Diabetologia (1999) 42: 856–864

Diabetologia

Rapid communication

Glucagon-like peptide-1 promotes DNA synthesis, activates phosphatidylinositol 3-kinase and increases transcription factor pancreatic and duodenal homeobox gene 1 (PDX-1) DNA binding activity in beta (INS-1)-cells

J. Buteau[1], **R. Roduit**[1], **S. Susini**[2], **M. Prentki**[1]

[1] Molecular Nutrition Unit, Department of Nutrition, University of Montreal and the CR-CHUM, Institute of Cancer, Montreal, Quebec, Canada
[2] Foundation for Medical Research, University of Geneva, Geneva, Switzerland

Abstract

Aims/hypothesis. Glucagon-like peptide-1 is a potent glucoincretin hormone and a potentially important drug in the treatment of Type II (non-insulin-dependent) diabetes mellitus. We have investigated whether it acts as a growth factor in beta (INS-1)-cells and have studied the signalling pathways and transcription factors implicated in this process.
Methods. Cell proliferation was assessed by tritiated thymidine incorporation measurements. We have examined the action of glucagon-like peptide-1 on the enzymatic activity of phosphatidylinositol 3-kinase. The DNA binding activity of transcription factors was investigated by electrophoretic mobility shift assay. Measurements of mRNA were done using the northern technique.
Results. Glucagon-like peptide-1 caused an increase in tritiated thymidine incorporation in beta (INS-1)-cells and phosphatidylinositol 3-kinase activity in a dose-dependent manner non-additively with glucose. The phosphatidylinositol 3-kinase inhibitors wortmannin and LY294002 blocked the effects of glucagon-like peptide-1 on DNA synthesis. Transcription factor pancreatic and duodenal homebox gene 1 (PDX-1) DNA binding activity was increased by glucagon-like peptide-1 at 3 or 11 mmol/l glucose and the phosphatidylinositol 3-kinase inhibitor LY294002 suppressed the action of glucagon-like peptide-1 on PDX-1 DNA binding activity. Glucagon-like peptide-1 and glucose alone did not change activating protein-1 DNA binding activity. They synergised, however, to increase the activity of activating protein-1. Glucagon-like peptide-1 also increased the expression of PDX-1, glucose transporter 2, glucokinase and insulin mRNAs. Finally, glucagon-like peptide-1 increased the incorporation of tritiated thymidine in isolated rat islets.
Conclusion/interpretation. The results suggest that glucagon-like peptide-1 may act as a growth factor for the beta cell by a phosphatidylinositol 3-kinase mediated event. Glucagon-like peptide-1 could also regulate the expression of the insulin gene and genes encoding enzymes implicated in glucose transport and metabolism through the phosphatidylinositol 3-kinase/PDX-1 transduction signalling pathway. [Diabetologia (1999) 42: 856–864]

Keywords Glucagon-like peptide-1, glucose, phosphatidylinositol 3-kinase, PDX-1, AP-1, DNA synthesis, pancreatic beta-cell, insulin secretion, cell growth

Received: 22 February 1999 and in revised form: 12 April 1999

Corresponding author: M. Prentki PhD, CHUM, Pavillon de Sève, 4e, 1560 Sherbrooke Est, Montreal, PQ H2L 4M1, Canada

Abbreviations: GLP-1, Glucagon-like peptide-1; PI3K, phosphatidylinositol 3-kinase; PDX-1, pancreatic and duodenal homeobox gene-1; AP-1, activating protein-1; IEG, immediate-early response genes; PMSF, phenylmethyl-sulphonyl fluoride; GLUT, glucose transporter.

Glucagon-like peptide-1-(7–36)amide (GLP-1) is secreted by the enteroendocrine L-cells in response to fat meals and carbohydrates [1, 2]. It is a potent glucoincretin hormone which acts as a competence factor in determining the ability of the beta-cell to respond to glucose by secretion of insulin [3], K/ATP channels closure [4] and the induction of immediate early response genes (IEG) [5]. It is a potentially important drug in the treatment of diabetes particularly

because of its ability to improve insulin secretion both in subjects with impaired glucose tolerance and Type II (non-insulin dependent) diabetes mellitus [6]. It is also an insulinotropic agent through its ability to stimulate insulin gene expression and proinsulin biosynthesis [7]. It is not known whether it also influences beta-cell growth. A beta-cell growth promoting action of GLP-1 is an attractive possibility because various pharmacological agents that are known to raise islet cAMP stimulate beta-cell replication [8] and because GLP-1 activates the cAMP/protein kinase A transduction system [2]. In addition, our recent work indicates that GLP-1 synergize with glucose to induce a number of IEG known to be implicated in cell growth and differentiation, in particular *c-fos*, *c-jun* and *junB* [5].

Little is known about the signalling pathways that regulate beta-cell replication by glucose and various peptide hormones. Some studies have implicated the protein kinase A and C transduction systems but more recent work has led to the emerging concept that some insulin-activated signalling molecules, in particular IRS-2 [9, 10], play a crucial part in this process. An important downstream effector of IRS-2 is phosphatidylinositol 3-kinase (PI3K), a family of proteins known to be activated in response to various growth factors in different cell types [11]. Glucose activates PI3K in pancreatic beta-cells, an action that is possibly causally implicated in insulin gene induction by the sugar by phosphorylation of the transcription factor pancreatic and duodenal homeobox gene 1 (PDX-1) [12]. Mutations in the transactivating domain of PDX-1 have been shown to cause a monogenic early onset form of diabetes (MODY4) [13] and it has been hypothesised that defective PDX-1 function could have pleiotropic effects on beta-cell function in particular abnormal cell growth and insulin gene expression [14].

In view of the possible importance of GLP-1 as a therapeutic agent [6] and of the crucial role of changed beta-cell replication/apoptosis in the aetiology of diabetes [8, 15] we have investigated the action of the incretin on beta-cell signalling and DNA replication.

Materials and methods

Materials. We purchased LY294 002 and wortmannin from Biomol (Plymouth Meeting, Pa., USA). Human glucagon-like peptide-1 fragment 7–36 amide was obtained from Sigma (Geneva, Switzerland). Anti p85α PI3K antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, Calif., USA). The RPMI 1640 and the supplements including fetal calf serum were purchased from Gibco BRL (Burlington, Ontario, Canada). Methyl [^{3}H] thymidine was from ICN (Costa Mesa, Calif., USA).

Cell culture and incubation. The INS-1 cells (passages 36–70) were grown in monolayer cultures as described previously [16] in regular RPMI-1640 medium supplemented with 10 mmol/l HEPES, 10 % heat-inactivated FCS, 2 mmol/l L-glutamine, 1 mmol/l sodium pyruvate, 50 μmol/l β-mercaptoethanol, 100 iU/ml penicillin and100 μg/ml streptomycin at 37 °C in a humidified (5 % CO_2, 95 % air) atmosphere. When cells reached 80 % confluence after approximately 7 days they were washed with 11 mmol/l phosphate buffered saline (PBS) and preincubated at 37 °C for 90 min in a Krebs-Ringer bicarbonate medium (KRB) containing 1 mmol/l $CaCl_2$, 5 mmol/l $NaHCO_3$, 25 mmol/l HEPES (pH 7.4) supplemented with 3 mmol/l glucose and 0.1 % defated BSA (Sigma, Fraction V). Cells were then washed with PBS and incubated in the same supplemented KRB medium containing the substances to be tested. Subsequently, electrophoretic mobility shift assay and PI3K activity measurements were carried out as described below.

Thymidine incorporation assay. The INS-1 cells were seeded 2 days before use in 96-well plates (8×10^4 cells per well) and cultured in regular RPMI medium as described above. Cells were then washed with PBS and preincubated for 24 h in minimal RPMI medium, i.e. without serum and glucose but with 0.1 % BSA. They were then incubated for 24 h in minimal RPMI medium with various test substances. Proliferation was determined by incorporation of [^{3}H]thymidine (37 Bq/well) during the final 4 h of the 24-h incubation period. Cells were then harvested with a PHD cell harvester from Cambridge technology (Watertown, Mass., USA) and the radioactivity retained on the dried glass fibre filters was measured.

Rat islets were isolated from 200-g Wistar rats as described before [17]. Isolated rat islets (50 islets per condition) were seeded in 12-well plates and cultured in regular RPMI containing 11 mmol/l glucose and 10 % FCS for 24 h. Islets were then washed with PBS and preincubated for 24 h in minimal RPMI medium. They were then incubated for 24 h in minimal RPMI medium containing 3 mmol/l glucose with or without GLP-1 and growth hormone. Tritiated thymidine (185 Bq/well) was added for the final 6-h incubation period. Then, islets were lysed and DNA precipitated in 10 % trichloroacetic acid using a published procedure [18]. DNA was rendered soluble in 0.4 mol/l NaOH and radioactivity measured.

Preparation of nuclear extracts and electrophoretic mobility shift assays. Nuclear extracts were isolated using a published procedure [19]. Briefly, cells (40×10^6 per condition) previously grown in 225 cm^2 petri dishes were harvested using a rubber policeman, washed in cold PBS, sedimented at $3500 \times g$ for 4 min and lysed in 1 ml of ice-cold buffer A (15 mmol/l KCl, 2 mmol/l $MgCl_2$, 10 mmol/l HEPES, 0.1 % phenylmethylsulphonyl fluoride (PMSF) and 0.5 % Nonidet P-40). After incubation for 10 min on ice, nuclei were collected by centrifugation ($100 \times g$ for 5 min) and washed with buffer A without Nonidet P-40. Nuclei were lysed in 10 μl of a buffer containing 2 mmol/l KCl, 25 mmol/l HEPES, 0.1 % EDTA, and 1 mmol/l dithiothreitol. After incubation for 15 min on ice, 300 μl of a buffer containing 25 mmol/l HEPES, 1 mmol/l dithiothreitol, 0.1 % PMSF, 2 μg/ml aprotinin, 0.1 mmol/l EDTA and 11 % glycerol was added to the nuclei preparation. Samples were centrifuged ($16000 \times g$, 20 min) and the supernatants containing the nuclear proteins was used for protein determination, subsequently aliquoted (50 μl) and kept frozen at –70 °C for further analysis. The oligonucleotides used to assess the binding activities of the transcription factors PDX-1 and activating protein-1 (AP-1) by electrophoretic mobility shift assays were purchased from ACGT (Toronto, Ontario, Canada). Respectively, a 30-mer oligonucleotide containing a sequence of the A1 box of the rat insulin I promoter was used to assess PDX-

1 activity [20] and a 28-mer oligonucleotide containing a consensus AP-1 recognition sequence has been used to measure AP-1 activity [21]. After annealing, the double-stranded oligonucleotides were labelled with [γ32P]-ATP using the DNA 5′-end-labelling kit from Boehringer Mannheim (Indianapolis, Ind., USA). Electrophoretic mobility shift assays were done as described previously [22]. Briefly, 5 μg of protein extracts were incubated with a radiolabelled probe (20000 cpm per sample) for 20 min at room temperature in a buffer containing 25 mmol/l HEPES, 10% glycerol, 50 mmol/l NaCl, 0.05% Nonidet P-40, and 1 mmol/l dithiothreitol. A 50-fold molar excess of cold oligonucleotide was added with the labelled probe to assess the specificity of nuclear protein binding of the electrophoretic mobility shift assays. Samples were analysed on 4% non-denaturing polyacrylamide gels containing 0.01% Nonidet P-40.

Phosphatidylinositol 3-kinase (PI3K) activity measurements. The INS cells were grown in 75 cm^2 petri dishes. After incubation for 5 min in KRB with various test substances, PI3K was assayed according to reference [23]. In brief, PI3K was immunoprecipitated from 2–3 mg of INS-1 cell total protein extracts and resuspended in 50 μl of a buffer containing 20 mmol/l TRIS-HCl (pH 7.5), 100 mmol/l NaCl, and 100 mmol/l EGTA. After preincubation for 10 min with 10 μg of L-α-phosphatidylinositol, 370 Bq of [γ32P]-ATP were added with 10 mmol/l $MgCl_2$. Reactions were stopped after 4 min at room temperature with 0.15 ml of $CHCl_3$-MeOH-HCl (100:200:2). Lipids were extracted and spotted on silica gel plates for thin layer chromatography [6].

RNA isolation and northern blot analysis. Total RNA was isolated by the guanidine isothiocyanate/phenol/chloroform method from cells incubated for 3 h in serum-free RPMI medium at 3 or 11 mmol/l glucose in 75 cm^2 petri dishes. The RNA (15 μg) was denatured with glyoxal and dimethylsulphoxide and subjected to electrophoresis in 1.2% agarose gels. The RNA was transferred to a nylon membrane by capillarity and detected by northern blot hybridisation with ^{32}P-labelled cDNA probes obtained by random priming. The inserts used were: a BamHI-XbaI rat insulin fragment subcloned in pBSKS; a glucokinase EcoRI 1–2216 EcoRI fragment from rat in plasmid pBSKS; a glucose transporter (GLUT) 2 EcoRI 18–882 BamHI fragment from rat subcloned in pGEM-4 (Promega, Madison, Wis., USA) and a 1.4 Kb EcoRI-EcoRI PDX-1 fragment subcloned in pBJ5. A human α-actin PstI 1–720 PvuII fragment in plasmid pSP65 and methylene blue staining were used to confirm equal loading of RNA on each lane of the agarose gels.

Calculations and statistics. Data are presented as means ± SEM. Statistical analyses were done with the SPSS for Windows system. Differences between two conditions were assessed with Student's *t* test for related samples. Differences were deemed to be significant when *p* was less than 0.05.

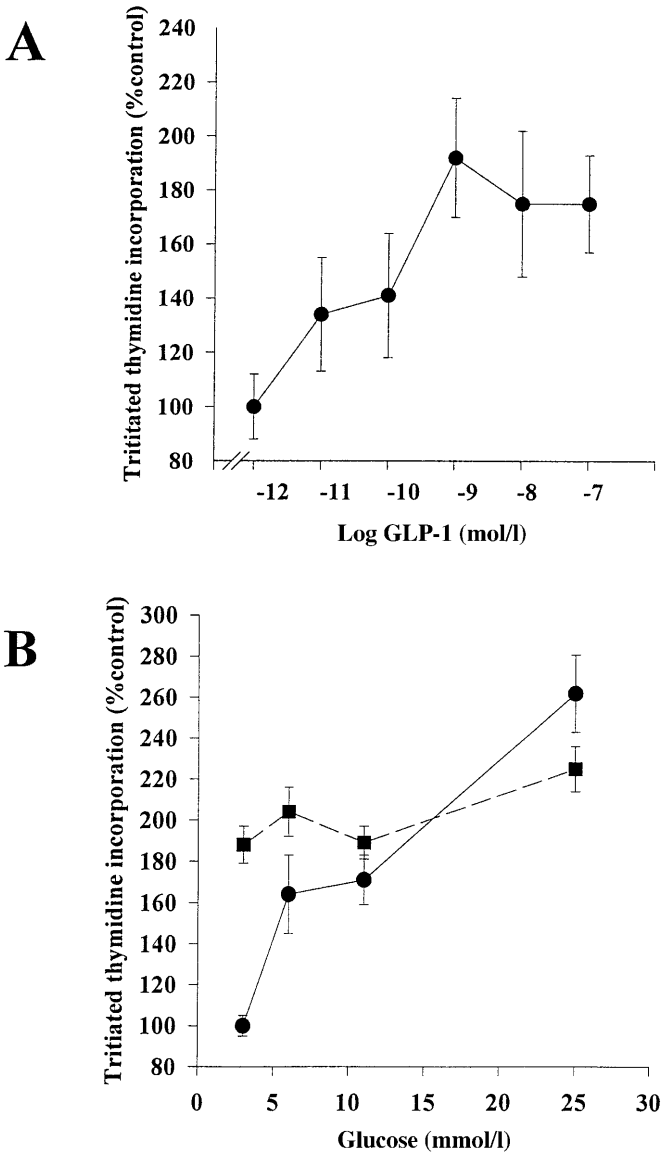

Fig. 1.A,B Effects of GLP-1 and glucose on DNA synthesis in INS-1 cells. DNA synthesis was determined by tritiated thymidine incorporation. **A** Dose-response characteristics for the effects of GLP-1 at 6 mmol/l glucose. **B** Dose-response characteristics for the effects of glucose in presence or absence of 10 nmol/l GLP-1. Cells were cultured for 24 h in serum-free RPMI medium containing 0.1% BSA and different concentrations of glucose in the presence or absence of GLP-1. 37 Bq/well of tritiated thymidine was added during the final 4 h of the 24 h incubation period. Data represent means ± SEM of four (**A**) or three (**B**) experiments (each comprising 3 to 4 wells). —■—, + GLP-1; —●—, –GLP-1

Results

Glucagon-like peptide-1 and glucose increase DNA synthesis in beta (INS-1)-cells in a non-additive manner. The effects of GLP-1 on beta-cell growth and DNA synthesis were initially examined in INS-1 cells cultured in a minimal RPMI medium (described in "Materials and methods") to avoid the effects of unknown factors present in the serum, particularly as serum is a potent growth factor for beta (INS-1)-cells [9].

Figure 1 shows the dose dependence of GLP-1 and glucose on beta (INS-1)-cell DNA synthesis. Figure 1A reports the effect of increasing GLP-1 concentration at a fixed concentration of glucose of 6 mmol/l. It is apparent that GLP-1 promotes tritiated thymi-

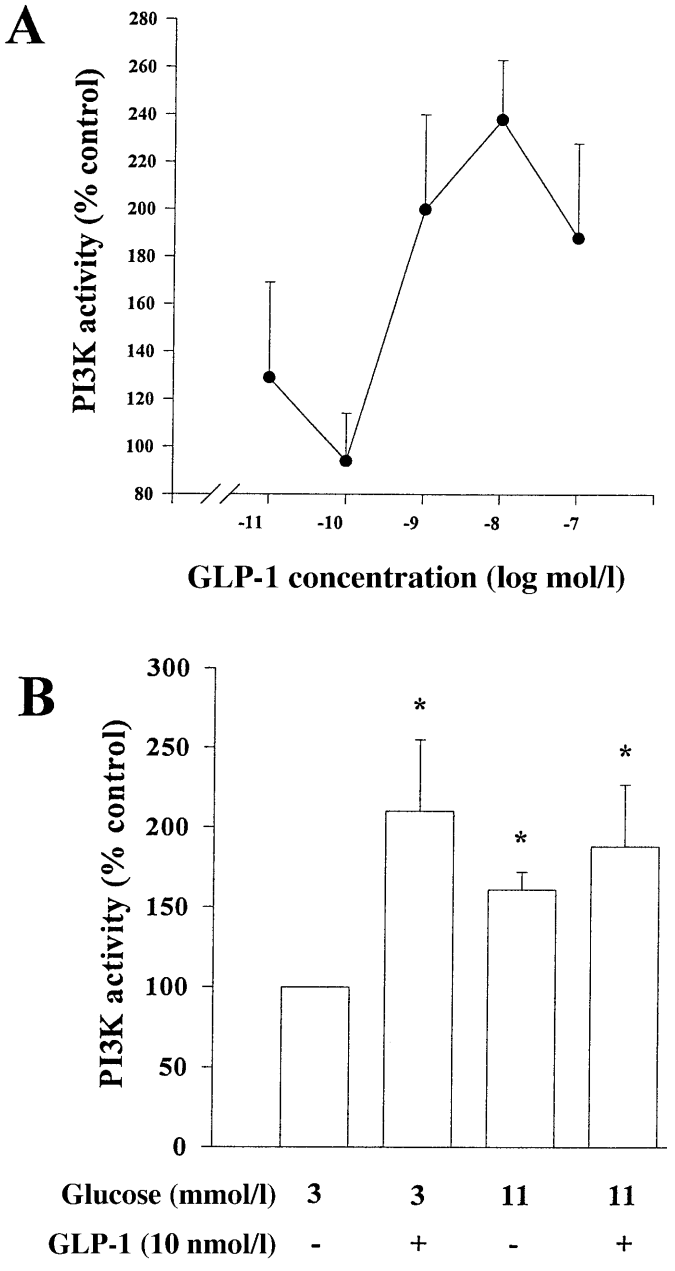

Fig. 2.A,B GLP-1 and glucose stimulate PI3K activity in INS-1 cells in a non-additive manner. **A** Cells were incubated in 6 mmol/l glucose KRB medium with different concentrations of GLP-1 for 5 min. PI3K was assayed as described in "Materials and methods". Data represent means ± SEM of three experiments. **B** Cells were incubated at different concentrations of glucose (3 mmol/l or 11 mmol/l) in the presence or absence of 10 nmol/l GLP-1. Proteins were extracted after 5 min to perform the assay. Means ± SEM of three experiments. $*p < 0.05$

dine incorporation in INS-1 cells in a dose-dependent manner. The threshold effective concentration is in the range 10^{-12} mol/l to 10^{-11} mol/l. The half maximum and maximum effective concentrations are respectively 10^{-10} and 10^{-9} mol/l. This underscores the extreme sensitivity of the action of GLP-1 on INS-1 cell proliferation. Figure 1B reports the dose dependence of the action of glucose ranging from 3 to 25 mmol/l in the absence and presence of GLP-1 at a maximally effective concentration of 10^{-8} mol/l. The results indicates that GLP-1 promotes maximum cell growth even at low (3 mmol/l) glucose and that the effects of glucose and GLP-1 are not additive, suggesting a possible common mechanism of the two agents (Fig. 1B). In subsequent experiments, we investigated the mode of action of GLP-1 at glucose concentrations ranging from 3 to 11 mmol/l.

Glucagon-like peptide-1 and glucose modulate PI3K activity in INS-1 cells. Phosphatidylinositol 3-kinase is an enzyme that has been implicated in many cellular processes including cell growth and cellular differentiation [11]. It is also thought to be involved in the mode of action of glucose on insulin gene expression [12]. We hypothesized that PI3K also participates in the mode of action of GLP-1 on beta-cell growth and wished to determine whether its activity in the presence of GLP-1 correlates with cell growth measurements. The activation of PI3K was studied after incubation for 5 min with increasing concentrations of GLP-1 (from 100 pmol/l to 100 nmol/l) at 6 mmol/l glucose (Fig. 2A). The results show that similar to cell growth a maximum activity is observed at 10^{-9} mol/l. Both GLP-1 and glucose (11 mmol/l) enhanced PI3K activity in a non-additive manner like they do for beta-cell growth (Fig. 1B).

We next investigated the action of PI3K inhibitors on the cell growth promoting action of GLP-1 and glucose to determine whether rises in DNA synthesis caused by GLP-1 and glucose involve the PI3K signal transduction pathway. Cells were incubated in 3 or 11 mmol/l glucose with or without 10 nmol/l GLP-1, in the presence or absence of the PI3K inhibitors (LY294003 and wortmannin). These agents blocked both GLP-1 and glucose-induced increases in DNA synthesis (Fig. 3). This provides pharmacological evidence of the implication of PI3K in the cell growth promoting action of the glucoincretin.

Much recent evidence has been obtained for a paracrine signalling pathway involving insulin in the beta-cell [24, 25]. To determine whether GLP-1 exerts its effect on PI3K by insulin, we investigated the action of 35 mmol/l KCl on the activity of the PI3K in beta (INS-1)-cells. Potassium chloride did not change PI3K activity even though it is well known to induce insulin secretion at this concentration (data not shown, $n = 4$). Furthermore, we measured PI3K activity in the presence of the large conductance (L-type) Ca^{2+} channel blocker nifedipine (100 nmol/l) to block insulin secretion. The effects of both glucose and GLP-1 singly or combined were preserved in the presence of the pharmacological agent (data not shown). These results suggest that the effects on PI3K and beta-cell proliferation mediated by GLP-1 and glucose are not due to a paracrine signalling pathway implicating insulin.

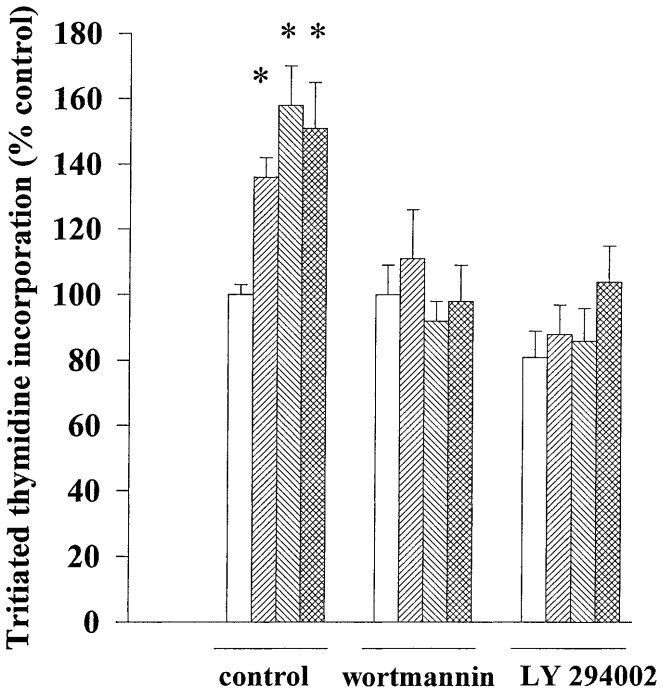

Fig. 3. LY 294002 and wortmannin inhibit the DNA synthesis in INS-1 cells which have been induced by GLP-1 and glucose. Cells were cultured for 24 h in serum-free RPMI medium as described in Fig. 1 in the presence or absence of the PI3K inhibitors LY-294002 (50 μmol/l) or wortmannin (50 nmol/l). During the last 4 h of the 24 h incubation period, 37 Bq of tritiated thymidine was added in each well. The data represent the mean values ± SEM obtained from four experiments (each comprising 4 wells). *$p < 0.005$. □ 3 mmol/l glucose; ▨ 3 mmol/l glucose + GLP; ▧ 11 mmol/l glucose; ▩ 11 mmol/l glucose + 10 nmol/l GLP

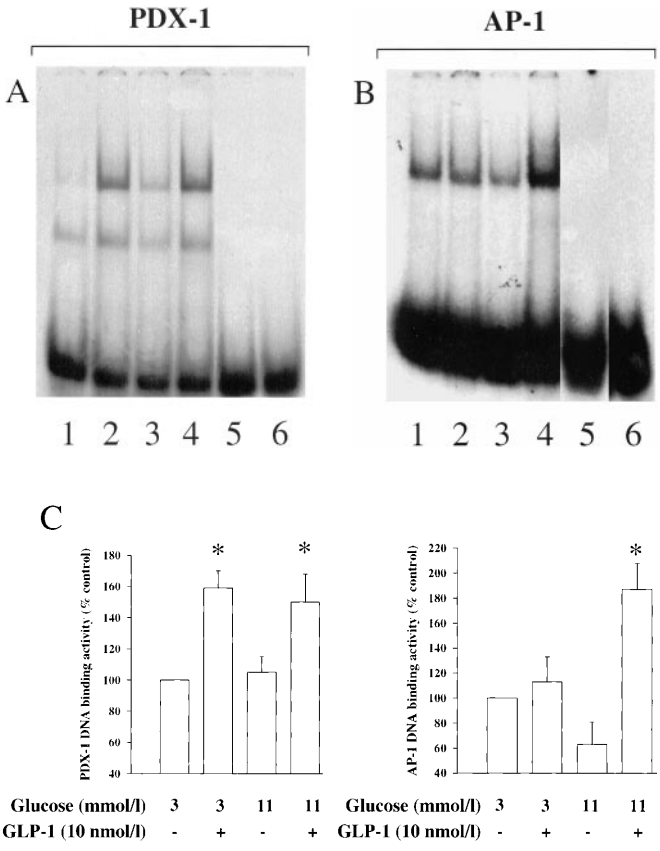

Fig. 4. Effects of GLP-1 and glucose on PDX-1 and AP-1 DNA binding activity. **A** Representative DNA retardation assay done with nuclear extracts of INS-1 cells cultured for 2 h at 3 or 11 mmol/l glucose in KRB medium in the presence or absence of 10 nmol/l GLP-1. A probe containing a consensus PDX-1 responsive element was used to assess PDX-1 DNA binding activity (top band) [20]. **B** The same experiment as in A was carried out using a probe containing an AP-1 binding site. Lane 1, 3 mmol/l glucose; lane 2, 3 mmol/l glucose and 10 nmol/l GLP-1; lane 3, 11 mmol/l glucose; lane 4, 11 mmol/l glucose and 10 nmol/l GLP-1; lane 5, no cell extract added; lane 6, replicate of lane 4 with a 100-fold excess of cold probe. **C** Quantification of the DNA retardation assays. Data represent the means ± SEM of five experiments for PDX-1 (left panel) and four experiments for AP-1 (right panel). *$p < 0.05$

Glucagon-like peptide-1 increases PDX-1 DNA binding activity and synergizes with glucose to induce AP-1 DNA binding activity. The transcription factor PDX-1 is beta-cell specific and important in regulating the development of the endocrine pancreas [14] as well as a number of beta-cell genes including insulin, glucokinase and the glucose transporter GLUT2 [26, 27]. We therefore wished to examine the possible action of GLP-1 on the activity of this transcription factor. We carried out DNA retardation assay using a double-stranded 30-mer oligonucleotide probe corresponding to a sequence within the human insulin gene enhancer region containing a PDX-1 binding site [20] (Fig. 4A). The quantification of five separate experiments is shown (Fig. 4C). It is apparent that GLP-1 promotes an increase in PDX-1 DNA binding activity at both 3 and 11 mmol/l glucose.

Previous work from our laboratory [5] indicated that both glucose (at the intermediate concentration of 11 mmol/l) and GLP-1 synergize to induce the expression of a number of immediate early response genes (IEG) and oncogenes like *c-fos*, *c-jun* and *junB* implicated in cell growth control and differentiation. We therefore aimed at determining whether the activity of the transcription factors AP-1 which consists of dimers of FOS, JUN and ATF proteins correlates with the induction of these IEG or cell growth measurements or both.

Figure 4B shows the DNA binding activity of AP-1 in a representative experiment as examined in a DNA retardation assay. Figure 4C shows the quantification of four different experiments. It is apparent that glucose at 11 mmol/l and GLP-1 alone did not statistically significantly modify AP-1 DNA binding activity. Glucose and GLP-1 synergise, however, to increase the DNA binding activity of AP-1. The data indicate that AP-1 DNA binding activity in the presence of GLP-1 does not correlate with either PI3K activity or cell growth measurements.

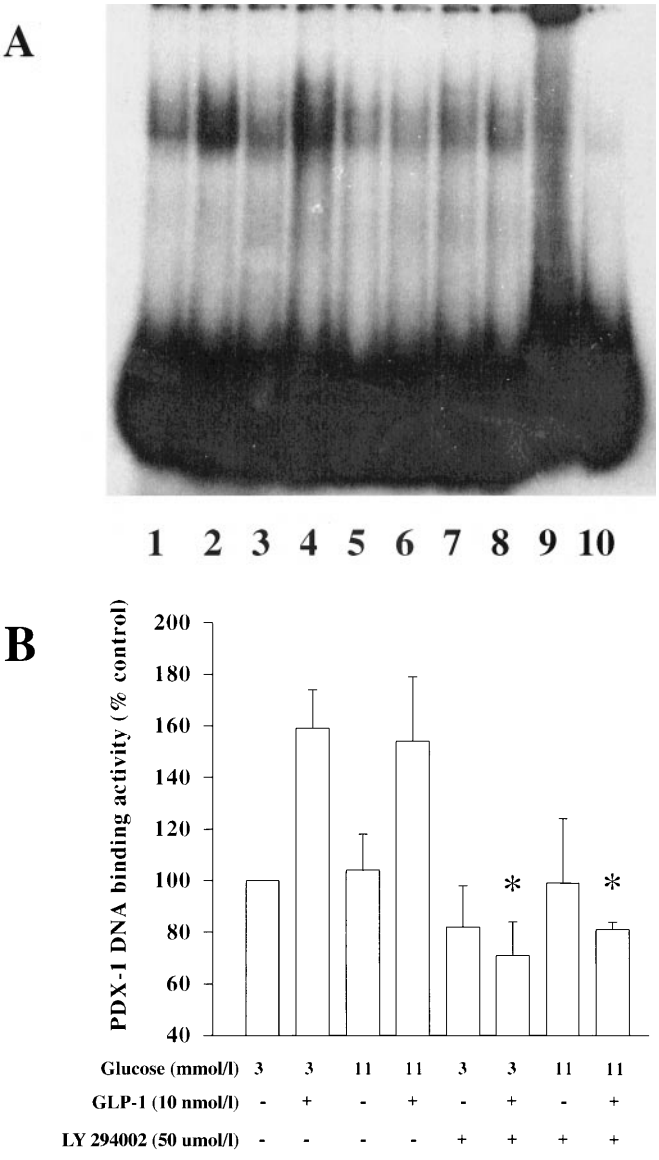

Fig. 5.A,B The PI3K inhibitor, LY 294002, inhibits GLP-1 modulation of PDX-1 DNA binding activity in INS-1 cells. **A** Representative DNA retardation assay done on nuclear extracts of INS-1 cells cultured as in Fig. 4 with or without 50 μmol/l LY-294002. Lane 1, 3 mmol/l glucose; lane 2, 3 mmol/l glucose and 10 nmol/l GLP-1; lane 3, 11 mmol/l glucose; lane 4, 11 mmol/l glucose and 10 nmol/l GLP-1; lanes 5–8, same as lanes 1–4 but in the presence of the inhibitor; lane 9, no cell extract added; lane 10, replicate of lane 4 with a 100-fold excess of cold probe. **B** Quantification of three different DNA retardation assays for PDX-1. Error bars indicate the SEM. $*p < 0.05$ compared with the control situation plus GLP-1 without the inhibitor

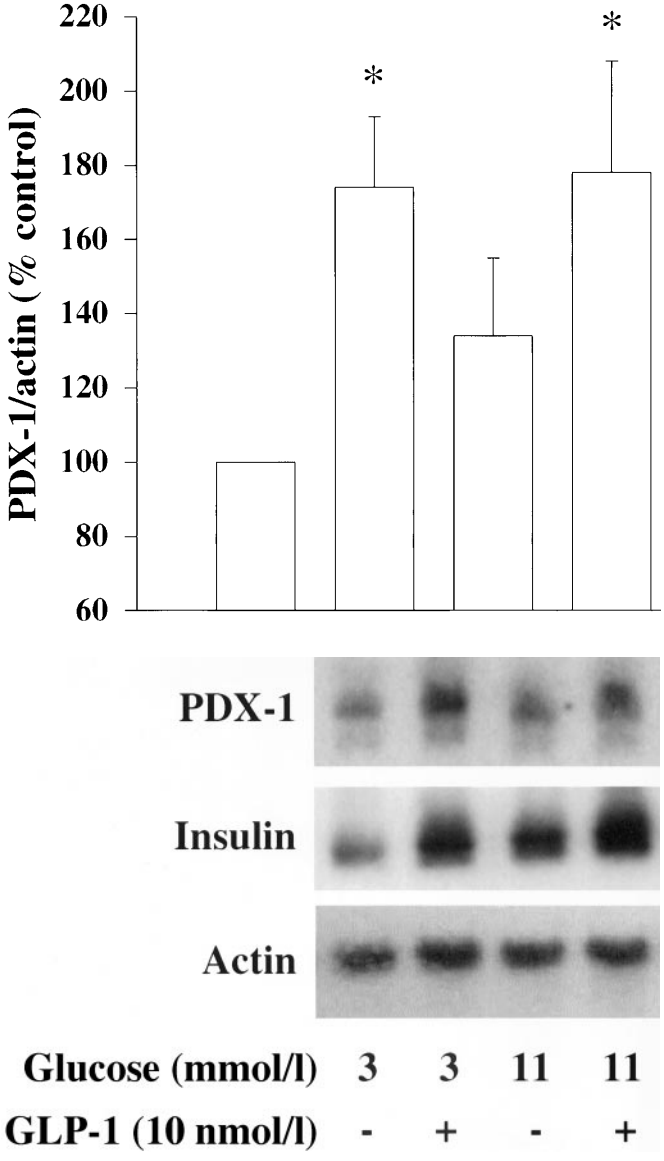

Fig. 6. Effect of GLP-1 on the expression of PDX-1 and insulin mRNAs. Representative results from northern blot. INS-1 cells were treated for 3 h in RPMI serum-free medium in the presence of glucose (3 mmol/l or 11 mmol/l) with or without 10 nmol/l GLP-1. Similar results were obtained in a second experiment for insulin. Means ± SEM of three experiments for PDX-1. $*p < 0.05$

To investigate whether GLP-1 modulation of PDX-1 DNA binding activity involves a PI3K mediated event, INS-1 cells were incubated in the presence or absence of the PI3K inhibitor LY294002 (Fig. 5). At the concentration used (50 μmol/l), LY2940002 is known to inhibit growth factor-stimulated PI3K in various cell types [28]. The results indicate that the PI3K inhibitor suppresses the activation of PDX-1 DNA binding activity at both 3 and 11 mmol/l glucose that is induced by GLP-1.

Glucagon-like peptide-1 increases PDX-1 and insulin mRNA expression in INS-1 cells. Figure 6 shows that GLP-1 causes an accumulation of the insulin and PDX-1 transcripts. This result correlates with the activity of the PDX-1 transcription factor as assayed by DNA retardation assay (Fig. 4A). As reported before in rat islets [29] GLP-1 also increases GLUT2 and glucokinase mRNA expression at 3 mmol/l glu-

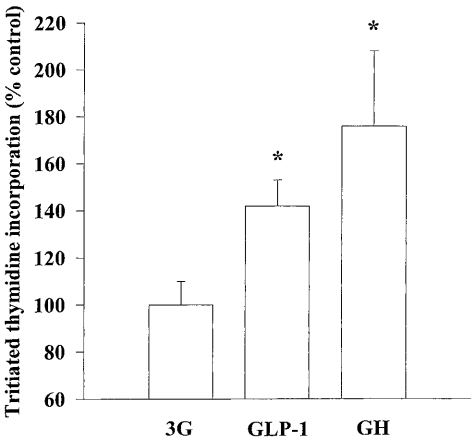

Fig. 7. Effects of GLP-1 and growth hormone on isolated rat pancreatic islets tritiated thymidine incorporation. Cells were incubated in 3 mmol/l glucose in the absence (3G) or in the presence of 10 nmol/l GLP-1 or 100 nmol/l growth hormone (GH) for 24 h. Then, 222 Bq/well of tritiated thymidine were added and cells further incubated for 6 h. Data represent the means ± SEM of 12 (3G, GLP-1) and six experiments (GH). *$p < 0.05$

cose by about twofold (data not shown, observed in four separate experiments).

Glucagon-like peptide-1 promotes tritiated thymidine incorporation in isolated rat islets. We next investigated whether the cell proliferation results obtained with the INS-1 cell line are applicable in a normal islet tissue. Figure 7 shows that GLP-1 promotes a 40% increase in tritiated thymidine incorporation into DNA. Growth hormone, which is a potent growth factor for the beta-cell [30], increased DNA synthesis by 80%.

Discussion

In this study, we provide evidence that GLP-1, a potent glucoincretin hormone and a potentially important drug in the treatment of diabetes [9, 10], acts as a growth factor in the beta (INS-1)-cell line as well as in isolated rat islet tissue. A beta-cell growth promoting action of GLP-1 in addition to its insulinotropic action reinforces the view of its usefulness as a therapeutic agent in the treatment of diabetes. Unlike secretion, however, the action of GLP-1 on DNA synthesis occurs at low glucose only, is not glucose dependent and does not increase with increasing glucose concentrations. This is different to the action of IGF-1 which is glucose dependent [31]. Thus, it can be speculated that GLP-1 is a growth factor under basal conditions and is important in early obesity where the beta-cell mass expands to compensate insulin resistance. In the diabetic state, GLP-1 might act as a growth factor when circulating glucose concentration has returned to close to its basal value after insulin or sulphonylurea treatment.

Phosphatidylinositol 3-kinase mediates nuclear activity by phosphorylation cascades leading to the activation of transcription factors [12, 32, 33]. It was found that glucose and GLP-1 alone do not statistically significantly modify AP-1 DNA binding activity. They synergise to induce AP-1 DNA binding activity, however, as they do for the induction of several IEGs [5]. This indicates that the activity of AP-1 is not essential to the proliferative action of GLP-1. Possibly, an increase in AP-1 activity is related to other functions which have been implicated for this transcription factor, such as differentiation and apoptosis [34]. To examine the possibility that another important candidate transcription factor (i.e. PDX-1) is implicated in the action of GLP-1 on DNA synthesis, we investigated PDX-1 DNA binding activity in the presence of GLP-1. It was found that GLP-1 causes an increase in PDX-1 DNA binding activity at 3 or 11 mmol/l glucose. Moreover, the PI3K inhibitor LY294002 suppressed the GLP-1 induced PDX-1 DNA binding activity and DNA synthesis, providing pharmacological evidence of the implication of PI3K in the GLP-1 induced PDX-1 response and DNA synthesis.

The cAMP analogue, chlorophenylthio-cAMP (0.5 mmol/l), was able to induce both PI3K and PDX-1 DNA binding activity (data not shown, $n = 3$ for each). In addition, high KCl and the Ca^{2+}-channel blocker nifedipine did not change the PI3K activity. Since GLP-1 causes a rise in both cAMP and Ca^{2+} in the beta-cell [2, 35], these results indicate that activation of the cAMP transduction system only and not Ca^{2+} is likely to be implicated in the reported action of GLP-1 on PI3K, PDX-1 and beta cell proliferation. The activation of PI3K by GLP-1 by a mechanism not involving calcium or the insulin receptor is intriguing. It can be speculated that the trimeric G-protein activated by GLP-1 directly interacts with PI3K or that the cAMP/PKA transduction system changes the phosphorylation state of the kinase resulting in enhanced enzymatic activity. Alternatively, glycogen breakdown induced by GLP-1 might result in enhanced glycolysis indirectly causing PI3K activation. Thus glucose itself, through its metabolism, activates PI3K [12]. The latter possibility is compatible with the observation that the actions of GLP-1 and glucose on PI3K and INS cell proliferation are not additive.

Our work could also be related to observations on IRS-2 knocked-out mice [36]. It is apparent that mice with disruption of IRS-2 have reduced beta-cell mass and an attenuation of insulin release induced by glucose. Knowing that PI3K is a direct downstream target of IRS-1/2, it is likely, as suggested by our data, that a possible decreased PI3K activation by this signalling pathway in response to GLP-1 and other growth factors would result in a reduced beta-

cell growth, metabolic enzyme gene expression and insulin biosynthesis.

In conclusion, our results indicate that GLP-1 at physiological concentrations increases INS-1 cell DNA synthesis non-additively with glucose by a mechanism which appears to implicate PI3K activation. Glucagon-like peptide-1 increases the expression of PDX-1 mRNA and activates the transcription factor PDX-1 non-additively with glucose as assessed by its DNA binding activity. It is possible that an increase in PDX-1 DNA binding activity promoted by GLP-1, involving a PI3K mediated event plays a part in the pleiotropic effects (DNA synthesis, metabolic enzyme gene expression, insulin biosynthesis) of the glucoincretin.

Acknowledgements. This work was supported by grants from the Medical Research Council of Canada, the Canadian Diabetes Association, the Cancer Research Society of Montreal, the Juvenile Diabetes Foundation (#197 047) and the Swiss National Science Foundation (#3200–050 879.97/1). R. Roduit is supported by a Swiss National Science Foundation Fellowship. M. Prentki is a Medical Research Council of Canada Scientist. We thank Dr. W. Schlegel and Dr. C. J. Rhodes for helpful discussions and critical reading of the manuscript.

References

1. Drucker DJ (1998) Glucagon-like peptides. Diabetes 47: 159–169
2. Widmann C, Burki E, Dolci W, Thorens B (1994) Signal transduction by the cloned glucagon-like peptide-1 receptor: comparison with signaling by the endogenous receptors of beta cell lines. Mol Pharmacol 45: 1029–1035
3. Fehmann HC, Goke R, Goke B (1995) Cell and molecular biology of the incretin hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide. Endocr Rev 16: 390–410
4. Holz GG, Kuhtreiber WM, Habener JF (1993) Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1 (7–37). Nature 361: 362–365
5. Susini S, Roche E, Prentki M, Schlegel W (1998) Glucose and glucoincretin peptides synergize to induce c-fos, c-jun, junB, zif-268, and nur-77 gene expression in pancreatic beta (INS-1) cells. FASEB J 12: 1173–1182
6. Gutniak M, Orskov C, Holst JJ, Ahren B, Efendic S (1992) Antidiabetogenic effect of glucagon-like peptide-1 (7–36) amide in normal subjects and patients with diabetes mellitus. N Engl J Med 326: 1316–1322
7. Fehmann HC, Habener JF (1992) Insulinotropic hormone glucagon-like peptide-I (7–37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma beta TC-1 cells. Endocrinology 130: 159–166
8. Sjoholm A (1996) Diabetes mellitus and impaired pancreatic beta-cell proliferation. J Intern Med 239: 211–220
9. Hugl SR, White MF, Rhodes CJ (1998) Insulin-like growth factor I (IGF-I)-stimulated pancreatic beta-cell growth is glucose-dependent. Synergistic activation of insulin receptor substrate-mediated signal transduction pathways by glucose and IGF-I in INS-1 cells. J Biol Chem 273: 17771–17779
10. Schuppin GT, Pons S, Hugl S et al. (1998) A specific increased expression of insulin receptor substrate 2 in pancreatic beta-cell lines is involved in mediating serum-stimulated beta-cell growth. Diabetes 47: 1074–1085
11. Vanhaesebroeck B, Leevers SJ, Panayotou G, Waterfield MD (1997) Phosphoinositide 3-kinases: a conserved family of signal transducers. Trends Biochem Sci 22: 267–272
12. Macfarlane WM, Smith SB, James RF et al. (1997) The p38/reactivating kinase mitogen-activated protein kinase cascade mediates the activation of the transcription factor insulin upstream factor 1 and insulin gene transcription by high glucose in pancreatic beta-cells. J Biol Chem 272: 20936–20944
13. Stoffers DA, Ferrer J, Clarke WL, Habener JF (1997) Early-onset type-II diabetes mellitus (MODY4) linked to IPF1. Nat Genet 17: 138–139 (Letter)
14. Oster A, Jensen J, Serup P et al. (1998) Rat endocrine pancreatic development in relation to two homeobox gene products (Pdx-1 and Nkx 6.1). J Histochem Cytochem 46: 707–715
15. Tanigawa K, Xu G, Nakamura S, Tamura K, Kawaguchi M (1996) Pancreatic B-cell replication in an animal model of insulin-resistant non-insulin-dependent diabetes mellitus. Pancreas 13: 326–327 (Letter)
16. Asfari M, Janjic D, Meda P et al. (1992) Establishment of 2-mercaptoethanol-dependent differentiated insulin-secreting cell lines. Endocrinology 130: 167–178
17. Gotoh M, Maki T, Satomi S et al. (1987) Reproducible high yield of rat islets by stationary in vitro digestion following pancreatic ductal or portal venous collagenase injection. Transplantation 43: 725–730
18. Strauss PR, Sheehan JM, Kashket ER (1977) Membrane transport by murine lymphocytes. II. The appearance of thymidine transport in cells from concanavalin A-stimulated mice. J Immunol 118: 1328–1334
19. Han JH, Beutler B, Huez G (1991) Complex regulation of tumor necrosis factor mRNA turnover in lipopolysaccharide-activated macrophages. Biochim Biophys Acta 1090: 22–28
20. Macfarlane WM, Read ML, Gilligan M, Bujalska I, Docherty K (1994) Glucose modulates the binding activity of the beta-cell transcription factor IUF1 in a phosphorylation-dependent manner. Biochem J 303: 625–631
21. Ausserer WA, Bourrat-Floeck B, Green CJ, Laderoute KR, Sutherland RM (1994) Regulation of c-jun expression during hypoxic and low-glucose stress. Mol Cell Biol 14: 5032–5042
22. Fried M, Crothers DM (1981) Equilibria and kinetics of lac repressor-operator interactions by polyacrylamide gel electrophoresis. Nucleic Acids Res 9: 6505–6525
23. Sun XJ, Rothenberg P, Kahn CR et al. (1991) Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. Nature 352: 73–77
24. Velloso LA, Carneiro EM, Crepaldi SC, Boschero AC, Saad MJ (1995) Glucose- and insulin-induced phosphorylation of the insulin receptor and its primary substrates IRS-1 and IRS-2 in rat pancreatic islets. FEBS Lett 377: 353–357
25. Xu GG, Rothenberg PL (1998) Insulin receptor signaling in the beta-cell influences insulin gene expression and insulin content: evidence for autocrine beta-cell regulation. Diabetes 47: 1243–1252
26. Watada H, Kajimoto Y, Miyagawa J et al. (1996) PDX-1 induces insulin and glucokinase gene expressions in alphaTC1 clone 6 cells in the presence of betacellulin. Diabetes 45: 1826–1831
27. Waeber G, Thompson N, Nicod P, Bonny C (1996) Transcriptional activation of the GLUT2 gene by the IPF-1/

STF-1/IDX-1 homeobox factor. Mol Endocrinol 10: 1327–1334
28. Vlahos CJ, Matter WF, Hui KY, Brown RF (1994) A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4- morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294 002). J Biol Chem 269: 5241–5248
29. Wang Y, Perfetti R, Greig NH et al. (1997) Glucagon-like peptide-1 can reverse the age-related decline in glucose tolerance in rats. J Clin Invest 99: 2883–2889
30. Bonner-Weir S, Smith FE (1994) Islet Cell Growth and the Growth Factors Involved. Trends Endocrinol Metab 5: 60–64
31. Tateishi K, Funakoshi A, Misumi Y, Matsuoka Y (1998) Jun and MAP kinases are activated by cholecystokinin in the pancreatic carcinoma cell line KP-1N. Pancreas 16: 499–504
32. Wang Y, Falasca M, Schlessinger J et al. (1998) Activation of the c-fos serum response element by phosphatidyl inositol 3-kinase and rho pathways in HeLa cells. Cell Growth Differ 9: 513–522
33. Montmayeur JP, Valius M, Vandenheede J, Kazlauskas A (1997) The platelet-derived growth factor beta receptor triggers multiple cytoplasmic signaling cascades that arrive at the nucleus as distinguishable inputs. J Biol Chem 272: 32670–32678
34. Karin M, Liu Z, Zandi E (1997) AP-1 function and regulation. Curr Opin Cell Biol 9: 240–246
35. Gromada J, Bokvist K, Ding WG et al. (1998) Glucagon-like peptide 1 (7–36) amide stimulates exocytosis in human pancreatic beta-cells by both proximal and distal regulatory steps in stimulus-secretion coupling. Diabetes 47: 57–65
36. Withers DJ, Gutierrez JS, Towery H et al. (1998) Disruption of IRS-2 causes type 2 diabetes in mice. Nature 391: 900–904