Prolactin induction of insulin gene expression: the roles of glucose and glucose transporter-2

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Abstract

Previous studies have shown that lactogenic hormones stimulate beta-cell proliferation and insulin production in pancreatic islets. However, all such studies have been conducted in cells incubated in medium containing glucose. Since glucose independently stimulates beta-cell replication and insulin production, it is unclear whether the effects of prolactin (PRL) on insulin gene expression are exerted directly or through the uptake and/or metabolism of glucose.

We examined the interactions between glucose and PRL in the regulation of insulin gene transcription and the expression of glucose transporter-2 (glut-2) and glucokinase mRNAs in rat insulinoma (INS-1) cells. In the presence of 5.5 mM glucose, the levels of preproinsulin and glut-2 mRNAs in PRL-treated cells exceeded the levels in control cells (1.7-fold, $P < 0.05$ and 2-fold, $P < 0.05$ respectively). The maximal effects of PRL were noted at 24–48 h of incubation. PRL had no effect on the levels of glucokinase mRNA. The higher levels of glut-2 mRNA were accompanied by an increase in the number of cellular glucose transporters, as demonstrated by a 1.4- to 2.4-fold increase in the uptake of 2-deoxy-D-³H]glucose in PRL-treated INS-1 cells ($P < 0.001$).

These findings suggested that the insulinotropic effect of PRL is mediated, in part, by induction of glucose transport and/or glucose metabolism. Nevertheless, even in the absence of glucose, PRL stimulated increases in the levels of preproinsulin mRNA (3.4-fold higher than controls, $P < 0.0001$) and glut-2 mRNA (2-fold higher than controls, $P < 0.01$). These observations suggested that PRL exerts glucose-independent as well as glucose-dependent effects on insulin gene expression. Support for this hypothesis was provided by studies of insulin gene transcription using INS-1 cells transfected with a plasmid containing the rat insulin 1 promoter linked to a luciferase reporter gene. Glucose and PRL, alone and in combination, stimulated increases in cellular luciferase activity. The relative potencies of glucose (5.5 mM) alone, PRL alone, and glucose plus PRL in combination were 2.2 ($P < 0.001$), 3.4 ($P < 0.01$), and 7.9 ($P < 0.0001$) respectively. Our findings suggest that glucose and PRL act synergistically to induce insulin gene transcription.

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Introduction

The second half of pregnancy is a diabetogenic state characterized by insulin resistance, glucose intolerance, and fasting and postprandial hyperinsulinemia. Studies in experimental animals indicate that the hyperinsulinemia of pregnancy derives from expansion of the mass of pancreatic beta cells, induction of insulin synthesis, and an increase in glucose-stimulated insulin secretion (Hellman 1960, Green & Taylor 1972, Bone & Taylor 1976, Parsons *et al.* 1995). Although the factors responsible for these adaptive changes in islet function during pregnancy remain poorly defined, a role for the lactogenic hormones prolactin (PRL) and placental lactogen is suggested by three lines of evidence: first, the increase in maternal

insulin production during mid- to late pregnancy coincides with increases in maternal plasma levels of placental lactogen and, in humans, PRL (Ogren & Talamantes 1988, Parsons *et al.* 1992); secondly, the number of islet cell binding sites for PRL and placental lactogen and the pancreatic levels of PRL receptor mRNA increase during pregnancy (Moldrup *et al.* 1993, Sorenson & Stout 1995); and thirdly, placental lactogen and PRL stimulate beta-cell proliferation, insulin production, and glucose-dependent insulin secretion in pancreatic islet cells in culture (Sorenson *et al.* 1987a, 1993, Brelje & Sorenson 1991, Hellerstrom *et al.* 1991, Brelje *et al.* 1993, 1994).

The cellular mechanisms by which lactogens stimulate insulin production in pancreatic beta cells are poorly

understood. All previous investigations of lactogen action in pancreatic islets have been conducted using culture medium that contains glucose. Since glucose independently stimulates beta-cell replication and insulin production (Docherty & Clark 1994, Philippe *et al.* 1994) it is unclear whether the effects of lactogens are exerted directly or through the uptake and/or metabolism of glucose. A role for glucose transport and/or glucose metabolism in lactogen action is suggested by studies in which prolonged exposure of islets to PRL increased cellular levels of immunoreactive glucose transporter 2 (glut-2) and glucokinase, as assessed by Western blot (Weinhaus *et al.* 1996).

To clarify the roles of glucose transport and glucose metabolism in lactogen action, we examined the interactions between glucose and PRL in the regulation of insulin gene transcription and the expression of glut-2 and glucokinase mRNAs in rat insulinoma (INS-1) cells. We also examined the effects of PRL on INS-1 cell uptake of 2-deoxyglucose. We selected INS-1 cells as an *in vitro* model of pancreatic beta cells because these cells are highly differentiated and resemble pancreatic beta cells both morphologically and functionally (Asfari *et al.* 1992). INS-1 cells have been used previously by many investigators to study the regulation of insulin gene expression, glut-2 gene expression, and insulin secretion in response to glucose and other physiological secretagogues (Philippe *et al.* 1994, Waeber *et al.* 1994, Asfari *et al.* 1995, Frödin *et al.* 1995, Sekine *et al.* 1996). Moreover, INS-1 cells express PRL receptor mRNA and lactogenic binding activity (Asfari *et al.* 1995), making them suitable for the study of PRL action.

Materials and Methods

Cell culture

INS-1 cells (kindly provided by M Asfari, INSERM, Paris, France) were incubated at 37 °C in RPMI 1640 medium (11.1 mM glucose; Life Technologies, Grand Island, NY, USA) supplemented with 10% fetal calf serum (FCS), 50 µM 2-mercaptoethanol, 1 mM pyruvic acid, 10 mM HEPES, and 1% antibiotic-antimycotic solution. When the cells were nearly confluent, the medium was changed to 'basal medium' (Dulbecco's modified essential medium (DMEM) 5.5 mM glucose supplemented with 0.1% human serum albumin, 10 µg/ml human transferrin, 0.1 nM tri-iodothyronine (T_3), 50 µM ethanolamine, 50 µM phosphoethanolamine, and 1% antibiotic-antimycotic solution; all from Sigma Chemical Co., St Louis, MO, USA) containing either PRL (ovine (o) PRL, 1 µg/ml; NIDDK-NIH, Bethesda, MD, USA) or diluent. The medium was changed daily.

To explore directly the role of glucose in PRL action, we employed two approaches: first, the cells were preincubated with cytochalasin B (5–50 µM), which inhibits

cellular glucose uptake. The cells were then incubated with PRL or diluent in the presence of cytochalasin B for periods ranging from 1 to 24 h. These experiments were impossible to interpret because the cytochalasin B, even at very low concentrations, proved to be highly toxic to the cells. In the second approach, the INS-1 cells were preincubated in 'basal medium' overnight; the medium was then replaced with serum-free, glucose-free DMEM (supplemented with 0.1% human serum albumin, 10 µg/ml human transferrin, 0.1 nM T_3 , 50 µM ethanolamine, 50 µM phosphoethanolamine, 1% antibiotic-antimycotic solution, and 1 mM sodium pyruvate as a source of carbohydrate). The cells were incubated in this glucose-free DMEM for 2 h prior to, and for up to 24 h after, the addition of oPRL or diluent. Under these conditions, the concentration of glucose in the culture medium was below the limits of detectability. All cell culture materials were obtained from Life Technologies, Frederick, MD, USA unless specified otherwise.

RNA extraction and Northern blot analysis

Total RNA was extracted from INS-1 cells using TRI REAGENT (Molecular Research Center, Inc., Cincinnati, OH, USA). For the experiments in glucose-containing medium, total RNA was isolated at various time-points between 3 and 72 h of incubation. For the experiments in glucose-free medium, total RNA was isolated at various time-points between 4 and 24 h of incubation. Gene expression was assessed by Northern blot analysis. Twenty micrograms of total RNA were electrophoresed on 1% agarose gels and blotted on nylon membranes. The cDNA probe for rat preproinsulin-I (kindly provided by R Liddle, Duke University, USA) was a 700 bp EcoRI–BamHI fragment containing the entire coding sequence for the peptide. The probe for rat glut-2 (kindly provided by G Bell, University of Chicago, IL, USA) was a 1.5 kb EcoRI insert encoding bases 105 through 1648 of the glut-2 cDNA. The probe encoding rat islet glucokinase (kindly provided by M Magnuson, Vanderbilt University, USA) was a KpnI–XbaI fragment of 1450 bp containing the entire coding sequence for the peptide. The probe for rat insulin-like growth factor-II (IGF-II; kindly provided by A J D'Ercole, University of North Carolina, NC, USA), complementary to 241 bp of the E-domain and 34 bp of the 3'-untranslated region, was derived from a 275 bp RsaI fragment of a rat IGF-II cDNA. The cDNA probes were labeled with digoxigenin using the Boehringer Mannheim High Prime kit (Boehringer Mannheim Co., Indianapolis, IN, USA). The probes were precipitated with ethanol, dried, and solubilized. The probes were added to the hybridization mixture, which contained 5 × SSC, 0.1% N-lauroylsarcosine, 0.02% SDS, and 1% blocking reagent (Boehringer Mannheim Co.). The hybridizations were performed at 60 °C overnight. The membranes were washed twice at

60 °C in $2 \times$ SSC with 0.1% SDS (15 min each), twice with $0.5 \times$ SSC with 0.1% SDS (15 min each), and once with $0.1 \times$ SSC with 0.1% SDS (10 min), and then rinsed with malate buffer. The membranes were blocked in blocking solution (Boehringer Mannheim Co.) for 1 h and then in the presence of anti-digoxigenin-alkaline phosphatase (1:5000) for 30 min. The membranes were washed in malate buffer for 15 min three times. Chemiluminescent detection of digoxigenin-labeled probes was performed by applying CSPD (10 µg/ml; Tropix, Bedford, MA, USA) diluted in buffer 3 (100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 1 mM MgCl₂) and exposing the membranes to Hyperfilm ECL (Amersham Life Science, Elk Grove, IL, USA) for 5–30 min. The films and the corresponding ethidium bromide (EtBr)-stained gels were scanned using an ARCUS II scanner (AGFA). The images were processed for densitometric analysis using the NIH image program from Scion Corporation. The levels of mRNA estimated by densitometric analysis of Northern blots were normalized for the amounts of total RNA loaded on the gels by calculating the ratio of specific mRNA to the corresponding total RNA as assessed by EtBr staining. Similar or identical results were obtained when the levels of insulin, glut-2, glucokinase, or IGF-II mRNAs were normalized to levels of cyclophilin mRNA, assessed by hybridizations using a digoxigenin-labeled cDNA encoding bases 66–629 of rat cyclophilin (Freemark *et al.* 1995).

Luciferase reporter plasmid construct

A portion of the rat insulin-I promoter (kindly provided by G Bell) beginning at –383 bases and extending through the first untranslated exon, the first intron, and the first 11 bp of the second exon (–383 to +172) was generated by PCR and cloned into the luciferase reporter plasmid pGL3 (Promega, Madison, WI, USA). The resulting plasmid was sequenced to confirm its identity with the known insulin promoter.

Transient luciferase assays

Circular reporter plasmid was electroporated into INS-1 cells using a Bio-Rad Gene Pulser (Bio-Rad Laboratories, Richmond, CA, USA) set at 270 mV, 960 µF, and infinite resistance. The transfected cells were allowed to recover for 24 h in RPMI containing 10% FCS. The medium was then changed to 'basal medium' (0–30 mM glucose) with or without oPRL. After an additional 24-h incubation the cells were trypsinized, washed with phosphate-buffered saline (PBS), resuspended in luciferase buffer (100 mM sodium phosphate pH 7.8, 3 mM MgCl₂, 1 mM dithiothreitol), and lysed upon the addition of 1% NP-40 (Sigma). The lysate was clarified by centrifugation. Luciferase activity was determined by incubating the cell extract with luciferin in a buffer containing 50 mM

glycylglycine, 20 mM MgSO₄, 7 mM ATP, and 0.7 mg/ml bovine serum albumin (BSA). The light produced was measured using a luminometer. Aliquots of cell extracts were analyzed for protein content using the Bio-Rad protein assay (Bio-Rad Laboratories). Transfection efficiency was assessed by measurement of beta-galactosidase activity in cell lysates (Sambrook *et al.* 1989) following cotransfection of INS-1 cells with an RSV-beta-galactosidase (RSV-beta-GAL) plasmid (kindly provided by S Langdon, Duke University, USA). Unless specifically noted, all chemicals used in the luciferase assay were obtained from Sigma Chemical Co.

Glucose transport assay

INS-1 cells were washed with PBS and preincubated in 'basal medium' with or without oPRL (1 µg/ml) for 48 h. The cells were then washed twice and incubated for 30 min at 37 °C in glucose-free PBS containing 1% BSA. After two additional washes with PBS, the cells were incubated with 2-deoxy-D-[³H]glucose (1 µCi/ml; Amersham Life Science) for 1, 2, 4, or 16 min. 2-deoxy-D-[³H]glucose uptake was also measured in the presence of unlabeled 2-deoxyglucose (Sigma Chemical Co.), which was added in 10 000-fold excess in order to block specific uptake of the labeled 2-deoxyglucose. The uptake was terminated by washing the cells rapidly with ice-cold PBS containing 0.2 mM phloretin (Sigma Chemical Co.). Cells were solubilized in 0.1% SDS, and aliquots were taken for counting (Tai *et al.* 1990). The amount of protein in each sample was assessed by using the Bio-Rad protein assay (Bio-Rad Laboratories). Counts obtained in the presence of excess 2-deoxyglucose were subtracted from those obtained in its absence to quantify specific uptake of the radiolabeled glucose.

Statistics

Data are presented as the means $\pm$ s.e. Each experiment was repeated at least three times with duplicate or triplicate values within each group. Statistical differences between sample means were assessed by ANOVA, followed by a post-hoc Tukey's multiple comparison test. The level of significance was determined as $P < 0.05$. The levels of mRNA estimated by densitometric analysis of Northern blots were normalized for the amounts of total RNA loaded on the gels by calculating the ratio of specific mRNA to the corresponding total RNA as assessed by EtBr staining. The ratios were then used for statistical analysis.

Results

Our initial experiments were designed to examine the effects of PRL on the expression of preproinsulin, glut-2,

Figure 1 (A) Effect of PRL on preproinsulin mRNA levels in INS-1 cells incubated in glucose-containing medium. INS-1 cells were grown in medium containing 10% FCS and 11.1 mM glucose. The medium was then replaced with serum-free 'basal medium' containing 5.5 mM glucose, and the cells were incubated for 48 h in the presence or absence of PRL (1 µg/ml). The figure shows a representative Northern blot and EtBr-stained RNA of the corresponding samples. Similar results were obtained in four independent experiments. (B) Graphic representation of the changes in the levels of insulin mRNA over time, normalized for total RNA as assessed by EtBr staining of the gels.

and glucokinase mRNAs. INS-1 cells were grown to 80% confluence in medium containing 10% FCS and 11.1 mM glucose. In the presence of 10% FCS and 11.1 mM glucose, INS-1 cells appeared to be maximally stimulated and no effect of PRL on preproinsulin, glut-2, or glucokinase mRNAs could be discerned. Therefore, in subsequent experiments the incubations were performed in serum-free 'basal' (supplemented) medium containing 5.5 mM glucose. The cells were incubated for 48 h in the presence or absence of PRL (1 µg/ml). The levels of the

various mRNAs in PRL-treated cells were compared with mRNA levels in the control cells at various time-points. The levels of preproinsulin and glut-2 mRNAs declined progressively in control cells after replacement of serum-containing medium with supplemented serum-free medium (Figs 1 and 2). However, the levels of preproinsulin and glut-2 mRNAs remained stable in serum-free medium containing PRL, and at 48 h of incubation the level of preproinsulin mRNA was 1.7-fold higher (1.71 ± 0.12 , $P < 0.05$) in PRL-treated cells than in control

Figure 2 (A) Effect of PRL on glut-2 mRNA levels in INS-1 cells incubated in glucose-containing medium. INS-1 cells were grown in medium containing 10% FCS and 11.1 mM glucose. The medium was then replaced with serum-free 'basal medium' containing 5.5 mM glucose, and the cells were incubated for 48 h in the presence or absence of PRL (1 µg/ml). The figure shows a representative Northern blot and EtBr-stained RNA of the corresponding samples. Similar results were obtained in four independent experiments. (B) Graphic representation of the changes in the levels of glut-2 mRNA over time, normalized for total RNA as assessed by EtBr staining of the gels.

cells (Fig. 1). Similarly, the level of glut-2 mRNA was 2.0-fold higher (2.04 ± 0.56 , $P < 0.05$) in PRL-treated cells than in control cells at 48 h of incubation (Fig. 2). Prolactin had no significant effect on the levels of glucokinase or IGF-II mRNAs (Fig. 3).

To determine whether the induction of glut-2 mRNA was accompanied by an increase in the number of cellular glucose transporters, we preincubated INS-1 cells in 'basal medium' for 48 h with or without PRL. The cells were washed with PBS and incubated with 2-deoxy-D-[3 H]glucose for periods of time ranging from 1 to 16 min.

In both control and PRL-treated cells, the specific uptake of glucose increased between 1 and 16 min. However, the rate of glucose uptake in PRL-treated cells exceeded the rate of glucose uptake in control cells. At 1–4 min and at 16 min the uptake of 2-deoxy-D-[3 H]glucose in PRL-treated cells was 2.4- and 1.4-fold higher respectively than the uptake of 2-deoxy-D-[3 H]glucose in diluent-treated cells ($P < 0.001$ by ANOVA; Fig. 4).

To explore the role of glucose in PRL action, we examined the time-course of the effects of PRL on preproinsulin, glut-2, and glucokinase mRNAs in INS-1

Figure 3 The effects of PRL on glucokinase (A) and IGF-II (B) mRNA levels. INS-1 cells were incubated for 48 h in serum-free 'basal medium' in the presence or absence of PRL (P; 1 µg/ml). The figure shows representative Northern blots (C=control). Normalization for total RNA revealed that PRL had no statistically significant effects on either glucokinase or IGF-II mRNAs. Similar results were obtained in four independent experiments.

cells in the absence of exogenous glucose (Fig. 5). The levels of mRNA encoding preproinsulin declined progressively in control cells during a 24-h incubation in glucose-free medium. The levels of mRNA encoding glut-2 declined more precipitously in glucose-free medium, reaching a nadir after 8 h of incubation. In PRL-treated cells, on the other hand, the levels of preproinsulin mRNA increased progressively during the incubation in glucose-free medium and by 24 h were 3.4-fold higher (3.38 ± 0.24 , $P < 0.0001$) than those in control cells. PRL also stimulated an increase in glut-2 mRNA in cells incubated in glucose-free medium. The maximal effects of PRL on glut-2 mRNA preceded the effects of the hormone on insulin mRNA and were noted within 8 h of incubation (2-fold increase, 2.01 ± 0.09 , $P < 0.01$). The effects of PRL on glucokinase expression are shown in Fig. 5C. The figure shows apparent inhibition of glucokinase expression by PRL; however, in an additional three experiments, PRL had no significant effect on the expression of glucokinase mRNA in the absence of glucose.

Figure 4 The effect of PRL on 2-deoxy-D-[³H]glucose uptake in INS-1 cells. INS-1 cells were preincubated in 'basal medium' for 48 h with or without PRL. The cells were washed with PBS and incubated with 2-deoxy-D-[³H]glucose for 1 to 16 min. The data are presented as the means $\pm$ S.E. of triplicate values. The effects of PRL were statistically significant at each time-point ($P < 0.01$).

Figure 5 Time-course of the effects of PRL on preproinsulin (A), glut-2 (B), and glucokinase (C) mRNAs in cells incubated in glucose-free medium. The cells were incubated in serum-free, glucose-free DMEM with 1 mM sodium pyruvate for 2 h prior to, and for 24 h after, the addition of PRL or diluent. The figures are graphic representations of the changes in the levels of preproinsulin, glut-2, and glucokinase mRNAs over time, normalized for total RNA as assessed by EtBr staining of the gels. The levels of preproinsulin, glut-2, and glucokinase mRNAs were compared with the levels of corresponding mRNAs 4 h after the addition of PRL. In other experiments there were no differences in the levels of the various mRNAs during the initial 4 h of incubation in control or PRL-containing media.

These findings suggested that PRL has glucose-independent as well as glucose-dependent effects on the expression of insulin mRNA. Since the levels of preproinsulin mRNA in control cells declined significantly from baseline during incubations in both glucose-replete and glucose-depleted media, the effect of PRL could be mediated by stabilization of preproinsulin mRNA and/or by the induction of preproinsulin gene transcription.

To determine whether the PRL effect on insulin mRNA levels results from induction of insulin gene transcription, we examined the effects of PRL on the activity of the insulin promoter (Fig. 6). To that end, we transfected INS-1 cells with a plasmid containing the rat insulin 1 promoter linked to a luciferase reporter gene. After an overnight incubation in RPMI containing 10% FCS, the cells were incubated for an additional 24 h with PRL or diluent in serum-free basal medium containing varying concentrations of glucose (0–11 mM).

In the absence of glucose (0 mM glucose), PRL stimulated a 3.4-fold increase in cellular luciferase activity (3.41 ± 0.34 , $P < 0.01$). This finding indicates that PRL stimulates insulin gene transcription directly, in a glucose-independent manner. Glucose alone at 5.5 mM stimulated a 2.2-fold increase in cellular luciferase activity (2.23 ± 0.07 , $P < 0.001$). However, the combination of PRL plus 5.5 mM glucose stimulated a 7.9-fold increase in luciferase activity (7.86 ± 0.36 , $P < 0.0001$). Thus, the combined effect of glucose plus PRL on cellular luciferase activity (7.9-fold greater than that of cells incubated in glucose-free, PRL-free medium) was 41.1% greater than the sum of the effects of glucose alone and PRL alone (2.2- and 3.4-fold increase respectively). Similar results were obtained in experiments in which cells were incubated in media containing 11 mM glucose. At a concentration of 11 mM, glucose alone stimulated a 1.6-fold increase in luciferase activity (1.64 ± 0.11 , $P < 0.05$). The effect of 11 mM glucose plus PRL on cellular luciferase activity (7.5-fold greater than that of cells incubated in glucose-free, PRL-free medium, 7.52 ± 0.07 , $P < 0.0001$) was 48.9% greater than the sum of the effects of 11 mM glucose alone (1.6-fold increase) and PRL alone (3.4-fold increase). These observations indicate that PRL and glucose act synergistically to stimulate insulin gene transcription.

Discussion

The lactogenic hormones placental lactogen and PRL stimulate beta-cell proliferation (Brelje & Sorenson 1991, Brelje *et al.* 1993, 1994), insulin biosynthesis (Nielsen 1982, Markoff *et al.* 1990, Galsgaard *et al.* 1996), glucose-stimulated insulin secretion (Sorenson *et al.* 1987a, Brelje *et al.* 1993), and gap junctional coupling among beta cells (Sorenson *et al.* 1987a,b). The results of our studies suggest that the effects of PRL on insulin gene expression are

Figure 6 Effects of glucose and PRL on insulin promoter activity. All INS-1 cells were transfected at the same time under identical conditions with a plasmid containing the rat insulin 1 promoter linked to a luciferase reporter gene. All cells were cotransfected with an RSV-beta-GAL construct. After an overnight incubation in serum-containing medium, the cells were incubated for 24 h in serum-free medium containing varying concentrations of glucose and PRL. Luciferase activity was normalized for cellular protein levels. The beta-galactoside levels in the control and PRL-treated cells were comparable and statistically indistinguishable at the various glucose concentrations. The data represent the means $\pm$ S.E. of triplicate samples. Similar results were obtained in five additional experiments. The luciferase activities in the various treatment groups were compared with the luciferase activity in cells incubated in glucose-free, PRL-free medium. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$.

mediated through both glucose-dependent and glucose-independent mechanisms. A role for glucose in PRL action is suggested by four lines of evidence: first, PRL increases the levels of glut-2 mRNA and stimulates cellular glucose uptake in INS-1 cells in culture; secondly, in the presence of glucose, the time-course of induction of glut-2 mRNA by PRL parallels that of insulin mRNA; thirdly, the effects of PRL on insulin gene transcription are blunted when cells are incubated in the absence of glucose; and finally, the effects of PRL are similar to those of glucose itself, which induces expression of glut-2 and insulin in pancreatic islets (Docherty & Clark 1994, Philippe *et al.* 1994, the current study). These observations suggest that the effects of PRL on insulin gene transcription may be mediated in part by induction of glucose transport and/or glucose metabolism.

The rate-limiting step in glucose metabolism in pancreatic beta cells is the activity of the enzyme glucokinase (Grupe *et al.* 1995). Previous studies have demonstrated the induction by PRL of immunoreactive glucokinase in cytosolic extracts of pancreatic islets (Weinhaus *et al.* 1996). However, we detected no

significant effect of PRL on the levels of glucokinase mRNA in INS-1 cells. It is possible that the effects of PRL on glucokinase immunoreactivity may be exerted at a post-transcriptional level.

While glucose may mediate, in part, the effects of PRL on glut-2 and insulin production, it is clear that PRL also exerts glucose-independent effects on glut-2 and insulin gene expression. In the absence of glucose, PRL increased the levels of glut-2 mRNA and insulin mRNA. In addition, PRL activated the insulin promoter under glucose-free conditions, albeit to a lesser extent than in cells incubated in 5.5 mM glucose. These observations indicate that the mechanisms by which PRL exerts its effects in islet cells must differ in part from the mechanisms by which glucose exerts its insulinotropic effects.

Support for this hypothesis comes from studies of insulin gene expression in which we compared directly the effects of glucose and PRL alone or in combination. Glucose (5.5 mM) alone stimulated a 2.2-fold increase in insulin promoter activity, while PRL alone stimulated a 3.4-fold increase in promoter activity. However, glucose and PRL in combination acted synergistically to stimulate a 7.9-fold

increase in promoter activity relative to diluent-treated cells incubated in the absence of glucose or PRL. Likewise, the effect of 11 mM glucose plus PRL on insulin promoter activity (7.5-fold increase relative to glucose-free, PRL-free controls) was approximately 50% greater than the sum of the effects of 11 mM glucose alone (1.6-fold increase) and PRL alone (3.4-fold increase). The synergism of glucose and PRL in combination indicates that the nutrient and the hormone induce insulin gene transcription, at least in part, through distinct mechanisms.

The effect of PRL on insulin gene transcription may be mediated by activation of the JAK-STAT pathway (Sorenson *et al.* 1987b, Rui *et al.* 1994, Galsgaard *et al.* 1996, Pezet *et al.* 1997, Bole-Feysot *et al.* 1998). PRL stimulates nuclear translocation of Stat5 in INS-1 cells (Stout *et al.* 1997), and human growth hormone (hGH), which binds to prolactin receptors as well as GH receptors, induces the binding of an immunoreactive Stat5 protein to gamma-interferon activated-like sequences in the insulin promoter of RIN-5AH rat insulinoma cells (Galsgaard *et al.* 1996). PRL also activates a number of other cellular kinases including FYN and the 85 kDa subunit of the phosphatidylinositol (PI)-3' kinase. The roles of the Jak, Fyn and PI-3 kinases in the interactions between PRL and glucose are unclear. Both glucose and PRL stimulate Ca^{2+} uptake by insulin-secreting cells (Sekine *et al.* 1996, Crepaldi *et al.* 1997), which may provide one common mechanism by which PRL and glucose stimulate insulin gene expression.

Clinical observations support a role for lactogenic hormones in the regulation of insulin gene expression. In pregnancy, high concentrations of PRL and placental lactogen in maternal serum are accompanied by increases in beta-cell mass, insulin production and glucose-dependent insulin secretion (Hellman 1960, Green & Taylor 1972, Bone & Taylor 1976, Ogren & Talamantes 1988, Parsons *et al.* 1992, 1995). Moreover, female patients with hyperprolactinemia have exaggerated insulin secretory responses to arginine (Maccario *et al.* 1996). Although the insulin response to glucose alone is not different from that observed in normal women, the combination of glucose and arginine in hyperprolactinemic women has an additive effect on insulin secretion.

The effects of lactogens on insulin production may be amplified by positive feedback effects of insulin on the production of lactogens. Insulin stimulates the secretion of placental lactogen in human placental explants and rat trophoblast cells in culture (Bhaumick *et al.* 1987, Kishi *et al.* 1993). In addition, insulin acts at the level of the pituitary to induce PRL gene transcription (Jacob & Stanley 1995). Thus, lactogenic and pancreatic hormones may interact at multiple levels to regulate carbohydrate metabolism in pregnancy and in various pathologic states.

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