Growth hormone (GH) and prolactin (PRL) binding to their receptors, which belong to the cytokine receptor superfamily, activate Janus kinase (JAK) 2 tyrosine kinase, thereby leading to their biological actions. We recently showed that GH mainly stimulated tyrosine phosphorylation of epidermal growth factor receptor and its association with Grb2, and concomitantly stimulated mitogen-activated protein kinase activity in liver, a major target tissue. Using specific antibodies, we now show that GH was also able to induce tyrosine phosphorylation of insulin receptor substrate (IRS)-1/IRS-2 in liver. In addition, the major tyrosine-phosphorylated protein in anti-p85 phosphatidylinositol 3-kinase (PI3-kinase) immunoprecipitate from liver of wild-type mice was IRS-1, and IRS-2 in IRS-1 deficient mice, but not epidermal growth factor receptor. These data suggest that tyrosine phosphorylation of IRS-1 may be a major mechanism for GH-induced PI3-kinase activation in physiological target organs of GH, liver. We also show that PRL was able to induce tyrosine phosphorylation of both IRS-1 and IRS-2 in COS cells transiently transfected with PRLR and in CHO-PRLR cells. Moreover, we show that tyrosine phosphorylation of IRS-3 was induced by both GH and PRL in COS cells transiently transfected with IRS-3 and their cognate receptors. By using the JAK2-deficient cell lines or by expressing a dominant negative JAK2 mutant, we show that JAK2 is required for the GH- and PRL-dependent tyrosine phosphorylation of IRS-1, -2, and -3. Finally, a specific PI3-kinase inhibitor, wortmannin, completely blocked the anti-lipolytic effect of GH in 3T3 L1 adipocytes. Taken together, the role of IRS-1, -2, and -3 in GH and PRL signalings appears to be phosphorylated by JAK2, thereby providing docking sites for p85 PI3-kinase and activating PI3-kinase and its downstream biological effects.

Growth hormone (GH) and prolactin (PRL) initiate their wide variety of biological effects by binding and dimerization of their membrane receptors (1, 2). The GH and PRL receptors belong to the cytokine/hematopoietin receptor superfamily, characterized by homologies in the extracellular domains and lack of intrinsic tyrosine kinase activity (1), however, ligands binding to their receptors activate JAK2 tyrosine kinase (3, 4). JAK2 tyrosine phosphorylates their receptors and JAK2 itself along with STATs (signal transducers and activators of transcription). The phosphorylated STAT proteins translocate into the nucleus and bind to DNA, thereby activating transcription of specific genes (5). In addition, a number of intracellular key proteins have been suggested to be involved in their signaling (6).

One of the signaling molecules known to be activated by GH and PRL (7, 8) is the mitogen-activated protein kinase (MAP kinase), which is believed to play a pivotal role in the regulation of cellular growth and differentiation (9). Association of tyrosine-phosphorylated proteins with Grb2 (growth factor receptor bound protein 2) is known to represent a crucial step in the activation of MAP kinase cascade (10). In the case of GH and PRL, epidermal growth factor receptor (EGFR) and Shc have been shown to be tyrosyl phosphorylated by JAK2 and bind Grb2, leading to MAP kinase activation (11, 12).

Another signaling molecule known to be activated by GH and PRL is phosphatidylinositol 3-kinase (PI3-kinase), which may play a role in initiating insulin-like effects of GH and PRL including glucose transport, glycogenesis, anti-lipolysis, and lipogenesis (13–15), because the selective PI3-kinase inhibitor wortmannin can block at least some of these effects such as anti-lipolysis and lipogenesis (16).

Insulin receptor substrate-1 (IRS-1) is the principal substrates of the insulin receptor kinase whose molecular mass is approximately 170 kDa and has many tyrosine phosphorylation sites (17), which provides binding sites for several distinct Src homology 2 (SH2) proteins (e.g. Grb2, the 85-kDa subunit of PI3-kinase (PI3-kinase p85), Syp, Nck, and Csk) and has been shown to mediate multiple signaling pathways (18, 19). IRS-1

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binds the PI3-kinase p85 when tyrosine phosphorylated, thereby activating PI3-kinase (20, 21), leading to exert metabolic effects of insulin such as glucose transport, glycogen synthesis, and anti-lipolysis (22–24).

To better understand the role of IRS-1 in vivo, we and others generated mice with a targeted disruption of the IRS-1 gene and demonstrated that they exhibited mild growth retardation and had partial resistance to the glucose-lowering effect of insulin, which was an unexpectedly mild phenotype, suggesting the presence of IRS-1 independent pathways (25, 26). In liver and also muscle extracts from IRS-1-deficient mice, tyrosine phosphorylation of IRS-2, whose molecular mass is approximately 180 kDa (25, 27, 28), was significantly induced in IRS-1-deficient mice compared with that in wild-type mice (29) and has been suggested to be the mechanism of compensation for IRS-1 deficiency in liver and muscle (30, 31). In another target tissue of insulin, adipocytes, both IRS-1 and IRS-3 (pp60) (32, 33) was suggested to play a major role in insulin-induced PI3-kinase activation, and IRS-3 (pp60) was suggested to be involved in regulating this process in the absence of IRS-1 (34).

Recently, IRS-1/IRS-2 have been shown to be tyrosine-phosphorylated and associated with PI3-kinase p85 in response to GH in primary rat adipocytes (35, 36) and in 3T3-F442A fibroblasts (37, 38). However, the following important issues remained unresolved. First, whether GH was able to induce tyrosine phosphorylation of IRS-1/IRS-2 in physiological target organ, liver, in vivo. Second, the relative contributions of IRS-1 and IRS-2 in the signaling pathways for GH to activate PI3-kinase. Third, the identity of the kinase responsible for the

Fig. 1. GH-stimulated tyrosine phosphorylation of IRS-1/IRS-2 in livers from wild-type (WT) and IRS-1 deficient mice (null). A and B, upper panel, liver extracts from wild-type or IRS-1 deficient mice untreated or treated with insulin for 2 min or treated with GH for 7.5 min were subjected to immunoprecipitation with αIRS-1 (A) or αIRS-2 (B) followed by Western blotting with αPY. A and B, lower panel, amount of tyrosine phosphorylation of IRS-1 (A) or IRS-2 (B) in livers. The amount of tyrosine phosphorylation was evaluated by densitometry and expressed as the percentage of the value of wild-type mice treated with insulin. Each bar represents the mean ± S.E. for livers from 5 to 7 mice. *, p < 0.05, N.S., the difference is not significant, GH injected mice versus untreated mice.

Fig. 2. GH-stimulated PI3-kinase activities in livers of wild-type (WT) and IRS-1 deficient mice (null). The αPY immunoprecipitates from the liver lysates of wild-type (□) or IRS-1-deficient mice (○) untreated or treated with insulin for 2 min or treated with GH for 7.5 min were subjected to PI3-kinase assay. The autoradiogram of the thin layer chromatograph is shown in the left panel. The radioactivity in the spots corresponding to phosphatidylinositol (PIP) was measured and the results are shown in the right panel, expressed as the ratio to the value of untreated wild-type mice. Each bar represents the mean ± S.E. for livers from five mice. *, p < 0.05, GH injected mice versus untreated mice.
GH-stimulated tyrosine phosphorylation of IRS proteins. Fourth, whether additional cytokine receptor superfamily/IRS proteins signaling pathways could also operate. Using specific antibodies, we show here that GH was able to induce tyrosine phosphorylation of IRS-1/IRS-2, and that tyrosine phosphorylation of IRS-1 may be a major mechanism for GH-induced PI3-kinase activation in physiological target organ, liver, using IRS-1-deficient mice. Finally, by using the JAK2-deficient cell lines or by expressing a dominant negative JAK2 mutant, we show that JAK2 is required for the GH- and PRL-dependent tyrosine phosphorylation of IRS-1, -2, and -3, their association with p85 PI3-kinase and activation of PI3-kinase. Taken together, the role of IRS-1, -2, and -3 in GH and PRL signalings is to be phosphorylated by JAK2, thereby providing docking sites for p85 PI3-kinase and activating PI3-kinase. A specific PI3-kinase inhibitor, wortmannin, completely blocked the antilipolytic effect of GH in 3T3 L1 adipocytes, as reported in isolated rat adipocytes (16). Thus our data may provide the biochemical fundamentals to understand the insulin-like effects of GH such as anti-lipolysis.

EXPERIMENTAL PROCEDURES

Materials and Mice—The polyclonal antibody against 85-kDa subunit of PI3-kinase was from Upstate Biotechnology Inc. The monoclonal antibody against hemagglutinin (HA), 12CA5, was from Boehringer Mannheim. All other materials were obtained from the sources described (12, 31). Mice were fasted overnight and then treated as described (31).

Expression of Wild-type and Kinase-inactive JAK2, Epitope-tagged IRS-3, GHR, and PRLR—The CHO (Chinese hamster ovary)-PRLR cells (approximately 1.0 × 10^6 receptors/cell) and expression vector of human PRLR were kindly provided by M. Takahashi, M. Wada, and M. Hon (21, 22). CHO-GHR, or CHO-PRLR cells were transfected with or without 3 μg of WT-JAK2 plasmids or dominant negative JAK2 (ΔJAK2) (40) that lacks the C terminus kinase domain under the SRα promoter in 6-cm dishes by the LipofectAMINE method with modification and the cells were stimulated as described (12, 39, 41). The rat IRS-3 cDNA in the pcDNA3 was modified to encode an additional 9-amino acid HA epitope (YPYDVPDYA), after the C terminus of IRS-3 (33). COS cells were transfected with 3 μg of GHR or PRLR, with or without HA-tagged IRS-3 and with or without 2 μg of WT-JAK2 or ΔJAK2 at 60–80% confluence in 3 ml of Dulbecco’s modified Eagle’s medium in 6-cm dishes by the calcium phosphate precipitation method and the cells were stimulated as described (12).

Immunoprecipitation, Immunoblotting, and PI3-kinase Activities—Immunoprecipitation and immunoblotting were performed as described (31). The amount of tyrosine phosphorylation or associated protein was evaluated by densitometry, as described (31). The significance of differences between the two groups was assessed using Student’s unpaired t test.

Lipolysis—3T3-L1 adipocytes were incubated in serum-free Dulbecco’s modified Eagle’s medium for 16 h, and then in modified Krebs-Ringer medium at pH 7.5 containing 24 mM HEPES, 119 mM NaCl, 4.95 mM KCl, 2.54 mM CaCl_2, 1.19 mM KHPO_4, 1.19 mM MgSO_4, 2 mM glucose for 3 h. Lipolysis, stimulated by norepinephrine (NE) (Sigma), was measured with or without pretreatment with 100 nM wortmannin for 20 min, as proportional to glycerol released to the medium during a 30-min incubation and determined as described (16).

RESULTS

GH Stimulates Tyrosine Phosphorylation of IRS-1/IRS-2 in Liver, in Vivo—To determine if IRS-1 and/or IRS-2 are tyrosine-phosphorylated following stimulation with GH in its physiological target organ, liver, in vivo, mice were injected with 5 μg/g body weight of GH and 7.5 min after the injection, solubilized proteins from livers of wild-type and IRS-1-deficient mice were immunoprecipitated with anti-IRS-1 antibody (aIRS-1) or anti-IRS-2 antibody (aIRS-2), and immunoblotted with aPY. IRS-1 (molecular mass 170 kDa) was tyrosine-phosphorylated in response to GH in wild-type mice (Fig. 1A, lane 3), however, to a much lesser degree than in response to insulin (Fig. 1A, lane 2). IRS-2 was tyrosine phosphorylated in response to GH most in IRS-1-deficient mice (Fig. 1B, lane 6), however, to a much lesser degree than in response to insulin (Fig. 1B, lane 5). GH-dependent tyrosine phosphorylation of IRS-1/IRS-2 reached the maximum level at 7.5 min (data not shown).

GH-stimulated PI3-kinase Activity in Liver—We measured...
PI3-kinase activity in livers from wild-type and IRS-1-deficient mice in the immunoprecipitates with the monoclonal antibody against phosphotyrosine (4G10). 7.5 min after GH injection and 2 min after insulin injection, maximal levels in kinase activity were reached (data not shown). In liver of both wild-type and IRS-1-deficient mice, insulin injection resulted in a 12–13-fold increase in PI-3 kinase activity in 4G10 immunoprecipitates, while GH caused an only 2–3-fold increase (Fig. 2).

Immunological Detection of GH-stimulated Tyrosine-phosphorylated Proteins Associated with PI3-kinase p85—The molecular mechanism by which GH activates PI3-kinase were determined by immunoblotting with the monoclonal anti-phosphotyrosine antibody (4G10) of anti-PI3-kinase p85 immunoprecipitates of livers 7.5 min after GH injection and 2 min after insulin injection. Interestingly, while the 180-kDa protein was mainly tyrosine phosphorylated and associated with Grb2 in wild-type mice when injected with GH (12), in op85 immunoprecipitates of GH-injected wild-type mice, a 170-kDa tyrosine-phosphorylated protein was mainly present (Fig. 3A, lane 4), indicating that tyrosine phosphorylation of IRS-1, but neither IRS-2 nor EGF, may be a major mechanism for GH-induced PI3-kinase activation in physiological target organ, liver. In wild-type mice, the amount of pp170 associated with PI3-kinase p85 in response to GH was much less than that in response to insulin, which was consistent with the data of the amount of pp180 (IRS-2) associated with PI3-kinase p85 in response to another cytokine superfamily member PRL, which lack JAK2 (39). GH was unable to induce these responses in y2A/GHR, whereas complementation with a WT-JAK2 expression plasmid restored them (Fig. 4). These data suggested that JAK2 is required for the GH-dependent tyrosine phosphorylation of IRS-1 and IRS-2, their association with PI3-kinase, and PI3-kinase activation are dependent on JAK2 by using y2A/GHR cells, which lack JAK2 (39). To confirm these observations, liver extracts of GH-treated wild-type mice for 7.5 min were incubated with the glutathione S-transferase fusion proteins containing the entire p85 PI3-kinase. Glutathione S-transferase-p85 only associated with pp170 (IRS-1) (data not shown). In IRS-1-deficient mice, the amount of pp180 (IRS-2) associated with PI3-kinase p85 in response to GH was also much less than that in response to insulin (Fig. 3A, lanes 5 and 6).

To further confirm these observations, we measured PI3-kinase activity in the immunoprecipitates with the polyclonal anti-IRS-1 antibody (aIRS-1) or anti-IRS-2 antibody (aIRS-2) (179C). In liver of wild-type mice, insulin injection resulted in a 11-fold increase of PI-3 kinase activity in aIRS-1 immunoprecipitates, while GH caused only a 2–3-fold increase (Fig. 3B, lanes 2 and 3), which was consistent with the data of the amount of PI3-kinase p85-associated pp170 (IRS-1) (Fig. 3A, lanes 3 and 4). In aIRS-2 immunoprecipitates from liver of wild-type mice, however, GH had little effect on an increase in PI3-kinase, and in IRS-1 deficient mice, GH caused only a 2–3-fold increase (Fig. 3C, lanes 2 and 4), which was also consistent with the data of the amount of pp180 (IRS-2) associated with PI3-kinase p85 (Fig. 3A, lanes 4 and 6).
PRLR cells treated with PRL for 5 min. We found that PRL was also able to induce tyrosine phosphorylation of IRS-2 (Fig. 5D, lane 2, and Fig. 6C, lane 6).

Complementation with a WT-JAK2 Expression Plasmid into JAK2-deficient Cell Line Restored PRL-dependent Tyrosine Phosphorylation of IRS-1/IRS-2, Their Association with p85 PI3-kinase, and Activation of PI3-kinase—We also examined whether PRL-induced tyrosine phosphorylation of IRS-1/IRS-2, IRS-1 association with p85 PI3-kinase, and PI3-kinase activation are dependent on JAK2 by using COS cells, in which the level of expression of JAK2 appears to be sufficiently low that some level of coexpression of wild-type (WT)-JAK2 and PRLR is required in order to detect activation of JAK2 by PRL (Fig. 5A). Neither expression of PRLR alone nor coexpression of kinase-inactive JAK2 (ΔJAK2) and PRLR, but coexpression of WT-JAK2 and PRLR restored these responses (Fig. 5B, C, D, and E, and data not shown). These data suggested that JAK2 is required for the PRL-dependent tyrosine phosphorylation of IRS-1 and -2, their association with p85 PI3-kinase and activation of PI3-kinase.

**Dominant Negative JAK2 Almost Completely Abolished GH- and PRL-dependent Tyrosine Phosphorylation of IRS-1/IRS-2 and Their Association with p85 PI3-kinase.** CHO-GHR or CHO-PRLR cells were transfected with or without dominant negative JAK2 (ΔJAK2) plasmids. Quiescent cells were stimulated for 5 min with GH or PRL (500 ng ml⁻¹). Upon lysis, precipitated IRS-1 (Fig. 6A, upper panel) or IRS-2 (Fig. 6B, upper panel) were immunoblotted with aPY. The p85 PI3-kinase immunoprecipitate was Western blotted with aIRS-1 (Fig. 6C, upper panel). The amount of tyrosine phosphorylation (A, B, and D, lower panel) was evaluated by densitometry and expressed as the percentage of the value of untreated cells transfected with WT-JAK2. Each bar represents the mean ± S.E. of three independent experiments. The aIRS-1 immunoprecipitates were subjected to PI3-kinase assay (E). The radioactivity in the spots corresponding to phosphatidylinositol (PIP) was measured and the results are shown in E, upper panel. The autoradiogram of the thin layer chromatograph is shown in E, lower panel, expressed as the ratio to the value of untreated cells transfected with vector alone in the presence of ligand. Each bar represents the mean ± S.E. of three independent experiments. *, p < 0.01, vector-transfected cells versus ΔJAK2-transfected cells.

[**Fig. 5.** Complementation with a WT-JAK2 expression plasmid into JAK2-deficient cell line restored PRL-dependent tyrosine phosphorylation of IRS-1/IRS-2, their association with p85 PI3-kinase, and activation of PI3-kinase. WT-JAK2 or ΔJAK2 plasmids were transfected to COS cells with PRLR plasmids. Quiescent cells were stimulated for 5 min with PRL (500 ng ml⁻¹). Upon lysis, precipitated JAK2 (A, upper panel), IRS-1 (B, upper panel), or IRS-2 (D, upper panel) was immunoblotted with aPY. The a85 PI3-kinase immunoprecipitate was Western blotted with aIRS-1 (C, upper panel). The amount of tyrosine phosphorylation (A, B, and D, lower panel) or IRS-1 (C, lower panel) was evaluated by densitometry and expressed as the percentage of the value of cells transfected with WT-JAK2 treated with PRL. Each bar represents the mean ± S.E. of three independent experiments. The aIRS-1 immunoprecipitates were subjected to PI3-kinase assay (E). The radioactivity in the spots corresponding to phosphatidylinositol (PIP) was measured and the results are shown in E, upper panel. The autoradiogram of the thin layer chromatograph is shown in E, lower panel, expressed as the percentage of the value of cells transfected with WT-JAK2 treated with PRL. Each bar represents the mean ± S.E. of three independent experiments. *, p < 0.01, WT-JAK2 transfected cells versus ΔJAK2 transfected cells or vector transfected cells.]

[**Fig. 6.** Dominant negative ΔJAK2 almost completely abolished GH- and PRL-dependent tyrosine phosphorylation of IRS-1/IRS-2 and their association with p85 PI3-kinase. CHO-GHR or CHO-PRLR cells were transfected with or without dominant negative JAK2 (ΔJAK2) plasmids. Quiescent cells were stimulated for 5 min with GH or PRL (500 ng ml⁻¹). Upon lysis, precipitated IRS-1 (A, upper panel) or IRS-2 (C, upper panel) were immunoblotted with aPY. The a85 PI3-kinase immunoprecipitate was Western blotted with aIRS-1 (B, upper panel). The amount of tyrosine phosphorylation (A and C, lower panel) or IRS-1 (B, lower panel) was evaluated by densitometry and expressed as the percentage of the value of cells transfected with vector alone in the presence of ligand. Each bar represents the mean ± S.E. of three independent experiments. *, p < 0.01, vector-transfected cells versus ΔJAK2-transfected cells.]
that the GH- and PRL-induced tyrosine phosphorylation of IRS-1/IRS-2, IRS-1 association with p85 PI3-kinase, and activation of PI3-kinase are largely dependent on JAK2.

GH and PRL Were Also Able to Induce Tyrosine Phosphorylation of IRS-3 and Its Association with p85 PI3-kinase via JAK2—Not only IRS-1 but also pp60 was shown to play a major role in insulin-induced activation of PI3-kinase in adipocytes (34). Recently, Lavan et al. (33) reported the isolation of the cDNA of pp60 which was designated as IRS-3. Thus we next examined whether GH and PRL were able to induce tyrosine phosphorylation of IRS-3 and its association with p85 PI3-kinase via JAK2 in COS cells transiently transfected with HA-tagged IRS-3 treated with GH or PRL for 5 min. We found that both GH and PRL were also able to induce tyrosine phosphorylation of IRS-3 and its association with p85 PI3-kinase via JAK2 (Fig. 7, A and B).

GH Effect on Anti-lipolysis Is Dependent on PI3-kinase—To study whether GH stimulation of PI3-kinase is linked to GH-induced insulin-like metabolic effect, GH-stimulated anti-lipolysis was measured with pretreatment with a specific PI3-kinase inhibitor, wortmannin. The anti-lipolytic effect of GH was measured as the ability to counteract lipolysis induced by NE at 100 nM in 3T3 L1 adipocytes. GH (0.5 μg/ml) inhibited lipolysis by 60 ± 10% (mean ± S.E., n = 3, p < 0.05), and wortmannin completely blocked this anti-lipolytic effect, as reported in isolated rat adipocytes (16). These data suggested that PI3-kinase activity was indeed required for anti-lipolysis in response to GH.

### DISCUSSION

**GH Stimulates Tyrosine Phosphorylation of IRS-1/IRS-2 in Liver, in Vivo**—We recently showed that GH mainly stimulated tyrosine phosphorylation of EGFR and its association with Grb2, and concomitantly stimulated MAP kinase activity in liver, a major target tissue (12). Using specific antibodies, we provided the first evidence that GH was also able to induce tyrosine phosphorylation of IRS-1 in liver, in vivo, although to a lesser extent as compared with that stimulated with insulin or tyrosine phosphorylation of EGFR stimulated with GH (Fig. 1 and Ref. 12). The degree of GH-stimulated activation of MAP kinase and PI3-kinase appeared to be correlated with the relative amount of GH-stimulated tyrosine-phosphorylated EGFR and IRS-1, respectively (Figs. 1 and 2 and Ref. 12).

**Tyrosine Phosphorylation of IRS-1, but Neither IRS-2 nor EGFR, May Be a Major Mechanism for GH-induced PI3-kinase Activation in Physiological Target Organ, Liver**—IRS-2 couple more sensitively to the IL-4 receptor system and less to the insulin receptor system than IRS-1 (28). In the case of the insulin receptor system, the degree of compensation for IRS-1 deficiency appears to be correlated with the relative amount of tyrosine-phosphorylated IRS-2 (in IRS-1 deficient mice) to that of IRS-1 (in wild-type mice) (31). Recently, using specific antibodies, IRS-1/IRS-2 have been shown to be associated with PI3-kinase p85 in response to GH in primary rat adipocytes (35, 36) and in 3T3-F442A fibroblasts (37, 38). There has been no study, however, which reported the relative contributions of
Tyrosine Phosphorylation of IRSs Induced by GH and PRL via JAK2

Tyrosine phosphorylation of IRS-1 and IRS-2 in the signaling pathways for GH to activate PI3-kinase. Interestingly, although EGFR was mainly tyrosine phosphorylated and associated with Grb2 when injected with GH (12), the major tyrosine-phosphorylated protein in anti-p85 PI3-kinase immunoprecipitate from liver of wild-type mice was IRS-1, and IRS-2 in IRS-1-deficient mice (Fig. 3 A) but not EGFR. Thus we provided the evidence that tyrosine phosphorylation of IRS-1, but neither IRS-2 nor EGFR, may be a major mechanism for GH-induced PI3-kinase activation in physiological target organ, liver, in vivo. The observations that GH had little effect on an increase in PI3-kinase activity in aIRS-2 immunoprecipitates from wild-type mice, while GH caused a 2–3-fold increase in IRS-1-deficient mice (Fig. 3C) may support the central role of IRS-1 in the control of PI3-kinase activation by GH.

Additional Cytokine Receptor Superfamily/IRS Proteins Signaling Pathways—Recently, PRL has been shown to be able to tyrosine phosphorylate IRS-1 in 293-PRLR cells (42). We show in this study for the first time that PRL was also able to induce tyrosine phosphorylation of IRS-2 in COS cells transiently transfected with PRLR (Fig. 5C, lane 2) and in CHO-PRLR cells (Fig. 6C, lane 6). In addition to IRS-1, pp60/IRS-3 has also been shown to play a major role in insulin-induced activation of PI3-kinase in adipocytes (34). In the present study, we also show that both GH and PRL were able to induce tyrosine phosphorylation of IRS-3 and its association with p85 PI3-kinase (Fig. 7). This is the first evidence that IRS-3 can be tyrosine phosphorylated by other tyrosine kinase(s) other than insulin receptor tyrosine kinase.

The Role of PI3-kinase in Mediating Insulin-like Effects of GH—PI3-kinase has been reported not to be required for glucose uptake stimulated by GH (48). GH-induced anti-lipolysis and lipogenesis, however, have been shown to be PI3-kinase-dependent in isolated rat adipocytes (16). We also showed that a specific PI3-kinase inhibitor, wortmannin, completely blocked the anti-lipolytic effect of GH in 3T3-L1 adipocytes (Fig. 8). Thus PI3-kinase may play an important role in initiating at least some insulin-like effects of GH, such as anti-lipolysis and lipogenesis.

In conclusion, 1) GH is able to induce tyrosine phosphorylation of IRS-1/IRS-2 in physiological target organ, liver, in vivo; 2) tyrosine phosphorylation of IRS-1, but neither IRS-2 nor EGFR, may be a major mechanism for GH-induced PI3-kinase activation in liver; 3) PRL is able to induce tyrosine phosphorylation of IRS-2 in COS cells transiently transfected with PRLR and in CHO-PRLR cells; 4) both GH and PRL are able to

 IRS-1 and IRS-2 in the signaling pathways for GH to activate PI3-kinase. Interestingly, although EGFR was mainly tyrosine phosphorylated and associated with Grb2 when injected with GH (12), the major tyrosine-phosphorylated protein in anti-p85 PI3-kinase immunoprecipitate from liver of wild-type mice was IRS-1, and IRS-2 in IRS-1-deficient mice (Fig. 3 A) but not EGFR. Thus we provided the evidence that tyrosine phosphorylation of IRS-1, but neither IRS-2 nor EGFR, may be a major mechanism for GH-induced PI3-kinase activation in physiological target organ, liver, in vivo. The observations that GH had little effect on an increase in PI3-kinase activity in aIRS-2 immunoprecipitates from wild-type mice, while GH caused a 2–3-fold increase in IRS-1-deficient mice (Fig. 3C) may support the central role of IRS-1 in the control of PI3-kinase activation by GH.

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In conclusion, 1) GH is able to induce tyrosine phosphorylation of IRS-1/IRS-2 in physiological target organ, liver, in vivo; 2) tyrosine phosphorylation of IRS-1, but neither IRS-2 nor EGFR, may be a major mechanism for GH-induced PI3-kinase activation in liver; 3) PRL is able to induce tyrosine phosphorylation of IRS-2 in COS cells transiently transfected with PRLR and in CHO-PRLR cells; 4) both GH and PRL are able to
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Tyrosine phosphorylation of IRS-3 and its association with p85 PI3-kinase; 5) the GH-and PRL-induced tyrosine phosphorylation of IRS-1, -2, and -3, their association with p85 PI3-kinase, and activation of PI3-kinase are largely dependent on JAK2; 6) a specific PI3-kinase inhibitor, wortmannin, completely blocked the anti-lipolytic effect of GH in 3T3.L1 adipocytes. Taken together, the role of IRSs in GH and PRL signaling appears to be phosphorylated by JAK2, thereby providing docking sites for p85 PI3-kinase and activating PI3-kinase and its downstream biological effects.

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