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Prolactin-Signal Transduction in Neonatal Rat Pancreatic Islets and Interaction with the Insulin-Signaling Pathway

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Abstract

During pregnancy, pancreatic islets undergo structural and functional changes in response to an increased demand for insulin. Different hormones, especially placental lactogens, mediate these adaptive changes. Prolactin (PRL) mainly exerts its biological effects by activation of the JAK2/STAT5 pathway. PRL also stimulates some biological effects *via* activation of IRS-1, IRS-2, PI 3-kinase, and MAPK in different cell lines. Since IRS-2 is important for the maintenance of pancreatic islet cell mass, we investigated whether PRL affects insulin-signaling pathways in neonatal rat islets. PRL significantly potentiated glucose-induced insulin secretion in islets cultured for 7 days. This effect was blocked by the specific PI 3-kinase inhibitor wortmannin. To determine possible effects of PRL on insulin-signaling pathways, fresh islets were incubated with or without the hormone for 5 or 15 min. Immunoprecipitation and immunoblotting with specific antibodies showed that PRL induced a dose-dependent IRS-1 and IRS-2 phosphorylation compared to control islets. PRL-induced increase in IRS-1/-2 phosphorylation was accompanied by an increase in the association with and activation of PI 3-kinase. PRL-induced IRS-2 phosphorylation and its association with PI 3-kinase did not add to the effect of insulin. PRL also induced JAK2, SHC, ERK1 and ERK2 phosphorylation in neonatal islets, demonstrating that PRL can activate MAPK. These data indicate that PRL can stimulate the IRSs/PI 3-kinase and SHC/ERK pathways in islets from neonatal rats.

Key words
Prolactin · Insulin · Neonatal Rat Islets · Insulin Receptors Substrates 1/2 · PI 3-Kinase · MAP-Kinase · Wortmannin

Introduction

Prolactin, a hormone that belongs to the growth hormone-placental lactogen family, signals through a transmembrane receptor (PRLR) of the cytokine receptor superfamily. Once bound to its receptor, PRL induces the phosphorylation and activation of the associated kinase JAK2 that leads to receptor dimerization and phosphorylation/activation of STAT5. Activated STAT5 forms homodimers that migrate to the nucleus, binding to specific DNA sequences and modulating gene transcription [1 – 5].

In addition to the JAK/STAT-signaling pathway, PRL also activates the SHC/GRB-2/SOS and Ras/Raf/MAP-kinase cascades [6,7]. SHC, an SH2-/plekstrin homology domain-containing protein, may be activated through JAK2 or through binding to proteins belonging to the insulin-receptor substrate (IRS) family [8]. Activation of these cascades targets the PRL signal to the nucleus and contributes to the pleiotropic actions of the hormone [9,10].

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In hepatocytes, PRL activates IRS-1/-2/-3, which in turn activates PI 3-kinase through JAK2 [8]. Activation of the IRS-PI 3-kinase pathways by insulin and IGF-1 [11 – 15] is important for regulating cell metabolism, apoptosis and mitogenesis [16].

During pregnancy, pancreatic islets undergo structural and functional changes in response to an increased peripheral demand for insulin [17 – 19]. The total islet mass increases, mainly as a consequence of B-cell hypertrophy and hyperplasia [20 – 23]. Insulin secretion in response to glucose is also enhanced, which is reflected by a shift to the left in glucose dose-response curves in isolated islets [19]. These adaptive changes are mediated by a variety of hormonal, chemical and neural signals to the islets [2, 3, 5, 24]. Placental lactogens, including PRL, play an important role in these adaptations [25 – 29]. The control of islet development and growth may also depend on the activity of the insulin/IGF-1 signaling pathway. Thus, targeted disruption of IRS-2 leads to diabetes as a result of increased peripheral insulin resistance and impairment of pancreatic B-cell function [30].

Since PRL and insulin/IGF-1 participate in the growth and maturation of pancreatic islet cells, we examined the crosstalk between the PRL and insulin/IGF-1-signaling pathways in neonatal rat islets.

Materials and Methods

Materials

The reagents and apparatus for SDS-PAGE and immunoblotting were obtained from Bio-Rad (Richmond, CA, USA). Tris, Triton-X 100, Tween 20, glycerol, acrylamide, bis-acrylamide, bovine serum albumin (BSA, fraction V), PMSF, sodium pyrophosphate, sodium fluoride, sodium vanadate, EDTA, EGTA, aprotinin, leupeptin, benzamidine, DTT, glycerol, Nonidet P-40, Ficoll, Hepes, RPMI-1640 medium, ATP, collagenase type V, and wortmannin were from Sigma (St. Louis, MO, USA). Rat PRL was from Dr. A. F. Parlow, Harbor University of California Los Angeles Medical Center and kindly provided by the National Hormone and Pituitary Program of the NIDDK). [^{125}I] Protein A, [^{125}I] insulin and nitrocellulose membranes (Hybond N, 0.45µm) were from Amersham (Buckinghamshire, UK), and protein A Sepharose 6 MB was from Pharmacia (Uppsala, Sweden). Anti-phosphotyrosine (mouse monoclonal), anti-IRS-1 (rabbit polyclonal), anti-IRS-2 (goat polyclonal), anti-SHC (rabbit polyclonal), and anti-JAK-2 (rabbit polyclonal) antibodies were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA); anti-PI 3-kinase p85 (rabbit polyclonal) was from UBI (Lake Placid, NY,USA) and anti-ERK (p44/42 MAPK mouse monoclonal) was from Biolabs Inc. (New England,USA). L-α-phosphatidylinositol (liver-sodium salt) was from Avanti Polar-Lipids (Alabaster, AL, USA) and human insulin from Biobras AS (Brazil).

Islet isolation and culture

For each set of experiments, islets from 80 – 100 neonatal rats (2 – 3 days old) were obtained as described [28, 31] and maintained in culture at 37 °C in a 5% CO_2/air atmosphere for 2 to 7 days. The culture medium consisted of RPMI – 1640 supplemented with 10% fetal bovine serum, 10 mmol glucose/l, 100 IU/ml penicillin, 100 µg/ml streptomycin, and PRL (0.1 µg/ml) and wortmannin (0.1 µg/ml) as required. After culture, the islets were separated from the remaining exocrine pancreatic debris by centrifugation in Ficoll gradients and distributed equally in three polypropylene Eppendorf tubes. The islets were incubated at 37 °C for 5 min or 15 min in Hanks solution containing 5.6 mmol glucose/l, 1 mg/ml BSA, and PRL and/or insulin as required.

Tissue extracts and immunoblotting

After incubation, the islets were homogenized in 300 µl of solubilization buffer (10% Triton-X 100, 100 mmol Tris/l (pH 7.4), 10 mmol sodium pyrophosphate/l, 100 mmol sodium fluoride/l, 10 mmol EDTA/l, 10 mmol sodium vanadate/l, 2 mmol PSMF/l, and 0.1 mg/ml aprotinin for 30 s using a Polytron PT 1200 C homogenizer (Brinkmann Instruments, NY, USA). The tissue extracts were centrifuged at 12,000 rpm at 4 °C for 20 min and the supernatant was used for the determination of protein, and for immunoprecipitation.

Immunoprecipitation was performed with 15 µl of anti-IRS-1/-2, anti-SHC, or anti-JAK2 antibodies at 4 °C overnight. The immune complexes were then precipitated with protein A-Sepharose 6 MB for 2 h. The pellets were washed three times in buffer containing 100 mmol Tris/l, 2 mmol sodium vanadate/l, 1 mmol EDTA/l, and 0.5% Triton X-100, resuspended in 18 µl of Laemmli sample buffer [32] and boiled for 5 min prior to loading onto polyacrylamide gels (8% for anti-IRS-1/-2 and anti-JAK2, 15% for anti-SHC). For total extracts, aliquots containing 180 µg of protein were run in 12% polyacrylamide gels SDS in a Bio-Rad miniature slab gel apparatus (Mini-Protean) [33].

The electrotransfer of proteins from the gel to nitrocellulose was undertaken for 2 h at 120 V using a Bio-Rad miniature transfer apparatus. Non-specific protein binding to nitrocellulose was reduced by preincubating the filter in blocking buffer (3% BSA, 10 mmol Tris/l, 150 mmol NaCl/l, and 0.02% Tween 20) for 2 h at 22 °C. The nitrocellulose membranes were then incubated for 2 h at 22 °C with antiphophotyrosine antibody, or anti-PI 3-kinase diluted in blocking buffer, and washed for 30 min in blocking buffer without BSA. The blots were then incubated with 2 µCi of [^{125}I] Protein A (30 µCi/µg) in 10 ml of blocking buffer for 1 h at 22 °C and washed again as described above for 2 h. [^{125}I] protein A bound to the antibodies was detected by autoradiography using pre-flashed Kodak film at – 80 °C for 24 – 60 h. Band intensities were quantified by optical densitometry (model GS 300; Hoefer Scientific Instruments, San Francisco, CA, USA) of the autoradiograph.

Phosphatidylinositol 3-kinase activity

PI 3-kinase activity was measured by phosphorylation of phosphatidylinositol *in vitro*. After isolation, the islets were incubated with or without of 2 µg/ml prolactin for 5 min or 15 min and then homogenized in 200 µl of ice-cold solubilization buffer (buffer A). The solubilization buffer A contained (in mmol/l) Hepes (pH 7.4) 50, NaCl 137, $MgCl_2$ 1,$CaCl_2$ 1, sodium vanadate 2, sodium pyrophosphate 10, sodium fluoride 100, EDTA 2, benzamidine 10, PMSF 2, 1% Nonidet P-40, 10% glycerol, 2 µg/ml aprotinin, and 5 µg/ml leupeptin. IRS-1 and 2 were immunoprecipitated from aliquots of the supernatant (1.5 mg/ml protein) by adding anti-IRS-1 and 2 antibodies followed by protein A Sepharose 6 MB and incubation for 2 h. The immunoprecipitates were washed

three times with phosphate-buffered saline containing 1% Nonidet P-40 and 100 µmol sodium vanadate/l, twice with buffer containing 100 mmol Tris/l (pH 7.5), 500 mmol $LiCl_2$/l, and 100 µmol sodium vanadate/l, and twice with buffer containing 10 mmol Tris/l (pH 7.5), 100 mmol NaCl/l, and 1 mmol EDTA/l. The pellets were resuspended in 50 µl of 10 mmol Tris/l (pH 7.5) containing 100 mmol NaCl/l, 1 mmol EDTA/l, and 100 µmol sodium vanadate/l. 10 µl of 100 mmol $MgCl_2$/l and 10 µl of phosphatidylinositol (2 µg/µl) previously sonicated in 10 mmol Tris buffer/l (pH 7.5) with 1 mmol EGTA/l were added to each pellet. The PI 3-kinase reaction was started by adding of 10 µl of 440 µmol ATP/l containing 30 µCi of [^{32}P] ATP. After 10 min at room temperature with constant shaking, the reaction was stopped by adding 20 µl of 8 N HCl and 160 µl of $CHCl_3$:methanol (1 : 1). The samples were centrifuged, and the lower organic phase was removed and applied to silica gel TLC (thin-layer chromatography) plates (Merck) coated with 1% potassium oxalate. The plates were resolved in $CHCl_3$:CH_3OH:H_2O:NH_4OH (60 : 47 : 11,3 : 2), dried, and visualized by autoradiography [11].

Insulin secretion

Groups of ten islets were first incubated for 45 min at 37 °C in Krebs-bicarbonate buffer containing 5.6 mM glucose and equilibrated with 95% O_2/5% CO_2 at pH 7.4. The solution was then replaced with fresh Krebs-bicarbonate buffer and the islets were incubated for a further hour with medium containing (in mmol/l) glucose 2.8 or 22.2 or 40 mmol K^+/l. The incubation medium contained (in mmol/l): NaCl 115, KCl 5, $NaHCO_3$ 24, $CaCl_2$ 2.56, $MgCl_2$ 1, and BSA 0.3% (w/v). The cumulative insulin release during one hour was quantified by radioimmunoassay using rat insulin as standard.

Statistical analysis

Experiments were always performed by comparing samples from islets treated with PRL and/or insulin in parallel with control islets. For comparisons, Student's *t*-test for unpaired samples was used. The level of significance was set at $p < 0.05$.

Results

Insulin secretion

In the presence of 2.8 mmol glucose/l, insulin secretion was 0.27 ± 0.06 ng/islet per hour (n = 8). The secretion was increased to 1.0 ± 0.25 and 1.5 ± 0.37 ng/islet per hour in the presence of 22.2 glucose/l and 40 mmol K+/l, respectively (n = 8; $p < 0.05$). The treatment of isolated islets with 2 µg/ml PRL for short period (5 – 15 min) did not change the insulin secretion compared to islets incubated with 2.8 or 22.2 mmol glucose/l in the absence of PRL (data not shown). However, glucose-induced insulin secretion in islets cultured for 7 days in the presence of 0.1 µg/ml PRL was significantly higher than control islets ($p < 0.05$). The presence of wortmannin in the culture medium abolished the potentiation effect of PRL ($p < 0.05$ PRL + Wor vs. PRL alone). Wortmannin per se did not affect insulin secretion induced by 22.2 mmol glucose/l after 7 days culture (Fig. **1**).

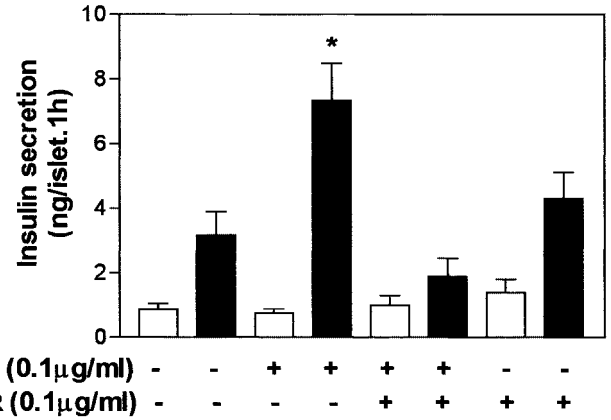

Fig. **1** Effect of PRL and wortmannin (Wor) on glucose-induced insulin secretion in 7 days cultured neonatal rat islets. Islets were cultured in RPMI medium containing or not 0.1 µg/ml PRL and/or wortmannin. Groups of 10 islets were then first incubated for 45 min at 37 °C in Krebs-bicarbonate buffer containing 5.6 mmol glucose/l and equilibrated with 95% O_2/5% CO_2, pH 7.4. The solution was then replaced with fresh Krebs-bicarbonate buffer and the islets were incubated for a further 1 h with medium containing 2.8 open bars or 22.2 mmol glucose/l closed bars. The bars represent cumulative insulin secretion release during 1 h. Data were mean ± SEM of 9 experiments. *$p < 0.05$ (PRL *vs.* PRL + Wor).

Phosphorylation of IRS-1, IRS-2 and their association with the p85 subunit of PI 3-kinase

To explore possible connections between PRL and the insulin-signaling pathway in neonatal islet cells, groups of 1,500 islets were incubated with or without different concentrations (0.02, 0.1 and 2 µg/ml) of PRL for 5 min or 15 min. As a whole, the protein levels of IRS-1 and IRS-2 were no different from control values (Fig. **2A**, upper and lower panels). Immunoprecipitation of islet extracts with anti-IRS-1 or anti-IRS-2 followed by immunoblotting with anti-phosphotyrosine antibodies revealed a dose-response increase in IRS-1 and IRS-2 phosphorylation induced by PRL. After 15 min incubation, physiological concentrations of 0.02 µg/ml PRL increased IRS-1 and IRS-2 phosphorylation by approximately 2.3-fold over basal values ($p < 0.05$). PRL-induced maximal IRS-1 and IRS-2 phosphorylation after 15 min exposure was obtained with 2 µg/ml PRL, reaching 4-fold and 6.8-fold increase over basal, respectively. Two µg/ml PRL also increased IRS-1 and IRS-2 phosphorylation by 2.2-fold and 3-fold over basal, respectively, after 5 min incubation (Fig. **2B**, upper and lower panels). The increase in the phosphorylation of IRS-1 and IRS-2 was accompanied by an association with the enzyme PI 3-kinase, as determined by immunoblotting of the complex with antibody to the regulatory subunit (p85) of the protein. A significant increase (2.2- and 2.8-fold) in the association of IRS-1 and IRS-2 with PI 3-kinase was observed after exposure to 2 µg/ml PRL for 5 min. The increased association between IRS-1/PI 3-kinase was 2.5-fold, 3.0-fold and 6.1-fold in the presence 0.02, 0.1 and 2 µg/ml PRL, respectively, after 15 min ($p < 0.05$ *vs.* basal values), whereas the association between IRS-2 and PI 3-kinase was 3.4-fold, 4-fold and 5.2-fold in the presence of the above PRL concentrations after 15 min ($p < 0.05$ compared with control values) (Fig. **2C**, upper and lower panels).

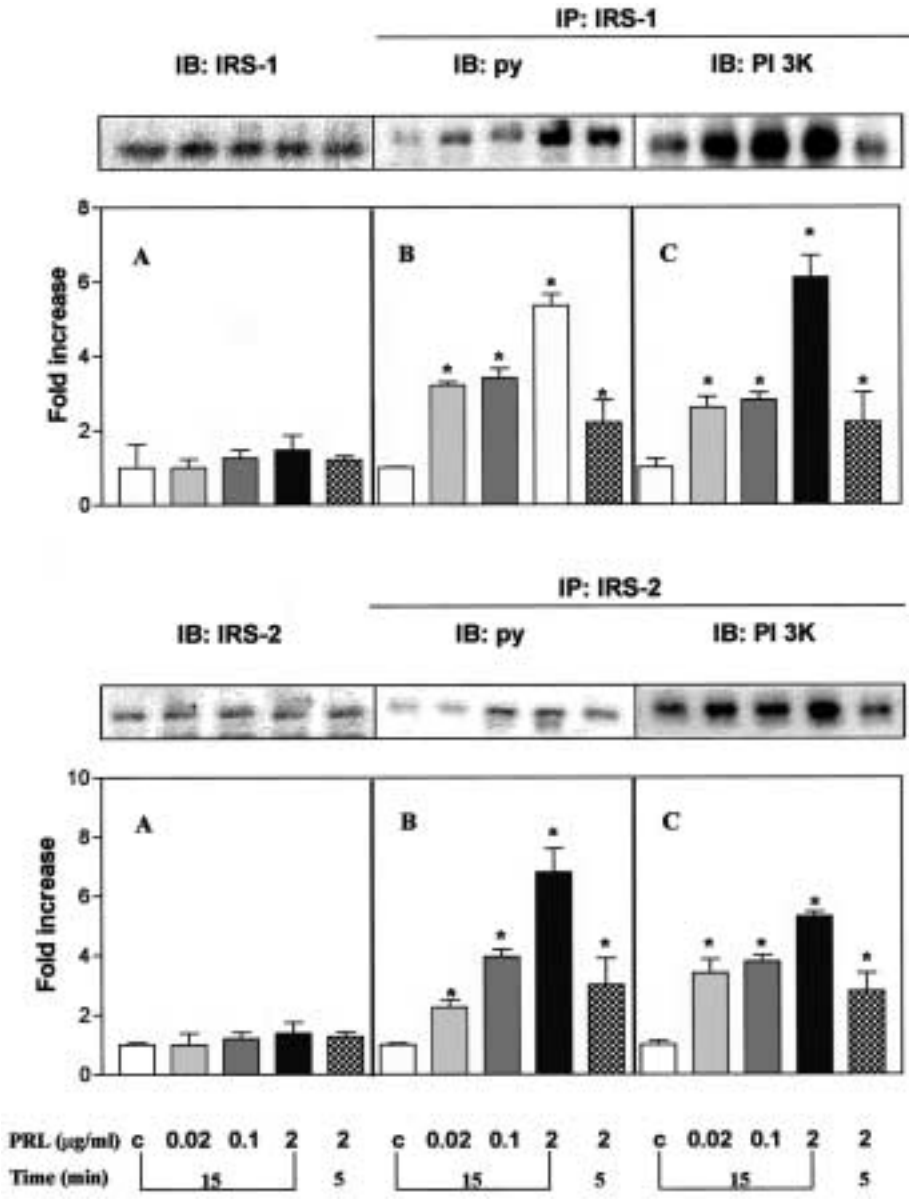

Fig. **2** PRL-induced tyrosine phosphorylation of IRS-1/2 and their association with PI 3-kinase in pancreatic islet cells. Extracts of islets incubated for 5 min and 15 min in Hanks solution containing 5.6 mmol glucose/l in the absence or presence of 0.02, 0.1 and 2 μg/ml PRL were immunoprecipitated (IP) with anti-IRS-1 and -2 antibodies and immunoblotted (IB) with anti-phosphotyrosine antibody (**B**, upper and lower panels). The nitrocellulose membrane transfers were stripped and probed with anti-p85 antibodies (**C**, upper and lower panels). To ascertain the protein amounts of IRS-1 and IRS-2, total protein extracts of neonatal pancreatic islets were submitted to SDS-PAGE and blotted with anti-IRS-1 and -2 antibodies (**A**, upper and lower panels). The bars represent the relative protein levels, phosphorylation of IRS-1/2 and the binding of p85 as determined by optical densitometry, and are the mean ± SEM of four experiments. $^{*}p < 0.05$ *vs*. basal values (c) after a 15 min incubation without PRL.

PRL and insulin induce phosphorylation of IRS-2 and its association with the p85 subunit of PI 3-kinase

PRL (0.1 μg/ml), insulin (10^{-7}mol/l) and the association of both hormones increased IRS-2 phosphorylation after 15 min by 6-fold, 10-fold and 6.5-fold over basal values, respectively ($p < 0.05$) (Fig. **3B**). The increase in IRS-2 phosphorylation was accompanied by an increase in the association of IRS-2 with the p85 subunit of PI 3-kinase by 6-fold, 8 fold and 5.8-fold over basal in the presence of 0.1 μg/ml PRL, 10^{-7} mol/l insulin and the association of both hormones, respectively ($p < 0.05$) (Fig. **3C**). No differences in the protein content of IRS-2 were detected (Fig. **3A**).

Induction of phosphatidylinositol 3-kinase activity by PRL treatment

Recent studies that evaluated the role of IRS-1 and IRS-2 in pancreatic islets by using knock-out mice with no IRS-1 or IRS-2 indicated that IRS-2 may participate in pathways that control islet growth. To assess the involvement of PRL in IRS-1/-2 dependent activation of PI 3-kinase, islets were incubated with or without PRL (2 μg/ml) and homogenized and immunoprecipitated with anti-IRS-1/-2 antibodies. PI 3-kinase activity was assayed as described in *Materials and Methods*. Incubation of islets with PRL for 15 min increased the IRS-1 associated PI 3-kinase activity by 5-fold (Fig. **4A**), and the IRS-2 associated PI 3-kinase activity by 7.3-fold (Fig. **4B**) compared to the control values.

PRL induces tyrosine phosphorylation of JAK2

The binding of PRL to its receptor leads to tyrosine phosphorylation and the activation of JAK2, followed by receptor dimerization, recruitment of STAT5 and the initiation of nuclear signaling. The incubation of 1,500 neonatal pancreatic islets with 2 μ/ml PRL for 5 min or 15 min resulted in 3-fold and 7-fold increases in tyrosine phosphorylation of JAK2, respectively (Fig. **5B**). No differences in the protein levels were detected (Fig. **5A**).

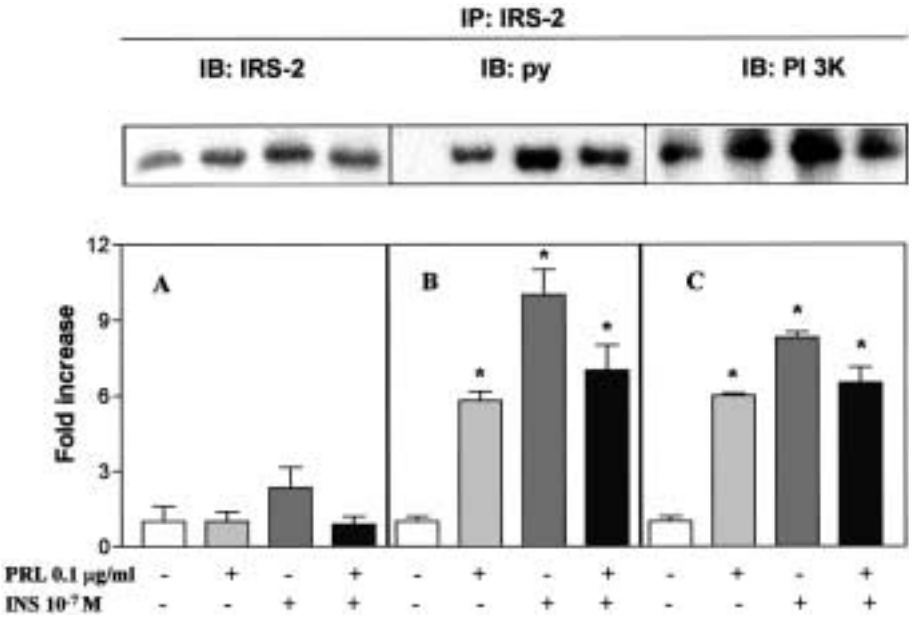

Fig. **3** PRL and insulin (INS) induces phosphorylation of IRS-2 and its association with the p85 subunit of PI 3-kinase in pancreatic islet cells. Extracts of islets incubated for 15 min in Hanks solution containing 5.6 mmol glucose/l in the absence or presence of 0.1 µg/ml PRL, 10^{-7}mol insulin/l or the association between both hormones were immunoprecipitated (IP) with anti-IRS-2 antibodies and immunoblotted (IB) with anti-phosphotyrosine antibody (**B**). The nitrocellulose membrane transfers were stripped and probed with anti-IRS-2 (**A**) and anti-p85 antibodies (**C**). The bars represent the relative protein levels, phosphorylation of IRS-2 and the binding of p85 as determined by optical densitometry, and are the mean ± SEM of four experiments. $^{*}p < 0.05$ *vs*. basal values (c) after a 15 min incubation without PRL and insulin.

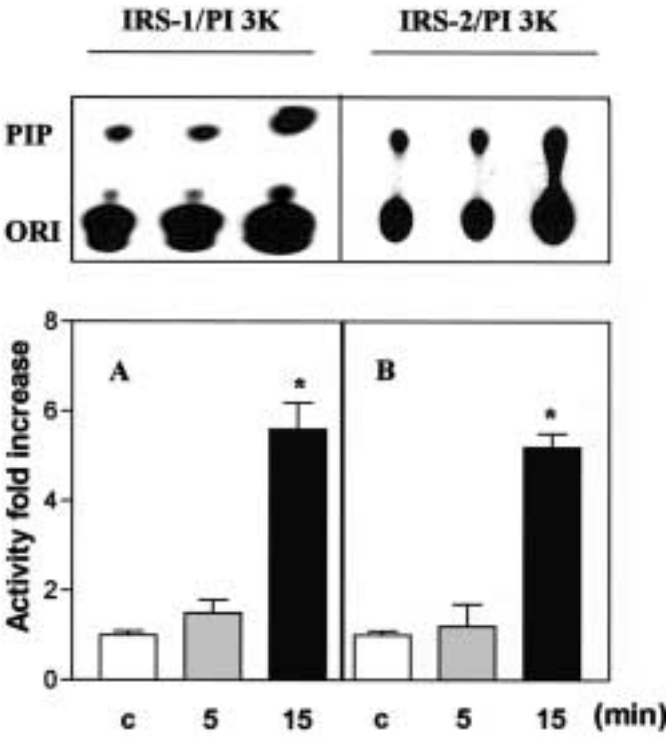

Fig. **4** PRL-induced PI 3-kinase activity associated with IRS-1/2 in pancreatic islet cells. Extracts of islets incubated for 5 min and 15 min in Hanks solution containing 5.6 mmol glucose/l and 2 µg/ml PRL (when required) were immunoprecipitated with anti-IRS-1/2 antibodies. PI 3-kinase in the immunoprecipitates was assayed as described in Methods. Autoradiographs show the silica TLC plate profiles of IRS-1 (**A**) and IRS-2 (**B**) associated PI 3-kinase activity. ORI, the origin; PIP, the migration of PI 3-P. The bars represent the relative incorporation of ^{32}P into PI 3-P (mean ± SEM) from three experiments. $^{*}p < 0.05$ *vs*. basal values (c) after a 5 min incubation without PRL.

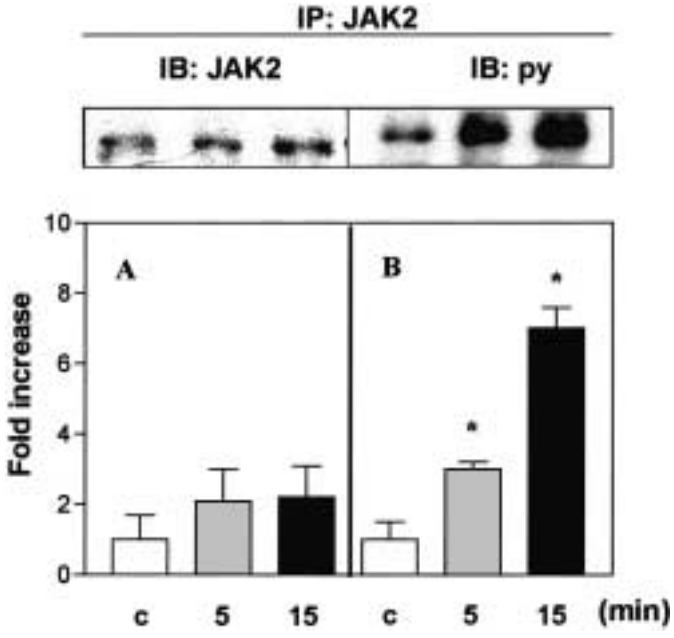

Fig. **5** PRL-induced tyrosine phosphorylation of JAK2 in pancreatic islet cells. Extracts of islets incubated for 5 min and 15 min in Hanks solution containing 5.6 mmol glucose/l and 2 µg/ml PRL (when required) were immunoprecipitated (IP) with anti-JAK2 antibody and immunoblotted (IB) with anti-JAK2 (**A**) and anti-phosphotyrosine antibodies (**B**). The bars represent the relative protein levels and phosphorylation of JAK2 as determined by optical densitometry, and are the mean ± SEM of three experiments. $^{*}p < 0.05$ *vs*. basal values (c) after 5 min incubation without PRL.

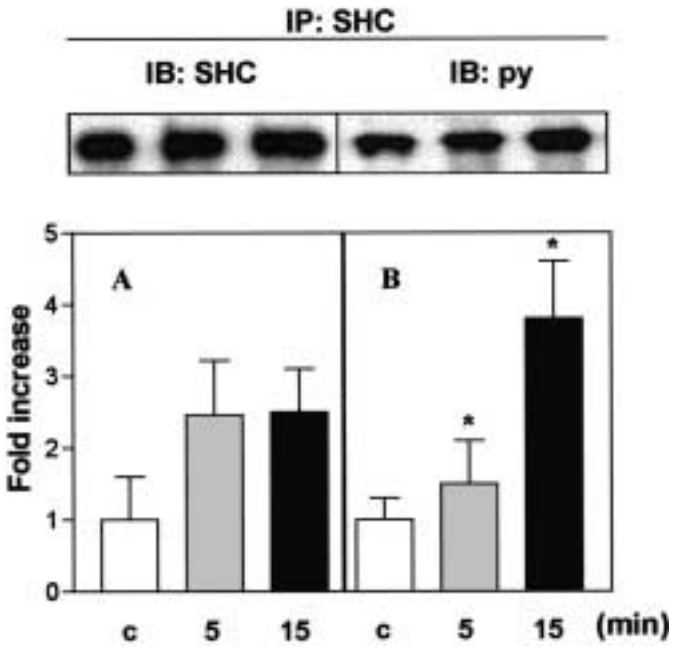

Fig. **6** PRL-induced tyrosine phosphorylation of SHC in pancreatic islet cells. Extracts of islets incubated for 5 min and 15 min in Hanks solution containing 5.6 mmol glucose/l and 2 μg/ml PRL (when required) were immunoprecipitated (IP) with anti-SHC antibody and immunoblotted (IB) with anti-SHC (**A**) and anti-phosphotyrosine antibodies (**B**). The bars represent the relative protein levels and phosphorylation of SHC as determined by optical densitometry, and are the mean ± SEM of three experiments. *$p < 0.05$ *vs.* basal values (c) after 5 min incubation without PRL.

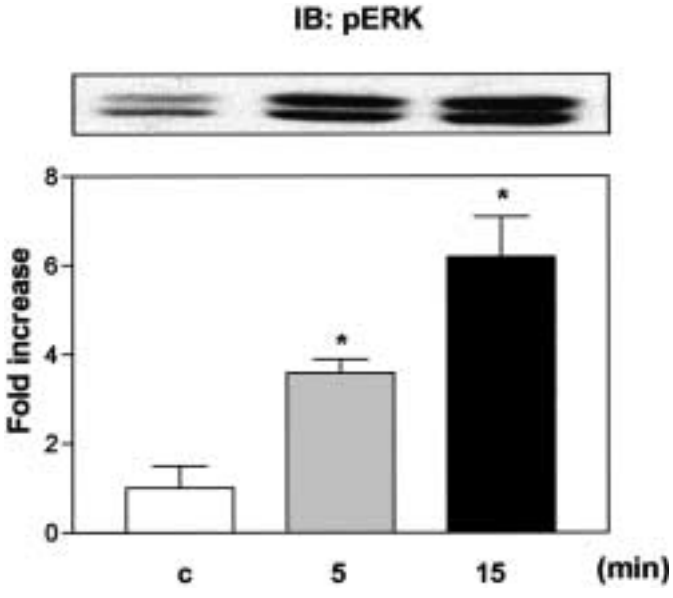

Fig. **7** PRL-induced tyrosine phosphorylation of ERK1/2 in pancreatic islet cells. Extracts of islets incubated for 5 min and 15 min in Hanks solution containing 5.6 mmol glucose/l and 2 μg/ml PRL (when required) were immunoblotted (IB) with anti-p-ERK antibody. The bars represent the relative phosphorylation of p-ERK1/2 as determined by optical densitometry, and are mean ± SEM of three experiments. *$p < 0.05$ *vs.* basal values (c) after 5 min incubation without PRL.

PRL activates SHC and ERK1/2 in neonatal pancreatic islets

In several signaling systems, SHC acts as a docking protein that participates in the activation of GRB-2/SOS/Ras and transmits the signal to the nucleus through the MAP kinase cascade. To investigate the participation of SHC and the MAP kinase cascade in PRL signaling in neonatal rat pancreatic islets, groups of approximately 1,500 islets were incubated with 2 μg/ml PRL for 5 min or 15 min. Islet extracts were either immunoprecipitated with anti-SHC antibody followed by SDS-PAGE and blotting with anti-phosphotyrosine antibodies, or were directly separated by SDS-PAGE, and blotted with anti-phospho-ERK1/2 antibody. PRL increased SHC tyrosine phosphorylation by 3.8-fold after 15 min compared to the control values (Fig. **6B**). Protein levels of SHC were not different between samples (Fig. **6A**). ERK1/2 serine/threonine kinase phosphorylation increased approximately 3.6-fold and 6.2-fold after 5 min and 15 min exposure to PRL, respectively (Fig. **7**).

Discussion and Conclusions

The activation of JAK/STAT is the major pathway involved in most of the cellular responses induced by the interaction of GH and PRL with their respective receptors in different cell types including pancreatic B-cells [9,10]. Signal transduction by GH and PRL also involves tyrosine phosphorylation of IRS proteins [8,34] with subsequent association/activation of PI 3-kinase. Activation of the MAP kinase cascade most probably occurs through the adapter protein complex SHC/GRB-2/SOS [6,7].

In the present report, intracellular crosstalk between the PRL and insulin-signaling systems was studied in neonatal rat pancreatic islets. We assessed the quality of the neonatal islet preparations by determining the insulin secretion response to glucose overload and high K^+. Insulin secretion was not altered by a 5 min or 15 min exposure of islets to PRL, thus indicating that the action of PRL on components of the insulin-signaling pathway did not involve insulin. However, exposure to PRL for prolonged period of time (7 days) strongly potentiated glucose-induced insulin secretion as already shown by many authors [18,26,28]. The PRL induced potentiation of insulin secretion by glucose was abolished by the concomitant treatment with wortmannin a specific PI 3-kinase inhibitor, suggesting a participation of this enzyme in the maturation of the glucose-sensing mechanism provoked by prolonged treatment with PRL (Fig. **1**). These data contrast with the observation that PI 3-kinase exerts a negative regulation of endocrine differentiation in human fetal cultured islet-like cell clusters [35]. However, we have to take into account that the tissues used in both experiments were not the same. In another series of experiments, we observed that both IRS-1 and IRS-2 were tyrosine-phosphorylated in response to physiological (0.02 and 0.1 μg/ml) and supraphysiological concentrations (2 μg/ml) of PRL for short period of time. Moreover, PI 3-kinase was also dose-dependently recruited to the IRS-1 and IRS-2 signaling complexes (Fig. **2**). The effects of 0.1 μg/ml PRL on IRS-2 phosphorylation and PI 3-kinase recruitment were not additive to the insulin (10^{-7} mol/l) effects as illustrated (Fig. **3**). At this moment, we have no explanation for such an effect. However, it is conceivable that both hormones compete for p85 subunit of PI

3-kinase binding as already demonstrated in other cross-talk systems [36,37].

In almost all systems tested, association of IRS-1 or IRS-2 with PI 3-kinase induced by hormones, growth factors or cytokines leads to activation of this kinase. Confirming these data, there was a significant increase in the activity of PI 3-kinase associated with IRS-1 (Fig. **4** A) and IRS-2 (Fig. **4** B). When activated by insulin, PI 3-kinase participates in the control of glucose uptake by muscle and fat in the control of glycogen and protein synthesis [38] and in the activation of anti-apoptotic elements [39].

Recent studies in pancreatic islets have shown that PI 3-kinase is involved in the control of several physiological functions in this tissue [40,41]. Moreover, insulin secretion induced by high glucose affects insulin and pyruvate kinase gene transcription by the activating PI 3-kinase [42]. Finally, PI 3-kinase and AKT activated by IGF-1 exert anti-apoptotic effects in pancreatic islets [43].

Significant tyrosine phosphorylation of JAK2 was observed within 5 min of exposure to PRL and maintained for at least 15 min. The intracellular kinases of the JAK family are usually constitutively associated with several members of the cytokine receptor superfamily. Accordingly, PRLR possess as JAK2 an associated kinase responsible for transducing the initial steps of the PRL signal. Receptors associated with JAKs deliver a rapid signal to the nucleus by inducing the activation of members of the signal transducer and activator of transcription (STAT) family. In PRL signaling, STAT5 is the isoform involved [24]. The activation of STAT5 via JAK2 mediates the growth and proliferation of primarily cultures of B-cells though a mechanism that is independent of PKC, PI 3-kinase and MAP kinase [44].

In addition to its classic signaling through JAK2, PRL activates the MAP-kinase cascade through SHC. In neonatal rat pancreatic islets, PRL induced early SHC tyrosine phosphorylation, which was followed by ERK1/2 phosphorylation. In several signaling systems, the activation of the MAP-kinase cascade leads to nuclear signaling and the control of cellular growth. As shown elsewhere [44], B-cell proliferation is not blocked by treatment with PD98059 or SB203580, inhibitors of different steps of the MAP-kinase cascade. It is possible that the disrupted architecture of the pancreatic islets (when working with isolated B-cells) may influence signaling events and the overall response to a given pathway of activation. Thus, results obtained in primary cultures of B-cells and insulin secreting cell lines may be not fully applicable to *in situ* or isolated islets. Another possibility is that the SHC/MAPK pathway, once activated by PRL in pancreatic islets may be involved in other cellular functions such as growth, apoptosis, morphogenesis, and cell repair [45].

Autocrine signaling of insulin apparently participates in the control of several functions that are important for islet homeostasis. As shown here, PRL, a hormone present at high concentrations during late embryonic and early extrauterine life, signals through elements classically involved in insulin signaling. No regulation of insulin secretion by PRL for short period of time was detected, but the activation of the IRS-1/-2 and PI 3-kinase pathway by PRL may be involved in morphogenesis, cell growth or ontogenetic apoptosis in pancreatic islets.

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