98059, and the immunosuppressant rapamycin did not affect basal levels of secreted leptin, but it markedly diminished both insulin- and dexamethasone-stimulated leptin secretion. PI 3-kinase is a key player in vesicle trafficking in adipocytes and other cell types. It transduces a myriad of growth and metabolic effects of insulin, including, but not limited to, stimulation of glucose transport, gene transcription, and insulin-stimulated protein synthesis (31). Thus, PI 3-kinase is likely to have a key role in mediating insulin-stimulated leptin synthesis and secretion at multiple steps. These data also indicate that both insulin- and dexamethasone-stimulated leptin secretion in freshly isolated rat adipocytes are regulated by PD98059- and rapamycin-sensitive signaling pathways. Insulin stimulates protein synthesis by increasing the rate of mRNA translation (32,33). This effect of insulin is dependent on phosphorylation of both ribosomal proteins and translation factors, including the eIF-4E-binding protein 4E-BP1 (also known as PHAS-I) (34–36). In the dephosphorylated state, 4E-BP1 is tightly bound to the mRNA cap-binding protein eIF-4E, effectively blocking translation of capped mRNA (37,38). Insulin-stimulated phosphorylation of 4E-BP1 promotes dissociation of the 4E-BP1/eIF-4E complex, which effectively enables eIF-4E to participate in the initiation of translation (38–41). Interestingly, both rapamycin and PD98059 have been shown to block insulin-mediated phosphorylation of 4E-BP1 (41–43). Rapamycin inhibits phosphorylation of 4E-BP1 presumably through selective inhibition of mTOR, the mammalian counterpart of the TOR1p and TOR2p proteins that are involved in regulating translation in *Saccharomyces cerevisiae* (44). A recent study reported that PD98059 can also block insulin-mediated phosphorylation of 4E-BP1, but the exact mechanism of this inhibition is unclear (43). Therefore, insulin- and

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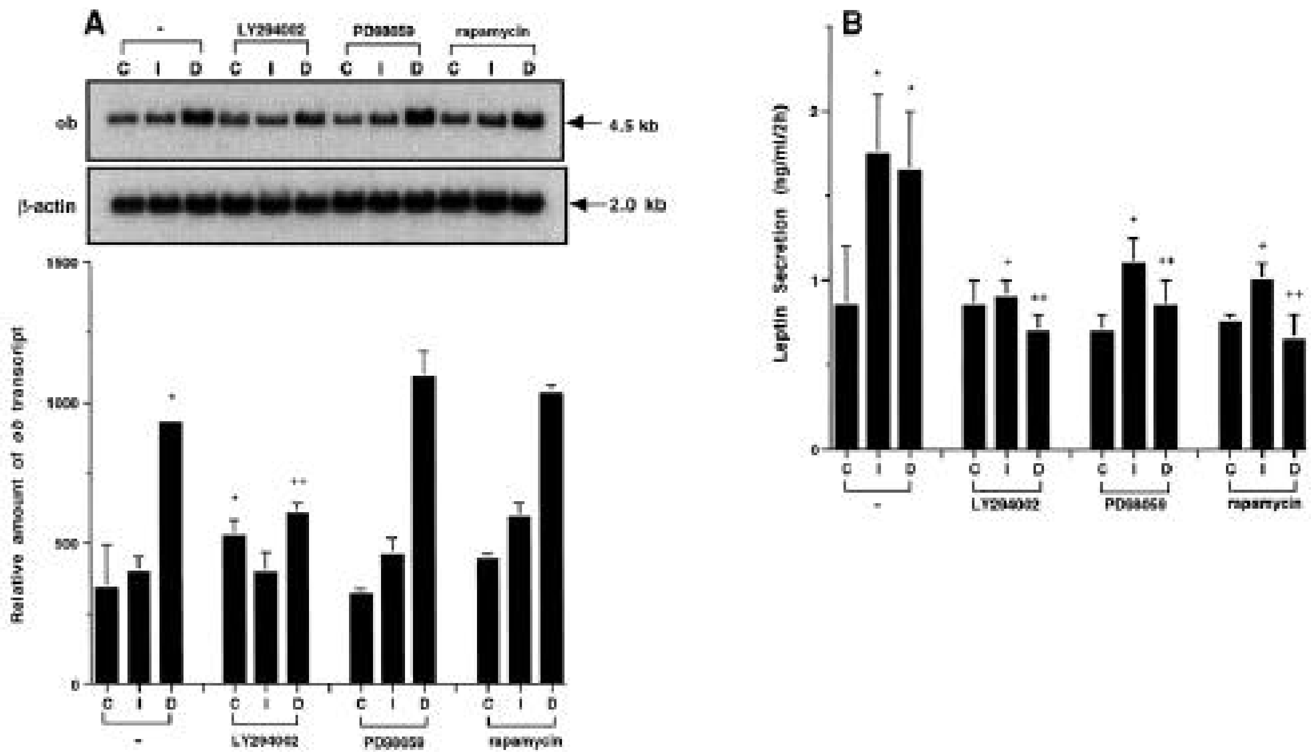

FIG. 4. ***A:*** **Effects of the PI 3-kinase inhibitor LY294002, the MEK inhibitor PD98059, and the immunosuppressant rapamycin on *ob* gene expression in control (C), insulin-treated (I), and dexamethasone-treated (D) rat adipocytes. Adipocytes were incubated in the absence or presence of 50 µmol/l LY294002, 10 µmol/l PD98059, or 20 nmol/l rapamycin for 30 min. The conditioned medium was changed. The adipocytes were then incubated with or without 100 nmol/l insulin or 100 nmol/l dexamethasone in the absence or presence of each inhibitor at the aforementioned concentrations. Each lane was loaded with 10 µg total RNA, and membranes were hybridized with a mouse *ob* cDNA probe. The relative amount of *ob* transcript was determined by densitometric scanning, and results are expressed as means ± SE. Membranes were stripped and reprobed with a human β-actin cDNA as a control for RNA integrity and loading.** ***B:*** **Effects of the PI 3-kinase inhibitor LY294002, the MEK inhibitor PD98059, and the immunosuppressant rapamycin on leptin secretion from freshly isolated adipocytes. Incubation conditions were those described above. Secreted leptin contained in the conditioned medium was measured by RIA. Results are means ± SE for three independent experiments (n = 14 per assay). *P < 0.05 vs. control (–); +P < 0.05 versus insulin-treated (–); ++P < 0.05 vs. dexamethasone-treated (–).**

dexamethasone-stimulated leptin secretion are potentially mediated via effects at the level of mTOR/4E-BP1. These possibilities are under current investigation.

In summary, the results presented here show that *ob* gene expression and leptin secretion from freshly isolated rat adipocytes are acutely regulated by insulin and dexamethasone. Dexamethasone-stimulated leptin secretion is mediated primarily by transcriptional mechanisms. By contrast, insulin's effects on leptin synthesis/secretion appear to be regulated in at least two posttranscriptional steps: *1*) by mobilizing preexisting pools of leptin and *2*) by stimulating leptin protein synthesis. These studies also implicate a role for a PI 3-kinase–dependent, a PD98059-sensitive, and/or a rapamycin-sensitive signaling pathway in mediating leptin synthesis and secretion. Additional studies are necessary to more precisely define the molecular components involved in this process.

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