Am J Physiol Endocrinol Metab 288: E1019–E1027, 2005.
First published December 7, 2004; doi:10.1152/ajpendo.00325.2004.

IGF-I downregulates resistin gene expression and protein secretion

Yen-Hang Chen, Pei-Fang Hung, and Yung-Hsi Kao

Department of Life Science, College of Science, National Central University, Chung-Li City, Taoyuan, Taiwan

Submitted 23 July 2004; accepted in final form 3 December 2004

Chen, Yen-Hang, Pei-Fang Hung, and Yung-Hsi Kao. IGF-I downregulates resistin gene expression and protein secretion. *Am J Physiol Endocrinol Metab* 288: E1019–E1027, 2005. First published December 7, 2004; doi:10.1152/ajpendo.00325.2004.—Resistin (Rstn) is known as an adipocyte-specific secretory factor that can cause insulin resistance and decrease adipocyte differentiation. Conversely, based on various studies, insulin-like growth factors (IGFs) can improve insulin resistance and stimulate adipocyte adipogenesis. Whether IGFs exert their effects through the control of Rstn's production or modulation of Rstn's action is unknown. This study was designed to examine the influence and the signaling of IGF-I on Rstn gene expression and protein secretion by 3T3-L1 adipocytes. We found that IGF-I suppressed Rstn mRNA expression and protein release in dose- and time-dependent manners. The IC_{50} of IGF-I was ~1 nM for a range of 6–10 h of treatment. Treatment with cycloheximide, but not with actinomycin D, prevented IGF-I-suppressed Rstn mRNA expression, suggesting that IGF-I destabilizes Rstn mRNA and that IGF-I's effect requires new protein, but not mRNA, synthesis. Pretreatment with IGF-I receptor (IGF-IR) antibody blocked IGF-I-altered IGF-IR activity and Rstn mRNA levels. Neither PD-98059, SB-203580, nor LY-294002 changed the IGF-I-decreased levels of Rstn mRNA, but they inhibited IGF-I-stimulated activities of MEK1, p38 MAPK, and phosphoinositide 3-kinase, respectively. However, SB-203580 antagonized the IGF-I-decreased Rstn protein release. These data demonstrate that IGF-I downregulates Rstn gene expression via IGF-IR-dependent and MEK1-, p38 MAPK-, and phosphoinositide 3-kinase-independent pathways and likely modifies the distribution of Rstn protein between the intracellular and extracellular compartments via a p38 MAPK-dependent pathway. Decreases in Rstn production and secretion induced by IGF-I may be related to the mechanism by which IGF-I modulates body weight and diabetes in animals.

insulin-like growth factor; 3T3-L1; adipocytes; mitogen-activated protein kinase

RESISTIN (Rstn), also known as an adipocyte-specific secretory factor (ADSF), is a cysteine-rich polypeptide that was first isolated from adipose tissues and found to be link between obesity and type 2 diabetes (45). In particular, Rstn mRNA expression in adipose tissues from obese humans is higher than that in normal subjects (10). In addition, a single nucleotide polymorphism in the Rstn gene promoter is associated with obesity (14) and diabetes (36), and plasma Rstn levels are elevated in patients with type 2 diabetes (50). Moreover, dominant inhibitory Rstn enhances adipogenesis and improves insulin sensitivity (28). Since its discovery, Rstn has also been found to possess other numerous actions. For example, Rstn inhibits the conversion of 3T3-L1 preadipocytes to adipocytes (27), regulates fasted blood glucose levels (4), suppresses insulin-stimulated glucose uptake in adipocytes (45) and muscle cells (32), and promotes endothelial cell activation (47).

The genomic DNAs of mouse and human Rstn contain five and four exons and four and three introns, respectively (19). The mRNA of Rstn, depending on the species and the number of polyadenylation signals, is composed of ~575–1,217 bp (9, 27, 45, 46). The open-reading frame encodes a protein of 108 and 114 amino acids with a calculated molecular mass of 10 and 12.5 kDa in humans and mice, respectively (9, 27, 45, 46). In mice, a 591-bp Rstn cDNA contains an 83-bp 5′-untranslated region, a 335-bp preproresistin coding region, and a 173-bp 3′-untranslated region (45). The mature derivative Rstn hormone is secreted as a homodimer protein, and its monomer contains an NH_2-terminal signal sequence, a variable middle portion, and a cysteine-rich COOH-terminal signature signal ($C\text{-}X_{11}\text{-}C\text{-}X_8\text{-}C\text{-}X\text{-}C\text{-}X_3\text{-}C\text{-}X_{10}\text{-}C\text{-}X\text{-}C\text{-}X\text{-}C\text{-}X_9\text{-}CC\text{-}X_{3-6}$-end), which is conserved in a family of Rstn-like molecules (46).

Despite the importance of Rstn, relatively little is known about the control of Rstn's production or modulation of Rstn's actions. Although a number of reports have shown that the expression of Rstn gene can be regulated by endocrine, nutritional, inflammatory, neurological, pharmacological, genetic, and developmental cues, several results appear contradictory (3, 17, 18, 23, 24, 26, 27, 33, 35, 41, 42, 45, 48, 49). For example, treatment of 3T3-L1 adipocytes with insulin decreased Rstn mRNA and protein levels (24, 26, 42) and increased secretion of Rstn protein (51). In contrast, insulin stimulated the expression of Rstn gene in T37i brown adipocytes (48) and in white adipose tissue of mice with streptozotocin-induced diabetes (27) and Zucker diabetic rats (49). In human white adipose tissue, insulin did not affect Rstn protein release (15). The fact that the signal element(s) responsible for transducing insulin's action on Rstn gene expression and secretion has not been identified (15, 26, 42, 51) and the fact that insulin can mimic insulin-like growth factors (IGFs) in modulating adipocyte function (39) have caused much controversy. Accordingly, a thorough examination of the signal element through which IGFs carry out their modulation of Rstn expression and secretion should help clarify these observations.

The structure of IGF-I is similar to that of proinsulin (39). Determining whether IGF-I has the same ability as the insulin hormone in reducing Rstn gene expression (24, 26, 42) and stimulating protein secretion (51) requires further investigation. IGFs have been reported to stimulate mitogenesis and adipogenesis of fat cells and improve insulin resistance and diabetes (39). Whether IGFs exert their effects through the control of Rstn's production or modulation of Rstn's action is unknown. On the other hand, a few reports have shown that growth hormone (GH) in vitro downregulated (16, 42) and in vivo upregulated gene expression of adipocyte Rstn (11). These results, together with the fact that GH is the major

Address for reprint requests and other correspondence: Y.-H. Kao, Dept. of Life Science, College of Science, National Central Univ., Chung-Li City, Taoyuan Taiwan 32054, (E-mail: ykao@cc.ncu.edu.tw).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

IGF-stimulating hormone, have created much controversy surrounding the possible role of IGFs in regulating Rstn gene expression and the possible involvement of IGFs in GH's regulation of Rstn gene expression. Further studies to determine whether IGFs affect Rstn gene expression and protein secretion should help clarify these notions.

In this study, we used 3T3-L1 adipocytes to examine the influence and the signaling of IGF-I on Rstn gene expression and protein secretion. We investigated whether IGF-I-regulated Rstn gene expression and protein secretion are dependent on the MAPK and phosphoinositide 3-kinase (PI3K) pathways. MAPK and PI3K were chosen because they have been reported to be essential signal transducers of IGF-I in the regulation of other genes (39) and because their overexpression has been reported to suppress Rstn expression (44).

MATERIALS AND METHODS

Chemical reagents. All materials (e.g., recombinant human IGF-I, IGF-II, insulin, and so forth) were purchased from Sigma (St. Louis, MO) unless otherwise stated. DMEM, penicillin-streptomycin, FBS, trypsin, agarose, the 1-kb plus DNA ladder marker, and the protein marker were purchased from GIBCO-BRL (New York, NY). Except for the Rstn antibody, which was obtained from Linco Research (St. Charles, MO), all other antibodies [P-ERKs, ERK1, ERK2, IGF-I receptor (IGF-IR), IGF-IRβ, P-p38, P-Akt, β-actin, P-Tyr, donkey anti-rabbit IgG-horseradish peroxidase (HRP), donkey anti-mouse IgG-HRP, donkey anti-goat IgG-HRP, and goat anti-guinea pig IgG-HRP] were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The 3′-rapid amplification of cDNA ends system, TRIzol, and T*aq* polymerase were purchased from Invitrogen Life Science Technologies (Carlsbad, CA).

Cell culture. 3T3-L1 cells (American Type Culture Collection, Manassas, VA) were grown in DMEM (pH 7.4) containing 10% FBS, 100 U/ml of penicillin, and 100 μg/ml streptomycin (GIBCO BRL) in a humidified atmosphere of 95% air-5% CO_2 at 37°C. 3T3-L1 cells were plated at a density of ~12,000–15,000/cm^2 on a 10-cm plate (25). Every 2 days, 10 ml of medium were replaced before these cells became confluent. To obtain 3T3-L1 adipocytes, 2-day postconfluent 3T3-L1 preadipocytes (3×10^6 cells/10-cm plate; designated *day 0*) were treated with DMEM containing a final concentration of 10 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, and 10% FBS for 48 h (42). The medium was then changed to DMEM containing 10% FBS for an additional 6–10 days (replaced every other day). With this protocol, >90% adipocyte differentiation was achieved, as indicated by phenotypical appearance and triglyceride accumulation (37). Differentiated adipocytes expressed more Rstn mRNA than did preadipocytes or differentiating preadipocytes.

For all experiments, differentiated adipocytes were serum starved for 12 h in DMEM containing 0.1% (fatty acid-free) BSA and then, unless noted otherwise, incubated with or without hormones at various concentrations for the indicated time periods. Actinomycin D (5 μg/ml), cycloheximide (CHX; 5 μg/ml), PD-98059 [a MEK1 inhibitor (1); 50 μM], SB-203580 [a p38 MAPK inhibitor (13); 10 μM], and LY-294002 [a PI3K inhibitor (8); 50 μM] were used to inhibit the transcriptional, translational, MEK1, p38 MAPK, and PI3K activities, respectively (26). In experiments, serum-starved 3T3-L1 adipocytes were pretreated with and without either actinomycin D for 30 min or other inhibitors for 90 min. Adipocytes were then stimulated with or without IGF-I (1 nM) for the indicated time period. After treatment, Rstn mRNA and protein levels were measured. Despite the high dose of some inhibitors used in the experiment, no adverse effects on cell viability of adipocytes for 24 h were noted, as examined by the trypan blue exclusion method (data not shown).

Digoxigenin-PCR ELISA for Rstn mRNA. We measured Rstn mRNA levels using a commercial PCR ELISA kit with digoxigenin labeling and detection (Roche Applied Science, Mannheim, Germany) (38). Total RNA was isolated from 3T3-L1 adipocytes with the TRIzol kit, and cDNA was then synthesized from equal amounts (5 μg) of RNA using Moloney murine leukemia virus RT (Invitrogen). PCR was performed under the following conditions: an initial denaturing cycle at 94°C for 5 min, followed by 30 cycles of amplification consisting of denaturation at 94°C for 1 min, annealing for Rstn at 60°C and for β-actin at 53°C for 90 s, and extension at 72°C for 90 s. A final extension at 72°C for 10 min was added after the last cycle. The PCR product was electrophoresed on 1.5% (wt/vol) agarose gel electrophoresis with 40 mM Tris-acetate buffer (pH 8.0) containing 1 mM EDTA and visualized by 0.5 μg/ml ethidium bromide. The forward and reverse primers were 5′-GTACCCACGGGATGAAGAACC-3′ and 5′-GCAGAGCCACAGGAGCAG-3′ for mouse Rstn (accession no. AF323080) and 5′-CCAGGGTGTGATGGTGGGAATG-3′ and 5′-CGCACGATTTCCCTCTCAGCTG-3′ for actin (accession no. X03672), respectively. The Rstn and actin PCR products, predicted to be 252 and 500 bp, respectively, were cloned into the pTargetT vector for subsequent nucleotide sequencing performed by Academia Sinica (Taiwan). Sample Rstn mRNA levels were determined by relation to a standard curve of Rstn cDNAs, ranging from 3 to 200 ng/well (optical density at 405 nm = 0.1141 + 0.0031 × ng DNA/well; $R^2 = 0.998$). An almost linear range in the number of PCR amplifications for Rstn was observed at between 20 and 40 cycles compared with the β-actin standard. Thus 30 cycles of PCR amplification were later used for all experiments. After normalization to β-actin mRNA, Rstn mRNA levels were expressed as percent of control.

Immunoprecipitation. IGF-IR was immunoprecipitated according to the method described by Kokontis et al. (29). After treatment experiments, adipocytes were washed twice in PBS and then lysed in 1 ml of buffer containing 20 mM Tris·HCl (pH 7.6), 1 mM EDTA, 1 mM Na_3VO_4, 0.2% Triton X-100, and 1 mM PMSF. Lysate was agitated for 15 min at 4°C and then centrifuged for 10 min to collect the supernatant. The protein content of the supernatant was determined in duplicate by the dye-binding method (7), using a Bio-Rad (Richmond, CA) microplate reader and BSA (Sigma) as a standard. An aliquot of the supernatant (1 mg of protein) was preincubated for 1 h at 4°C with mouse monoclonal IGF-IR (3B7) antibody (which does not cross-react with the insulin receptor) or preimmunized normal mouse serum (NMS; as the control). The mixture was incubated with 20 μl of protein A-agarose (Santa Cruz Biotechnology) overnight at 4°C. The total amounts of IGF-IR and phosphotyrosine-IGF-IRβ (P-Tyr-IGF IRβ) indicative of IGF-IR activation (39) in the immunoprecipitates were measured by Western blot analysis with IGF-IR, IGF-IRβ (C-20), or P-Tyr antibodies, respectively.

Western blot analysis. Western blot analysis was performed on supernatant fractions of adipocytes (50 μg of protein) separated by 12.5% SDS-PAGE with a loading buffer (100 mM Tris·HCl, pH 6.8, 4% SDS, 20% glycerol, 0.2% bromophenol blue, and 10% β-mercaptoethanol) (29). Gel protein was then blotted onto Immobilon-NC transfer membrane (Millipore, Bedford, MA). The immunoblots were blocked for 1 h at room temperature with 10 mM PBS containing 0.1% Tween 20 and 5% defatted milk. After the wash with PBS-Tween 20, immunoblot analyses with primary antibody were performed. All primary antibodies (ERK1, ERK2, P-ERKs, P-p38, P-Akt, IGF-IR, phosphotyrosine, β-actin, and Rstn antisera) were used at a dilution concentration of 1:1,000 (~0.2 μg/ml). Donkey anti-rabbit IgG, donkey anti-mouse IgG, donkey anti-goat IgG, or goat anti-guinea pig conjugated with HRP was used as the secondary antibody at a dilution of 1:2,000 (~0.2 μg/ml). We visualized the immunoblots using Western Lightning chemiluminescence reagent plus kit (Perkin-Elmer Life Science, Boston, MA) for 3 min; this was followed by exposure to Fuji film for 2–3 min. We quantified blots using a Molecular Imager (Bio-Rad Laboratories, Hercules, CA). After normalization to β-actin protein, levels of the intracellular Rstn

protein and kinases were expressed as a percent of the control, unless noted otherwise.

ELISA for extracellular Rstn protein. A homologous ELISA procedure was adapted from Eilertson and Sheridan (12) and Maebuchi et al. (30) to analyze secreted Rstn protein. The primary antiserum (guinea pig anti-mouse Rstn serum; cross-reactive with tissue and serum Rstn from rats and mice) was used at a dilution of 1:1,000, and the secondary antiserum (donkey anti-guinea pig IgG serum) was used at a dilution of 1:3,000. The lowest detectable level of Rstn was 5 ng/well. The interassay and intra-assay coefficients of variation in the ELISA were ~7–9 and ~3–4%, respectively. The reproducible results were obtained in the range of Rstn from 5 to 80 ng/well (optical density at 405 nm = 0.1269 + 0.0044 × ng/well; $R^2 = 0.979$). The resulting dilution curve from the culture medium for testing the specificity of the antiserum was parallel to the Rstn standard. Sample Rstn in the culture medium was calculated from the standard curve; 0.1% BSA-DMEM and Rstn hormone, as expressed in *Escherichia coli* and purified with an Ni-column (Amersham Biosciences, Taiwan Branch, Taipei, Taiwan) were used as the blank control and standard, respectively.

Construction, expression, and purification of hexahistidine-tagged Rstn. Similar to that reported by Maebuchi et al. (30), the cDNA of mouse Rstn, prepared from 3T3-L1 adipocytes, was primed with oligo(dT). It was then amplified by PCR with the primers 5′-ATGAAGAACCTTTCATTTCCC-3′ and 5′-GGCCACGCGTCGACTAGTAC-3′ under the following conditions: an initial denaturing cycle at 94°C for 3 min, followed by 30 cycles of amplification consisting of denaturation at 94°C for 45 s, annealing for Rstn at 53°C for 40 s, and extension at 72°C for 90 s. A final extension at 72°C for 10 min was added after the last cycle. The Rstn PCR product (predicted as 500 bp) was cloned into the pTargetT vector. The resulting plasmid, as the template, and the primers 5′-GGTACCTCAGGAAGCGACCTGCAGCTTAC-3′ and 5′-GGATCCATGAAGAACCTTTCATTTCCC-3′ were introduced between the sites of *Bam*HI and *Kpn*I. The amplified sequence was cut, ligated, and cloned between the sites of *Bam*HI and *Kpn*I of the pQE31 expression plasmid. The nucleotide sequence of the insert was verified before expression of the Rstn protein. To express and purify the Rstn protein, pQE31-Rstn cDNA was transfected into *E. coli* SG13009 cells. Cells were grown at 37°C in Luria-Bertani medium containing 1% tryptone, 0.5% yeast extract, 0.5% NaCl, 100 μg/ml ampicillin, and 25 μg/ml kanamycin. At the midexponential growth phase (optical density at 600 nm = 0.5–0.7), isopropyl-1-thio-β-D-galactopyranoside was added to a final concentration of 1 mM, and the incubation was continued for another 4 h at 37°C to induce the expression of $(His)_6$-tagged Rstn. The harvested cells were lysed with *buffer A* (10 mM imidazole, pH 8.0, 300 mM NaCl, 1 mg/ml lysozyme, 8 M urea, and 50 mM NaH_2PO_4) and sonicated at 4°C. After centrifugation (10,000 *g* for 30 min at 4°C), the supernatant was loaded onto an Ni-NTA column equilibrated with *buffer A* (except lysozyme). The column was washed with *buffer B* (30 mM imidazole, pH 8.0, 300 mM NaCl, 8 M urea, and 50 mM NaH_2PO_4) and eluted with *buffer C* (250 mM imidazole, pH 8.0, 300 mM NaCl, 8 M urea, and 50 mM NaH_2PO_4). The fraction containing the $(His)_6$-tagged Rstn was dialyzed with 10 mM PBS overnight at 4°C to remove the salt. After dialysis, the $(His)_6$-tagged Rstn solution was dried with a Labconco lyophilizer and stored at −70°C. Using Western blot analysis with the Rstn antibody, we verified the molecular mass of the expressed Rstn protein to be ~12 kDa, similar to that reported by Steppan et al. (45).

Statistical analysis. Data are expressed as means ± SE. Unpaired Student's *t*-test was used to examine differences between the control and IGF-I-treated groups. One-way ANOVA followed by the Student-Newman-Keuls multiple-range test was used to examine differences among multiple groups. Differences were considered significant at $P < 0.05$. We performed all statistics using SigmaStat (Jandel Scientific, Palo Alto, CA).

RESULTS

Effects of IGF-I on Rstn mRNA expression. IGF-I inhibited the steady-state levels of Rstn mRNA in concentration-dependent (Fig. 1*A*) and time-dependent (Fig. 1*B*) manners. The IC_{50} of IGF-I was ~1 nM after 6–10 h.

Effects of IGF-I on Rstn mRNA stability. The possibility that the IGF-I-induced reduction in Rstn mRNA expression resulted from increased Rstn mRNA degradation was also examined (Fig. 1*C*). 3T3-L1 adipocytes were pretreated with the transcriptional inhibitor actinomycin D and then treated with or without 1 nM IGF-I. The basal half-life of Rstn mRNA was more than 12 h, but it shifted to <10 h in the presence of IGF-I (Fig. 1*C*).

Effects of IGF-I on Rstn protein expression and secretion. To determine whether IGF-I-suppressed Rstn gene expression also occurred at the level of translation, changes in the intracellular Rstn protein content as well as release of Rstn into culture medium were measured (Fig. 2). Intracellular Rstn protein slightly, but not significantly, decreased in the presence of 1–10 nM IGF-I within 24 h (Fig. 2, *A* and *C*). IGF-I significantly reduced Rstn protein release in a concentration-dependent fashion (Fig. 2, *B* and *D*). IGF-I at 1 nM significantly decreased Rstn protein secretion from 225 to 150 ng/ml after 6 h and from 405 to 300 ng/ml after 24 h (Fig. 2*D*).

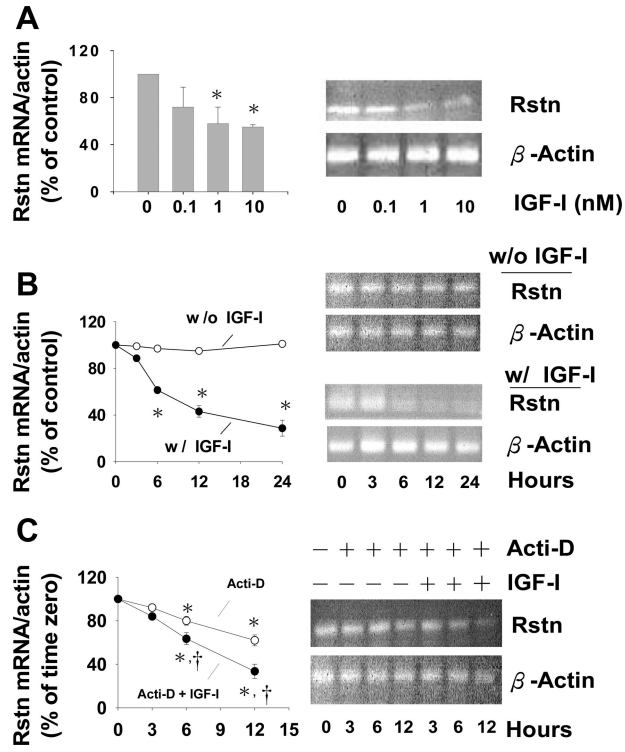

Fig. 1. Inhibitory effects of insulin-like growth factor (IGF)-I and actinomycin D (Acti-D) on resistin (Rstn) mRNA expression in 3T3-L1 adipocytes. *A*: dose-dependent effect of IGF-I was observed 6 h after treatment. *B*: time-dependent effect of IGF-I (1 nM) was observed. *C*: IGF-I decreased Rstn mRNA stability. Data are expressed as means ± SE from replicates of 3 experiments. SE bars are too small to be seen in *B* and *C*. In *A* and *B*, control experiments were those not treated with IGF-I at a given time, whereas, in *C*, control was set at *time 0* when IGF-I was added in the beginning. *$P < 0.05$ vs. control at a given dose (*A*) and time (*B*). In *C*, *$P < 0.05$ vs. the control at a given time; †$P < 0.05$, Acti-D vs. Acti-D + IGF-I at a given time. *Right*: RT-PCR. *Left*: digoxigenin-PCR ELISA.

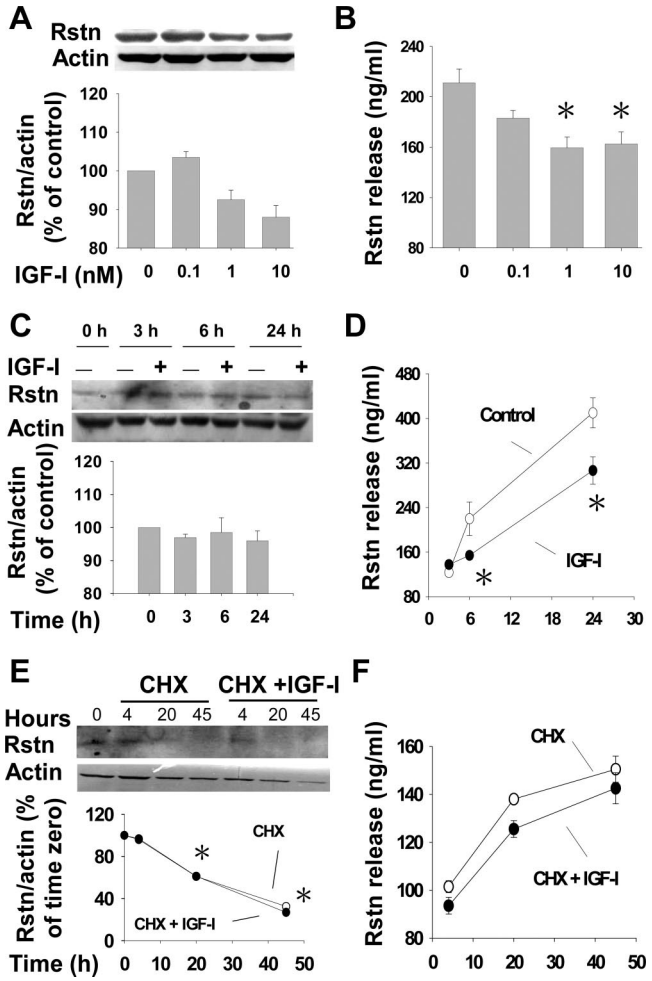

Fig. 2. Effects of IGF-I and cycloheximide (CHX) on the intracellular (*A*, *C*, and *E*) and extracellular (*B*, *D*, and *F*) Rstn protein levels in 3T3-L1 adipocytes. *A* and *B*: dose-dependent effects of IGF-I were observed 6 h after treatment. *C* and *D*: time-dependent effects of IGF-I (1 nM) were observed. *E* and *F*: IGF-I did not affect Rstn protein stability during the 45-h treatment. Data are expressed as means ± SE from replicates of 3 experiments. SE bars are too small to be seen in *D–F*. In *A–D*, control experiments were those without IGF-I treatment; in *E* and *F*, control was set at *time 0* when IGF-I was added in the beginning. *$P < 0.05$ vs. control at a given dose (*B*) and time (*D* and *E*).

Effects of IGF-I on Rstn protein stability. The possibility that IGF-I-inhibited release of Rstn protein was related to changes in Rstn protein stability was examined (Fig. 2, *E* and *F*). 3T3-L1 adipocytes were pretreated with the translational inhibitor CHX and then treated with or without 1 nM IGF-I (Fig. 2, *E* and *F*). The basal half-life of the intracellular Rstn protein was ~20 h, and IGF-I had no effect on protein stability (Fig. 2*E*). Similarly, IGF-I did not affect the half-life of Rstn protein released into the extracellular medium (Fig. 2*F*).

Effect of CHX on IGF-I-induced decreases in Rstn mRNA expression. To further examine whether IGF-I-suppressed expression of the adipocyte Rstn gene is mediated via other proteins, adipocytes were pretreated with or without CHX for 90 min and then cells were stimulated with or without 1 nM IGF-I for 6 h (Fig. 3). Treatment with CHX alone did not alter Rstn mRNA expression of adipocytes compared with control. CHX, however, prevented IGF-I-induced decreases in Rstn gene expression.

Changes in IGF-IR activity. The effect of IGF-I on its receptor activity was assessed by changes in the amount of tyrosine phosphorylation of the IGF-IR β-subunit (Fig. 4). Adipocytes were pretreated with mouse monoclonal IGF-IR antiserum or preimmunized NMS for 90 min. Adipocytes were then incubated in the presence and absence of 1 nM IGF-I either for 20 min to determine IGF-IR activity (Fig. 4, *C* and *D*) or for 6 h to determine Rstn mRNA expression (Fig. 4, *A* and *B*). IGF-IR inactivation via the IGF-IR antibody (Fig. 4*A*), but not via NMS (Fig. 4*B*), tended to increase the basal Rstn mRNA level and prevented an IGF-I-induced decrease in Rstn mRNA expression. Concomitant with this observation, IGF-IR antiserum (Fig. 4*C*), but not NMS (Fig. 4D), resulted in significant decreases in the basal IGF-IR activity and prevented IGF-I-stimulated IGF-IR activity, as indicated by decreased P-Tyr-IGF-IR.

Changes in ERK MEK, p38 MAPK, and PI3K activities. Whether IGF-I-induced downregulation of Rstn mRNA expression depended on the MAPK or PI3K pathways was assessed by changes in the activities of ERK MEK, p38 MAPK, and PI3K (Fig. 5). Adipocytes were pretreated with either the ERK MEK inhibitor PD-98059, the p38 MAPK inhibitor SB-203580, or the PI3K inhibitor LY-294002 and then treated with 1 nM IGF-I for 6 h. Activities of ERK MEK and PI3K were assessed by changes in the amount of the phosphorylated form of their own protein substrates, ERK1, ERK2, and Akt, respectively, whereas the activity of p38 MAPK was assessed by changes in the amount of the tyrosine^{182}-phosphorylated form of p38. IGF-I alone had no effect on ERK1 and ERK2 proteins but increased the amounts of P-ERK1 and P-ERK2 proteins by 80% (Fig. 5*A*) and the amounts of P-p38 (Fig. 5*B*) and P-Akt proteins by 50–70% (Fig. 5*C*).

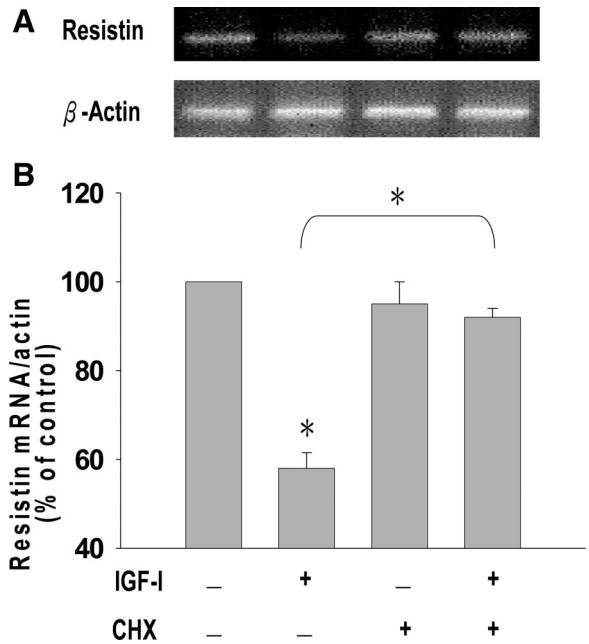

Fig. 3. CHX prevented an IGF-I-induced decrease in Rstn mRNA. Starved 3T3-L1 adipocytes were pretreated with or without CHX (5 μg/ml) for 90 min and then treated with or without 1 nM IGF-I for 6 h. *A*: RT-PCR. *B*: digoxigenin-PCR ELISA. Control experiments were those treated with neither IGF-I nor CHX. Data are expressed as means ± SE from replicates of 3 experiments. *$P < 0.05$ vs. control, IGF-I vs. CHX + IGF-I.

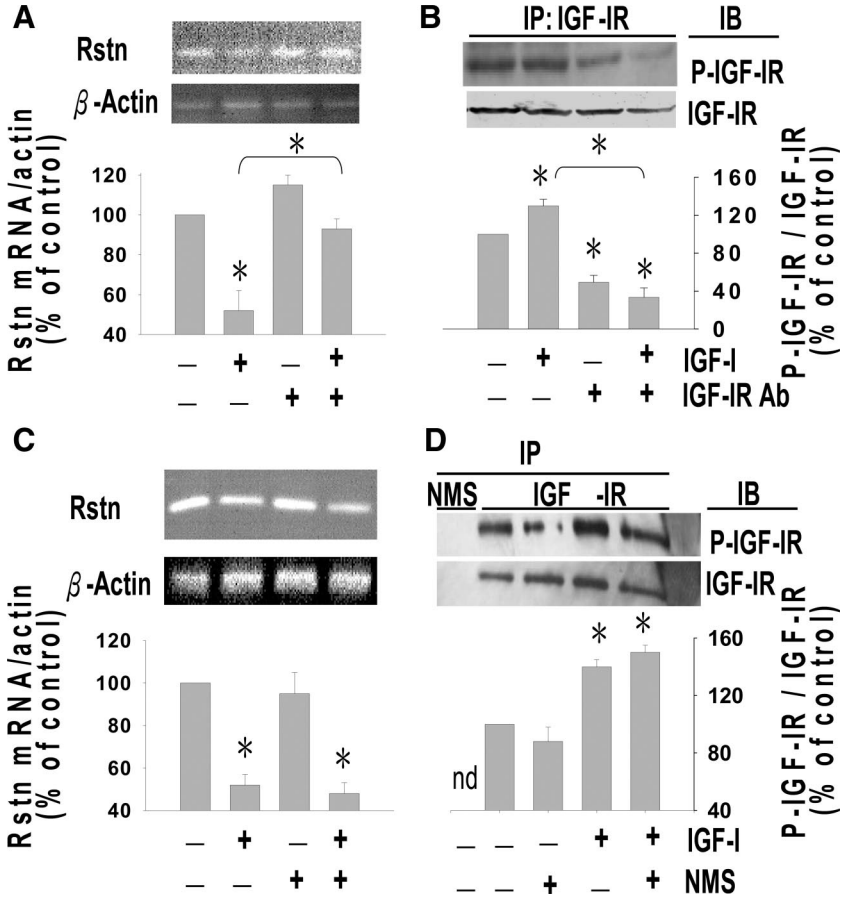

Fig. 4. IGF-I receptor antibody (IGF-IR Ab) blocked IGF-I-altered Rstn mRNA expression and IGF-IR activity. Serum-starved 3T3-L1 adipocytes were pretreated with or without either 5 μg/ml IGF-IR Ab (*A* and *C*) or 5 μg/ml normal mouse serum (NMS; *B* and *D*) for 90 min and then stimulated with 1 nM IGF-I. After 6 h of IGF-I treatment (*A* and *B*), Rstn mRNA levels were analyzed by RT-PCR (*top*) and by the quantitative digoxigenin-PCR ELISA (*bottom*). In *C* and *D*, IGF-IR protein content and activity (as indicated by amount of phosphotyrosine-IGF-IR) were determined 20 min after IGF-I treatment. Total cell lysates were immunoprecipitated (IP) with IGF-IR Ab (*C*) or NMS (*D*), and the amounts of IGF-IR and phosphotyrosine-IGF-IR were then measured by Western blot analysis (IB; *top*) with IGF-IR or phosphotyrosine antibodies, respectively. IGF-IR activity was corrected with IGF-IR protein. Data are expressed as means ± SE from replicates of 3 experiments. nd, Nondetectable; P-IGF-IR, phosphotyrosine-IGF-IR. **P* < .05 vs. control, IGF-I vs. IGF-I + IGF-IR Ab (brackets).

In contrast, PD-98059 alone reduced the basal activity of ERK MEK; in the presence of IGF-I, it suppressed IGF-I-increased levels of P-ERK proteins (Fig. 5*A*). Compared with the control, PD-98059 did not alter the total amounts of ERK1 and ERK2 proteins or the levels of Rstn mRNA. Compared with the IGF-I-treated group, PD-98059 did not alter IGF-I-suppressed expression of Rstn mRNA. Regardless of the presence of IGF-I, SB-203580 pretreatment (Fig. 5B) significantly decreased the amount of P-p38 protein in adipocytes relative to the control. SB-203580 did not alter decreases in Rstn gene expression induced by IGF-I. LY-294002 significantly reduced the amount of P-Akt protein in adipocytes treated with or without IGF-I compared with the control (Fig. 5*C*). Similar to PD-98059 and SB-203580, LY-294002 neither changed Rstn gene expression alone nor significantly affected IGF-I-induced decreases in Rstn mRNA levels.

Whether IGF-I-induced alterations in Rstn protein production and secretion were dependent on the MAPK or PI3K pathways was also examined (Fig. 6). There was a trend for SB-203580 to decrease the basal content of intracellular Rstn protein (Fig. 6*A*) and to increase the basal release of Rstn protein (Fig. 6*B*) after 6 h of treatment. In addition, this p38 MAPK inhibitor decreased IGF-I-retained levels of the intracellular Rstn protein and antagonized IGF-I-induced decreases in Rstn protein release. The effects of PD-98059 and LY-294002 differed from those of SB-203580. There was a trend for PD-98059 and LY-294002 to increase the basal content of the intracellular Rstn protein (Fig. 6*A*) and to decrease the basal release of the Rstn protein (Fig. 6*B*) after 6 h of treatment. Neither PD-98059 nor LY-294002 affected IGF-I-induced alterations in the intracellular or extracellular Rstn protein contents. These data indicate that SB-203580, but not PD-98059 or LY-294002, significantly modified IGF-I-induced changes in Rstn protein contents between the intracellular (Fig. 6*A*) and extracellular (Fig. 6*B*) compartments.

Effects of IGF-I, IGF-II, and insulin on Rstn mRNA expression. Differences among IGF-I, IGF-II, and insulin in regulating Rstn mRNA expression were assessed (Fig. 7). IGF-I, IGF-II, and insulin all decreased the Rstn mRNA levels. The IC_{50} for IGF-I was ~1 nM after 6 h of treatment. Although IGF-I and IGF-II were ~10–15% more effective than insulin, there was no significant difference among all the factors in their potency to inhibit Rstn expression ($P < 0.16$, IGF-I vs. insulin; $P < 0.07$, IGF-II vs. insulin).

DISCUSSION

The present study demonstrates that IGF-I inhibits Rstn gene expression in adipocytes. The effects of IGF-I were dose and time dependent. These effects are similar to those observed previously for insulin (24, 26, 42), although we showed that, at 1 nM, IGF-I or IGF-II tended to be more effective than insulin. It is likely that IGF-I downregulates Rstn mRNA levels by destabilizing Rstn mRNA. This conclusion is supported by our findings that IGF-I shortens the basal half-life of Rstn mRNA induced by actinomycin D alone and that the 12-h decreasing rate of adipocyte Rstn mRNA induced by IGF-I and actinomycin D is similar to that induced by IGF-I alone. The basal

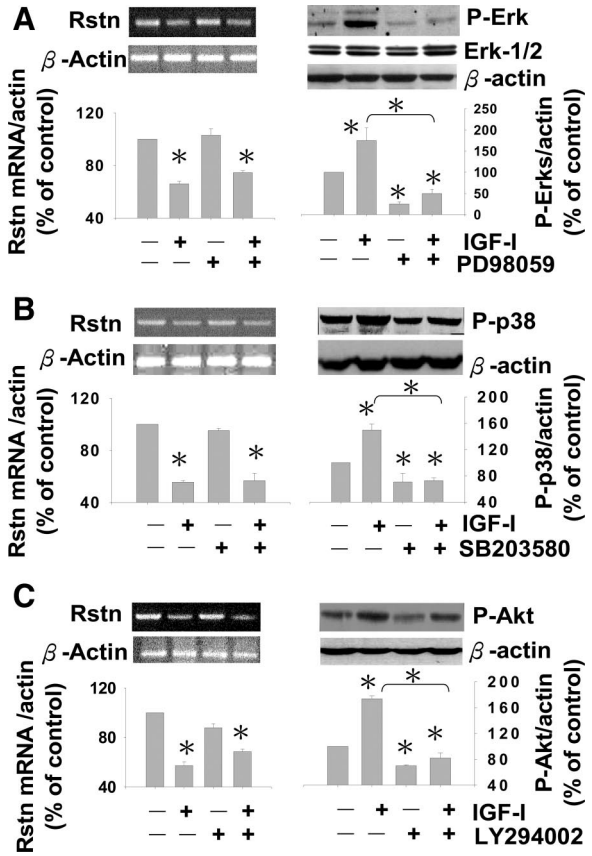

Fig. 5. Effect of PD-98059, SB-203580, and LY-294002 on the IGF-I-induced decrease in Rstn mRNA expression. Starved 3T3-L1 adipocytes were pretreated with or without 50 μM PD-98059 (*A*), 10 μM SB-203580 (*B*), or 50 μM LY-294002 (*C*) for 90 min and then stimulated with IGF-I (1 nM) for 6 h. *Top left*: representative RT-PCR products. *Top right*: representative Western blots. Data are expressed as means ± SE from replicates of 3 experiments. $^*P < 0.05$ vs. control, IGF-I vs. IGF-I + inhibitor (brackets).

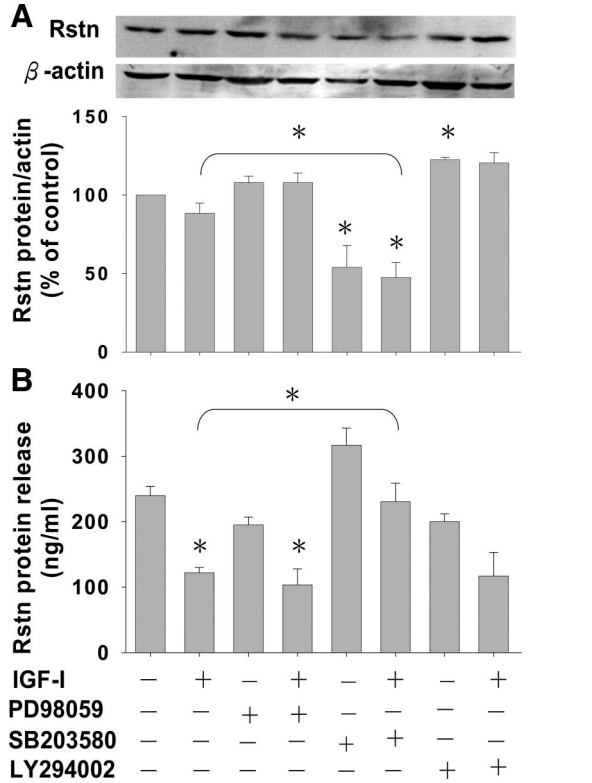

Fig. 6. Effect of PD-98059, SB-203580, and LY-294002 on IGF-I-induced changes in Rstn protein expression (*A*) and secretion (*B*). Starved 3T3-L1 adipocytes were pretreated with or without 50 μM PD-98059, 10 μM SB-203580, or 50 μM LY-294002 for 90 min and then stimulated with 1 nM IGF-I for 6 h. *A*, *top*: representative Western blots. Data are expressed as means ± SE from replicates of 3 experiments. $^*P < 0.05$ vs. control, IGF-I vs. IGF-I + inhibitor (brackets).

half-life of Rstn mRNA demonstrated by actinomycin D treatment was more than 12 h, which is consistent with that reported by Kawashima et al. (26). Interestingly, the absence of an effect of actinomycin D on IGF-I-induced degradation of Rstn mRNA may argue for the presence of preexisting mRNA. This appears somewhat different from the reported actinomycin D-sensitive effect of insulin (26), suggesting that insulin and IGF-I work differently in 3T3-L1 adipocytes to decrease Rstn stability. In higher eukaryotes, the control of mRNA stability is the result of a complex set of events, dependent on the stability of the poly(A) tail, polysomes, and the 3′ untranslated region with adenosine- and uridine-rich elements, as well as on the activities of adenosine- and uridine-rich element-binding proteins, coding region-binding proteins, and RNases (40). Determining whether factors such as IGF-I and insulin influence the half-life of adipocyte Rstn mRNA in distinct ways by changing the characteristics of these factors requires further study.

To our knowledge, the half-life of Rstn protein has not been reported previously. We treated 3T3-L1 adipocytes with CHX alone and estimated the basal half-life of Rstn protein as being ~20 h. The greater stability of Rstn protein compared with mRNA may favor targeting of the Rstn protein from the endomembrane system of adipocytes to their extracellular

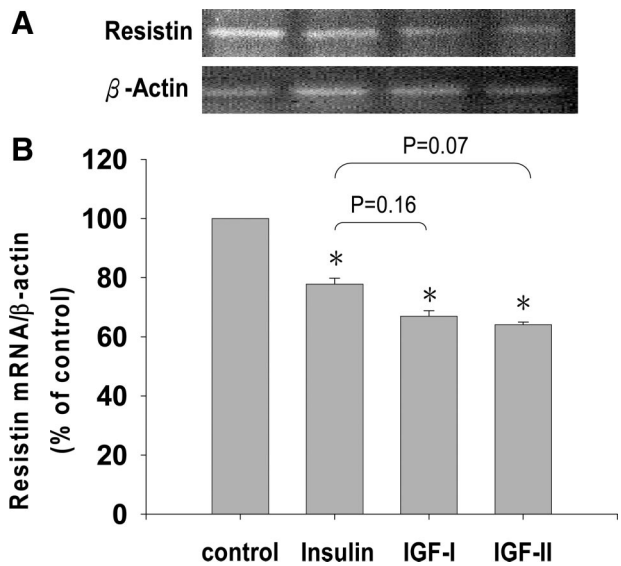

Fig. 7. Assessment of differences among IGF-I, IGF-II, and insulin in reducing Rstn mRNA expression. Starved 3T3-L1 adipocytes were incubated in the presence or absence (control group) of 1 nM human IGF-I, IGF-II, or insulin for 6 h. *A*: RT-PCR. *B*: digoxigenin-PCR ELISA. Data are expressed as means ± SE from replicates of 3 independent experiments. $^*P < 0.05$ vs. control. The statistical probability values between insulin and IGF-I or between insulin and IGF-II are shown above the brackets.

compartment. In addition to being a translational inhibitor involved in the inhibition of Rstn protein expression, CHX can prevent IGF-I-induced degradation of Rstn mRNA expression. Our data indicate that new protein synthesis is required for IGF-I's effect. The fact that the decrease in Rstn mRNA occurred at ~6–24 h, but not at 3 h, after IGF-I stimulation also supports this observation. These findings are similar to observations reported for insulin (26).

The signaling protein required for insulin-inhibited Rstn gene expression is still unknown (24, 26, 42). Although many actions of IGF-I may be mediated by phosphorylation-dephosphorylation mechanisms dependent on IGF-IR, MAPK, or PI3K (21, 39), there has been no report on IGF-I signaling involved with Rstn biosynthesis/secretion. It is evident from these data that IGF-IR inactivation via IGF-IR antiserum prevented the IGF-I-induced decrease in Rstn mRNA and increase in IGF-IR activity. This demonstrates that the specific interaction of IGF-I with IGF-IR is necessary for IGF-I's effect. We attempted to search for the downstream signaling transducers of IGF-IR involved with the suppression of Rstn mRNA expression. We observed that none of the specific inhibitors of ERK MEK, p38 MAPK, and PI3K, such as PD-98059, SB-203580, and LY-294002, significantly changed the IGF-I-decreased levels of Rstn mRNA, but they did prevent IGF-I-induced increases in the activities of these enzymes, respectively. This observation suggests that the downregulatory effect of IGF-I on Rstn mRNA expression may be mediated via ERK MAPK-, p38 MAPK- and PI3K-independent pathways. These IGF-I data are consistent with the published PD-98059-, SB-203580-, and LY-294002-independent effects of insulin (26). To support these observations, Kawashima et al. (26) found that the overexpression of dominant-negative PI3K did not block insulin-suppressed Rstn mRNA expression, and we found that overexpression of MEK1 and its constitutively active mutant in 3T3-L1 adipocytes did not change any Rstn expression (unpublished observations). In contrast, Song et al. (44) reported that overexpression of PI3K 110α, Akt, MEK1, MKK6, or MKK7 suppressed Rstn gene expression in 3T3-L1 adipocytes. One possible explanation for these disparate findings is the presence of a variety of serum growth factors and hormones (i.e., TNF-α) in the reported experimental condition of transfected 3T3-L1 adipocytes (44). Another explanation is that a different signal transducer besides MAP and PI3 kinases may be responsible for IGF-I's reduction of Rstn gene expression. This contention is indirectly supported by the observations that overexpression of PTEN (a lipid phosphatase) has been reported to stimulate Rstn expression of adipocytes (44) and that other signaling mechanisms, i.e., insulin receptor substrates (39), JAK/STAT transducers (20), Src family tyrosine kinases (6), and PTEN (31), have been reported for IGF-I's effects on different genes. Determining whether any of these mechanisms links IGF to Rstn mRNA stability requires further exploration.

Because the promoter activity and expression of the Rstn gene are regulated by a variety of nuclear receptors and by coactivator systems (3, 17, 18, 23, 33, 41, 45, 49), we measured the amounts of C/EBPα and PPARγ proteins in 3T3-L1 adipocytes after they were treated with 1 nM IGF-I for 3, 6, or 24 h. IGF-I did not significantly change the amount of these proteins (unpublished observations). Whether C/EBPα and PPARγ gene expressions and their DNA binding activities are altered by IGF-I was not determined in this study. Various hormones and cytokines (3, 17), including dexamethasone, androgen, thyroid hormones, epinephrine, endothelin-1, and TNF-α, have been reported to regulate Rstn gene expression. Further study is required to determine whether any of these component receptors is responsible for the effect of IGF-I on Rstn gene expression.

Expression and secretion of Rstn protein are differently regulated by certain hormones, such as insulin, GH, dexamethasone, and endothelin-1 (42, 51). We report herein that IGF-I at 0.1–10 nM did not significantly alter the amount of intracellular Rstn protein within 24 h but significantly decreased Rstn protein release in dose- and time-dependent manners (Fig. 2, *A–D*). Decreased Rstn protein release was observed after 6 and 24 h, but not 3 h, of IGF-I treatment. These observations suggest that IGF-I transiently modifies the distribution of Rstn protein between the intracellular and extracellular compartments. This is consistent with the observation that IGF-I can regulate the translocation of the estrogen receptor (43). The lack of observed changes in the intracellular Rstn protein levels by IGF-I over the 24-h incubation period may have been due to the acute effect of IGF-I in decreasing Rstn protein release. In contrast, insulin significantly decreased the intracellular protein and stimulated Rstn protein secretion of 3T3-L1 adipocytes (42, 51). Interestingly, in human adipose tissues, insulin has no effect on Rstn protein release (15). These findings suggest that insulin and IGF-I may act through different mechanisms or through species-specific pathways to regulate Rstn protein secretion. Because Rstn is an ADSF and because the falling time (~6 h) of Rstn protein release initiated by IGF-I parallels the decreases in Rstn mRNA levels caused by IGF-I, the effect of IGF-I on decreasing Rstn protein release may be partially explained by decreased Rstn mRNA stability and levels. An alternative explanation is that IGF-I may modulate the respective splicing and processing events of Rstn mRNA and protein and thereby lead to translocation of the Rstn protein, accumulation of the nonsecretable Rstn isoform, and a decrease in Rstn protein release. This assumption can be indirectly supported by recent evidence that a novel nonsecretable Rstn isoform lacking the exon 2 component has been reported in adipose tissues of Wistar rats (2), mice, and humans (34).

Knowledge of the mechanism regulating Rstn protein secretion is emerging (42, 51). Our study implies the involvement of kinase-dependent pathways in the distribution of Rstn protein between the intracellular and extracellular compartments. This conclusion is based on the observation that SB-203580 differed from PD-98059 and LY-294002 in increasing the basal release of Rstn protein and decreasing the basal content of intracellular Rstn protein after 6 h of treatment. The reciprocal effects of kinase inhibitors on these parameters were not due to cell membrane damage, since the concentrations used in this study were not toxic to the cells when examined by the trypan blue dye exclusion method. Several proteins involved with the exocytosis of secretory vesicles, such as synapsin, have been reported to be associated with phosphorylation pathways (5). Whether any of these attaching and docking proteins is dependent on the MAPK and PI3K pathways and whether any would allow adipocytes to secrete the Rstn protein remain unknown. However, we linked the ERK, p38, and PI3K pathways to the underlying IGF-I signaling in Rstn protein secretion. We found

that SB-203580 inhibited IGF-I-stimulated p38 MAPK activity, reduced the IGF-I-retained intracellular Rstn protein, and antagonized IGF-I-decreased Rstn protein release. Neither PD-98059 nor LY-294002 changed the IGF-I-modified distribution of Rstn protein, although they did inhibit IGF-I-stimulated activities of ERK MEK and PI3K, respectively. These data suggest that the way in which IGF-I signaling decreases Rstn protein release differs from that by which it decreases Rstn mRNA levels; the former is likely mediated through a p38 MAPK-dependent pathway. This contention is consistent with a recent report showing that IGF-I enhancement of estrogen receptor-α translocation is related to the phosphorylation of kinases (43).

In conclusion, we have shown that IGF-I suppression of Rstn gene expression in 3T3-L1 adipocytes is dependent on protein synthesis and is likely mediated through decreased mRNA stability. Although these actions were shown to be mediated via the IGF-IR, signaling was demonstrated to be largely independent of the ERK MEK, p38 MAPK, and PI3K pathways. However, inhibitors of p38 MAPK, but not of ERK MEK or PI3K, modified the IGF-I-stimulated distribution of the Rstn protein between the intracellular and extracellular compartments. Further studies on identifying and characterizing some unknown signal transducers are needed to elucidate the basis for the distinct and overlapping mechanisms of IGF-I and insulin actions on Rstn mRNA expression and protein secretion. Because Rstn functions to inhibit adipogenesis of fat cells and cause insulin resistance (27, 28, 45), decreases in Rstn production and secretion induced by IGF-I in this study may be related to the mechanism by which IGF-I operates to modulate body weight, insulin resistance, and diabetes in animals (39).

ACKNOWLEDGMENTS

We are grateful to Dr. Mark A. Sheridan and his associates at the Department of Zoology of the North Dakota State University (Fargo, ND) for editing of the manuscript and for polishing the English. We also thank Yin-Huei Lai, Shen-Jun Liu, Ming-Hua Wu, and Yin-Ju Chen for technical assistance.

GRANTS

This work was supported by grants from the National Science Council, Taiwan (NSC 91-2311-B-008-004, NSC 92-2311-B-008-005, and NSC 93-2311-B-008-005) and Brain Research Center, Taiwan to Y.-H. Kao.

REFERENCES

1. **Alessi DR, Cuenda A, Cohen P, Dudley DT, and Saltiel AR.** PD98059 is a specific inhibitor of the activation of mitogen-activated protein kinase kinase in vitro and in vivo. *J Biol Chem* 270: 27489–27494, 1995.
2. **Arco AD, Peralta S, Carrascosa JM, Ros M, Andres A, and Arribas C.** Alternative splicing generates a novel non-secretable resistin isoform in Wistar rats. *FEBS Lett* 555: 243–249, 2003.
3. **Banerjee RR and Lazar MA.** Resistin: molecular history and prognosis. *J Mol Med* 81: 218–226, 2003.
4. **Banerjee RR, Rangwala SM, Shapiro JS, Rich AS, Rhoades B, Qi Y, Wang J, Rajala MW, Pocai A, Scherer PE, Steppan CM, Ahima RS, Obici S, Rossetti L, and Lazar MA.** Regulation of fasted blood glucose by resistin. *Science* 303: 1195–1198, 2004.
5. **Becker WM, Kleinsmith LJ, and Hardin J.** *The World of the Cell*. San Francisco, CA: Pearson Education, 2003.
6. **Boney CM, Sekimoto H, Gruppuso PA, and Frackelton AR.** Src family tyrosine kinases participate in insulin-like growth factor I mitogenic signaling in 3T3-L1 cells. *Cell Growth Differ* 12: 379–386, 2001.
7. **Bradford MM.** A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248–254, 1976.
8. **Brunn GJ, Sabers WJ, Wiederrecht G, Lawrence JC, and Abraham RT.** Direct inhibition of the signaling functions of the mammalian target of rapamycin by the phosphoinositide 3-kinase inhibitors, wortmannin and LY294002. *EMBO J* 15: 5256–5267, 1996.
9. **Cepica S, Rohrer GA, Masopust M, Kubickova S, Musilova P, and Rubes J.** Partial cloning, cytogenetic and linkage mapping of the porcine resistin (RSTN) gene. *Anim Genet* 33: 381–383, 2002.
10. **Degawa-Yamauchi M, Bovenkerk J, Juliar BE, Watson W, Kerr K, Jones R, Zhu Q, and Considine RV.** Serum resistin (FIZZ3) protein is increased in obese humans. *J Clin Endocrinol Metab* 88: 5452–5455, 2003.
11. **Delhanty PJ, Mesotten D, Mcdougall F, and Baxter RC.** Growth hormone rapidly induces resistin gene expression in white adipose tissue of spontaneous dwarf (SDR) rats. *Endocrinology* 143: 2445–24448, 2002.
12. **Eilertson CD and Sheridan MA.** Pancreatic somatostatin-14 and somatostatin-25 release in rainbow trout is stimulated by glucose and arginine. *Am J Physiol Regul Integr Comp Physiol* 269: R1017–R1023, 1995.
13. **Engelman JA, Lisanti MP, and Scherer PE.** Specific inhibitors of p38 mitogen-activated protein kinase block 3T3-L1 adipogenesis. *J Biol Chem* 273: 32111–32120, 1998.
14. **Engert JC, Vohl MC, Williams SM, Lepage P, Loredo-Osti JC, Faith J, Dore C, Renaud Y, Burtt NP, Villeneuve A, Hirschhorn JN, Altshuler D, Groop LC, Despres JP, Gaudet D, and Hudson TJ.** 5′-Flanking variants of resistin are associated with obesity. *Diabetes* 51: 1629–2002, 2002.
15. **Fain JN, Cheema PS, Bahouth SW, and Hiler ML.** Resistin release by human adipose tissue explants in primary culture. *Biochem Biophys Res Commun* 300: 674–678, 2003.
16. **Fasshauer M, Klein J, Neumann S, Eszlinger M, and Paschke R.** Tumor necrosis factor α is a negative regulator of resistin gene expression and secretion in 3T3-L1 adipocytes. *Biochem Biophys Res Commun* 288: 1027–1031, 2001.
17. **Fasshauer M and Paschke R.** Regulation of adipocytokines and insulin resistance. *Diabetologia* 46: 1594–1603, 2003.
18. **Felipe F, Bonet ML, Ribot J, and Palou A.** Modulation of resistin expression by retinoic acid and vitamin A status. *Diabetes* 53: 882–889, 2004.
19. **Ghosh S, Singh AK, Aruna B, Mukhopadhyay S, and Ehtesham NZ.** The genomic organization of mouse resistin reveals major differences from the human resistin: functional implications. *Gene* 305: 27–34, 2003.
20. **Gual P, Baron V, Lequoy V, and Obberghen EV.** Interaction of Janus kinases JAK-1 and JAK-2 with the insulin receptor and the insulin-like growth factor-1 receptor. *Endocrinology* 139: 884–893, 1998.
21. **Gutierrez J, Parrizas M, Maestro MA, Navarro I, and Plisetskaya EM.** Insulin and IGF-I binding and tyrosine kinase activity in fish heart. *J Endocrinol* 146: 35–44, 1995.
22. **Hana V, Silha JV, Justova V, Lacinova Z, Stepan JJ, and Murphy LJ.** The effects of GH replacement in adult GH-deficient patients: changes in body composition without concomitant changes in the adipokines and insulin resistance. *Clin Endocrinol (Oxf)* 60: 442–450, 2004.
23. **Hartman HB, Hu X, Tyler KX, Dalal CK, and Lazar MA.** Mechanisms regulating adipocyte expression of resistin. *J Biol Chem* 277: 19754–19761, 2002.
24. **Haugen F, Jorgensen A, Drevon CA, and Trayhurn P.** Inhibition by insulin of resistin gene expression in 3T3-L1 adipocytes. *FEBS Lett* 507: 105–108, 2001.
25. **Kao YH, Hippakka RA, and Liao S.** Modulation of obesity by a green tea catechin. *Am J Clin Nutr* 72: 1232–1241, 2000.
26. **Kawashima J, Tsuruzoe K, Motoshima H, Shirakami A, Sakai K, Hirashima Y, Toyonaga T, and Araki E.** Insulin down-regulates resistin mRNA through the synthesis of protein(s) that could accelerate the degradation of resistin mRNA in 3T3-L1 adipocytes. *Diabetologia* 46: 231–240, 2003.
27. **Kim KH, Lee K, Moon YS, and Sul HS.** A cysteine-rich adipose tissue-specific secretory factor inhibits adipocyte differentiation. *J Biol Chem* 276: 11252–11256, 2001.
28. **Kim KH, Zhao L, Moon Y, Kang C, and Sul HS.** Dominant inhibitory adipocyte-specific secretory factor (ADSF)/resistin enhances adipogenesis and improves insulin sensitivity. *Proc Natl Acad Sci USA* 101: 6780–6785, 2004.
29. **Kokontis JM, Hay N, and Liao S.** Progression of LNCaP prostate tumor cells during androgen deprivation: hormone-independent growth, repression of proliferation by androgen, and role for p27Kip1 in androgen-induced cell cycle arrest. *Mol Endocrinol* 12: 941–953, 1998.

30. **Maebuchi M, Machidori M, Urade R, Ogawa T, and Moriyama T.** Low resistin levels in adipose tissues and serum in high-fat fed mice and genetically obese mice: development of an ELISA system for quantification of resistin. *Arch Biochem Biophys* 416: 164–170, 2003.
31. **Meyer GE, Shelden E, Kim B, and Feldman EL.** Insulin-like growth factor I stimulates motility in human neuroblastoma cells. *Oncogene* 20: 7542–7550, 2001.
32. **Moon B, Kwan JJ, Duddy N, Sweeney G, and Begum N.** Resistin inhibits glucose uptake in L6 cells independently of changes in insulin signaling and GLUT4 translocation. *Am J Physiol Endocrinol Metab* 285: E106–E115, 2003.
33. **Nogueiras R, Gualillo O, Caminos JE, Casanueva FF, and Dieguez C.** Regulation of resistin by gonadal, thyroid hormone, and nutritional status. *Obes Res* 11: 408–414, 2003.
34. **Nohira T, Nagao K, Kameyama K, Nakai H, Fukumine N, Okabe K, Kitano S, and Hisatomi H.** Identification of an alternative splicing transcript for the resistin gene and distribution of its mRNA in human tissue. *Eur J Endocrinol* 151: 151–154, 2004.
35. **Oliver P, Pico C, Serra F, and Palou A.** Resistin expression in different adipose tissue depots during rat development. *Mol Cell Biochem* 252: 397–400, 2003.
36. **Pizzuti A, Argiolas A, Paola RD, Baratta R, Rausea A, Bozzali M, Vigneri R, Dallapiccola B, Trischitta V, and Frittitta L.** An ATG repeat in the 3′-untranslated region of the human resistin gene is associated with a decreased risk of insulin resistance. *J Clin Endocrinol Metab* 87: 4403–4406, 2002.
37. **Ramirez-Zacarias JL, Castro-Munozledo F, and Kuri-Harcuch W.** Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil red O. *Histochemistry* 97: 493–497, 1992.
38. **Reischl U, Ruger R, and Kessler C.** Nonradioactive labeling and high sensitive detection of PCR products. *Mol Biotechnol* 1: 229–240, 1994.
39. **Rosenfeld RG and Roberts CT.** *The IGF System: Molecular Biology, Physiology, and Clinical Applications.* Totowa, NJ: Humana, 1999.
40. **Ross J.** Control of messenger RNA stability in higher eukaryotes. *Trends Genet* 12: 171–175, 1996.
41. **Seo JB, Noh MJ, Yoo EJ, Park SY, Park J, Lee IK, Park SD, and Kim JB.** Functional characterization of the human resistin promoter with adipocyte determination-and differentiation-dependent factor 1/sterol regulatory element binding protein 1c and CCAAT enhancer binding protein-α. *Mol Endocrinol* 17: 1522–1533, 2003.
42. **Shojima N, Sakoda H, Ogihara T, Fujishiro M, Katagiri H, Anai M, Onishi Y, Ono H, Inukai K, Abe M, Fukushima Y, Kikuchi M, and Oka Y, and Asano T.** Humoral regulation of resistin expression in 3T3-L1 and mouse adipose cells. *Diabetes* 51: 1737–1744, 2002.
43. **Song RX, Barnes CJ, Zhang Z, Bao Y, Kumar R, and Santen RJ.** The role of Shc and insulin-like growth factor 1 receptor in mediating the translocation of estrogen receptor a to the plasma membrane. *Proc Natl Acad Sci USA* 101: 2076–2081, 2004.
44. **Song H, Shojima N, Sakoda H, Ogihara T, Fujishiro M, Katagiri H, Anai M, Onishi Y, Ono H, Inukai K, Fukushima Y, Kikuchi M, Shimano H, Yamada N, Oka Y, and Asano T.** Resistin is regulated by C/EBPs, PPARs, and signal-transducing molecules. *Biochem Biophys Res Commun* 299: 291–298, 2002.
45. **Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, and Lazar MA.** The hormone resistin links obesity to diabetes. *Nature* 409: 307–312, 2001.
46. **Steppan CM, Brown EJ, Wright CM, Bhat S, Banerjee RR, Dai CY, Enders GH, Silberg DG, Wen X, Wu GD, and Lazar MA.** A family of tissue-specific resistin-like molecules. *Proc Natl Acad Sci USA* 98: 502–506, 2001.
47. **Verma S, Li SH, Wang CH, Fedak PWM, Li RK, Weisel RD, and Mickle DAG.** Resistin promotes endothelial cell activation further evidence of adipokine-endothelial interaction. *Circulation* 108: 736–740, 2003.
48. **Viengchareun S, Zennaro M, Tallec LP, and Lombes M.** Brown adipocytes are novel sites of expression and regulation of adiponectin and resistin. *FEBS Lett* 532: 345–350, 2002.
49. **Way JM, Gorgun CZ, Tong Q, Uysal KT, Brown KK, Harrington WW, Oliver WR, Willson TM, Kliewert SA, and Hotamisligil GS.** Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor γ agonists. *J Biol Chem* 276: 25651–25653, 2001.
50. **Youn BS, Yu KY, Park HJ, Lee NS, Min SS, Youn MY, Cho YM, Park YJ, Kim SY, Lee HK, and Park KS.** Plasma resistin concentrations measured by enzyme-linked immunosorbent assay using a newly developed monoclonal antibody are elevated in individuals with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 89: 150–156, 2004.
51. **Zhong Q, Lin CY, Clarke KJ, Kemppainen RJ, Schwartz DD, and Judd RL.** Endothelin-1 inhibits resistin secretion in 3T3-L1 adipocytes. *Biochem Biophys Res Commun* 16: 383–387, 2002.