Specific Regulation of IRS-2 Expression by Glucose in Rat Primary Pancreatic Islet β-Cells*

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Insulin receptor substrate 2 (IRS-2) plays a critical role in pancreatic β-cells. Increased IRS-2 expression promotes β-cell growth and survival, whereas decreased IRS-2 levels lead to apoptosis. It was found that IRS-2 turnover in rat islet β-cells was rapid, with mRNA and protein half-lives of ~90 min and ~2 h, respectively. However, this was countered by specific glucose-regulated IRS-2 expression mediated at the transcriptional level. Glucose (≥2 mM) increased IRS-2 mRNA and protein levels in a dose-dependent manner, reaching a maximum 4-fold increase in IRS-2 mRNA and a 5–6-fold increase in IRS-2 protein levels at ≥12 mM glucose (p < 0.01). Glucose (15 mM) regulation of islet β-cell IRS-2 gene expression was rapid, with a significant increase in IRS-2 mRNA levels within 2 h that reached a maximum 4-fold increase by 4 h. IRS-2 protein expression in β-cells followed that of IRS-2 mRNA. Glucose metabolism was necessary for increased IRS-2 expression in β-cells. Moreover, inhibition of a glucose-induced rise in islet β-cell cytosolic [Ca2+]2, prevented an increase in IRS-2 expression, indicating this was Ca2+-dependent. The glucose-induced rise in IRS-2 levels correlated with increased IRS-2 tyrosine phosphorylation and downstream activation of protein kinase B. These data indicate that fluctuations of glucose in the normal physiological range (5–15 mM) promote β-cell survival via regulation of IRS-2 expression and a subsequent parallel protein kinase B activation. Given that the onset of type-2 diabetes is marked by loss of β-cells, these data further the idea that controlled IRS-2 expression in β-cells could be a therapeutic means to promote β-cell survival and delay the onset of the disease.

Pancrteatic β-cells are the sole source of insulin production in adults, and regulated insulin secretion is a primary control of mammalian glucose homeostasis. It is now widely acknowledged that the onset of type-2 diabetes is not only due to insulin secretory dysfunction (1, 2) but also significant loss of β-cell mass that no longer compensates for the metabolic load and/or acquired insulin resistance (3, 4). The loss of functional β-cell mass is common to both type-1 and -2 diabetes (3). As such, there is current interest in molecular mechanisms that promote β-cell survival as a potential therapeutic means to delay the onset of diabetes.

Members of the insulin receptor substrate (IRS) family are adaptor proteins that act as an interface for transmitting certain signal transduction pathways (5, 6). In β-cells, IRS-2 plays a critical role in promoting growth and especially survival (3, 7–11). In insulin target tissues, particularly the liver, IRS-2 also plays a pivotal role in insulin signal transduction pathways that mediate the anabolic and metabolic effects of insulin (8). As such, in IRS-2−/− mice, there is inherent insulin resistance that cannot be balanced by a compensatory increase in β-cell mass (12, 13). Indeed, without IRS-2 expression, β-cells undergo spontaneous apoptosis, markedly reducing the net β-cell mass so that frank diabetes ensues in IRS-2−/− mice (12). This is in marked contrast to IRS-1−/− mice, where the inherent insulin resistance is compensated by an increase in β-cell mass (13). This illustrates the crucial role that IRS-2 plays in maintaining adequate β-cell growth and survival. Indeed, genetically engineering an increase in IRS-2 expression specifically in β-cells is protective against β-cell apoptosis and can even prevent diabetes in vivo in experimental models (9, 10). Thus, there is potential that a controlled specific expression of IRS-2 in β-cells can promote β-cell survival, maintain adequate β-cell mass, and delay the onset of diabetes. In this regard, it has been shown that GLP-1 analogs regulate IRS-2 expression in β-cells, which in turn could be a major contributory mechanism as to how GLP-1 promotes β-cell growth and survival (14–16). However, it is quite likely that other factors, independent of GLP-1, control IRS-2 expression in β-cells and consequently promote β-cell growth and survival. In this regard, glucose and certain other nutrients have been shown to promote β-cell growth and survival (17–19). It should be noted, however, that this is pertinent to a relatively short term (<24 h) exposure limited to the physiologically relevant range of glucose concentrations (5–15 mM) (17). When β-cells are chronically exposed (>24 h) to higher glucose concentrations (>20 mM), apoptosis can be induced, a process often referred to as glucotoxicity (20, 21). In this study, in rat primary islet β-cells, we have shown that IRS-2 can be specifically regulated by physiological glucose concentrations (5–15 mM) relatively rapidly (>2 h) at the transcriptional level. This suggests a mechanism whereby the β-cell mass can effectively adapt to changes in the metabolic status via glucose-induced control of IRS-2 expression.

EXPERIMENTAL PROCEDURES

Materials—The IRS-2 antibody was provided by Dr. M. White (Children’s Hospital and Harvard Medical School, Boston, MA) or purchased

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4The abbreviations used are: IRS, insulin receptor substrate; PI3K (p85), phosphatidylinositol-3-kinase 85-kDa regulatory subunit; Erk-1/2, extracellular signal-regulated protein kinases 1 and 2; PKA, protein kinase-A; PKB, protein kinase B (also known as Akt); RT, reverse transcription; T1/2, half-life; BAPTA-AM, 1,2-bis(2-aminophenoxy)ethane-N,N',N' triacetonic tetras (acetoxymethyl ester).
from Upstate Biotechnology, Inc. (Lake Placid, NY). The antibodies to the p85 subunit of PI3′K and to β-catenin were also from Upstate Biotechnology, Inc., and the antibodies to total and phospho-Erk-1/2 were from Promega Corporation (Madison, WI). Phosphospecific antisera against phospho-Thr 

[32P]dCTP (3000 Ci/mmol) was obtained from Amersham Biosciences. Cell culture medium RPMI 1640, fetal calf serum, glutamine, and antibiotics were from Invitrogen. All other biochemicals were purchased from Sigma or Fisher.

Animals—Wistar rats (150–200 g) were obtained from Charles River Laboratories (Wilmington, MA). All animal care, use, and experiment protocols were submitted and approved by the Institutional Animal and Use Committee (IACUC) of Pacific Northwest Research Institute.

Pancreatic Islet Isolation—Pancreatic islets were isolated from normal male Wistar rats by collagenase digestion and Histopaque density gradient centrifugation as previously described (22). After isolation, the islets were handpicked using a stereomicroscope and incubated for 16 h in RPMI 1640 without serum but containing 3 mM glucose, 0.1% fatty acid-free bovine serum albumin, and 100 units/ml penicillin/100 mg/ml streptomycin.

Cell Culture—The pancreatic β-cell line INS-1 (23) was maintained in the complete medium RPMI 1640 (11 mM glucose) containing 10% (v/v) fetal calf serum, 50 μM ω-mercaptoethanol, 10 mM HEPES, 2 mM glutamine, 1 mM sodium pyruvate, 100 units/ml penicillin, and 100 mg/ml streptomycin and incubated at 37 °C, in 5% CO2 as defined previously (17, 18, 24). Prior to experimental use, the INS-1 cells were incubated in RPMI 1640 without serum but containing 3 mM glucose, 0.1% fatty acid-free bovine serum albumin, and 100 units/ml penicillin/100 mg/ml streptomycin for 16 h.

Real-time Fluorescence-based Reverse Transcription (RT)-PCR—Total RNA was extracted from 120 isolated rat islets as described previously (25, 26). One-step reverse RT-PCR was carried out using the Taqman™ PCR core reagent kit from Applied Biosystems and an ABI Prism 7700 sequence detector equipped with a thermocycler (Taqman Technology) and a cooled charge-coupled device camera to detect fluorescence emission over a range of wavelengths (500–650 nm), as described previously (27). Briefly, reverse transcription was first performed using specific oligonucleotides and the Multiscribe™ reverse transcriptase at 48 °C for 30 min. Samples were then PCR-amplified using GoldTaq™ polymerase and oligonucleotide primers for IRS-2, insulin, and β-actin (control for RNA quantity) as described below for 40 cycles under the following conditions: denaturation at 95 °C for 15 s, annealing, and extension at 60 °C for 1 min. Comparative analysis was based upon the cycle number at which a significant increase in the amplification signal above the baseline line was detected. Standard curves were constructed for each experiment with R values > 0.98. Primer and probe sequences (5′–3′) were as follows: IRS-2 probe, 6FAM-TGCT-CACAATTTCCACGCGCACAAT-TAMRA; IRS-2 forward primer, CCCAGGTGGCCCATCCTC; IRS-2 reverse primer, TTTCCTGAGGAGACGCTTTTCCA. Insulin probe, 6FAM-AGCTTCACACCA-GTGAGACACACGAGACGTTTTCCA. Insulin forward primer, GCCCAGCTTTGGTCAAA; Insulin reverse primer, CTTCCTGAGGAGACGCTTTTCCA. Insulin probe, 6FAM-AGCTTCACACCA-GTGAGACACACGAGACGTTTTCCA. Insulin forward primer, GCCCAGCTTTGGTCAAA; Insulin reverse primer, CTTCCTGAGGAGACGCTTTTCCA. β-actin probe, TGGTTGTTAATGCGGTGTTCA.

Northern Blot Analysis—Total RNA was isolated from ~4 × 106 INS-1 cells using an RNeasy mini-kit (Qiagen, Valencia, CA). Between 5 and 10 μg of total RNA was loaded onto a formaldehyde gel (1% agarose) and subjected to electrophoresis. The RNA was transferred to a nylon membrane (Amersham Biosciences) and hybridized to the IRS-2 or β-actin probe. The random primers labeling system purchased from Invitrogen was utilized to generate the following [32P]dCTP probes: a 0.4-kb NotI-HindIII fragment of mouse IRS-2 cDNA subcloned into pShuttleCMV and a 0.6-kb EcoRI fragment of β-actin cDNA subcloned into topo-pCR2.1. Hybridization of probes was conducted at 65 °C for 2–4 h. Membranes were exposed for autoradiography at ~70 °C using film (Amersham Biosciences). IRS-2 mRNA (7 kb) and β-actin mRNA (1.1 kb) were visualized in 1–7 days. IRS-2 mRNA was quantitated with Optiquest image software (n ≥ 3).

Immunoblot Analysis—Either 100 isolated rat islets or 1 × 106 INS-1 cells per observation were incubated in RPMI 1640 medium without serum at various glucose concentrations (3–15 mM) for times between 0.5 and 24 h at 37 °C as indicated. Thereafter, the islets or cells were washed in phosphate-buffered saline and placed in a lysis buffer containing, 50 mM HEPES, pH 7.5, 1% Triton X-100, 2 mM sodium vanadate, 50 mM sodium fluoride, 10 mM sodium pyrophosphate, 4 mM EDTA, 10 μM leupeptin, 10 μg/ml aprotinin, and 100 μM phenylmethylsulfonyl fluoride. Cell lysates were generated by sonication (25 watts for 10 s) on ice and centrifuged at 10,000 × g for 10 min to remove insoluble material and then normalized for equivalent total protein content. Immunoblot analysis on cell lysates was then performed as previously described (18, 24, 28).

Co-immunoprecipitation Analysis—INS-1 cells (grown on 20-cm plates) were preincubated at basal 3 mM glucose and then at a stimulatory 15 mM glucose for between 0 and 8 h. The cells were then lysed in lysis buffer and centrifuged at 10,000 × g for 10 min. The supernatant was collected and protein content between samples normalized to contain 5 mg of total protein/ml. The samples were then subjected to immunoprecipitation with an antibody against IRS-2 and immune complexes resolved by SDS-PAGE as described previously (10, 11, 29). The extent of IRS-2 tyrosine phosphorylation and association of PI3′K(p85) was determined by subsequent immunoblot analyses as described previously (10, 11, 29).

Immunofluorescent Analysis—INS-1 cells were subcultured on glass coverslips within 6-well plates to 60–70% confluence and, after incubation at basal 3 mM glucose or stimulatory 15 mM glucose as indicated, were washed with ice-cold phosphate-buffered saline, fixed to coverslips with 4% paraformaldehyde/phosphate-buffered saline, rehydrated, and then rewarmed with phosphate-buffered saline. The immunofluorescence analysis was conducted as previously described (29).

Other Procedures—Protein assay was by the bicinchoninic acid method (Pierce). Where appropriate, data are presented as means ± S.E. Statistically significant differences between groups were analyzed using one-way analysis of variance, where p < 0.05 was considered a statistically significant difference.

RESULTS

IRS-2 mRNA and Protein Have a Rapid Turnover in Rat Pancreatic Islet β-Cells, Independent of the Glucose Concentration—The half-life (T1/2) of IRS-2 mRNA was determined in isolated rat islets and INS-1 cells. Essentially, islets or INS-1 cells were incubated in the presence of the transcriptional blocker actinomycin-D (1 μg/ml) at either basal 3 mM or stimulatory 15 mM glucose for up to 8 h at 37 °C, and then IRS-2, β-actin, and preproinsulin mRNA levels were assessed at different time points by quantitative RT-PCR (in isolated islet samples because of the limited amount of tissue available) (Fig. 1A) or Northern blot analysis (in INS-1 cells) (Fig. 1B) as described under “Experimental Procedures.” The IRS-2 mRNA levels decayed much more rapidly in islets (Fig. 1A) and INS-1 cells (Fig. 1B) relative to that for β-actin and preproinsulin.
The estimated T_{1/2} value for IRS-2 mRNA in islets at basal 3 mM glucose was 85.6 ± 7.8 min (n = 6) and at stimulatory 15 mM glucose 92.1 ± 7.3 min (n = 6). Similar T_{1/2} values for IRS-2 mRNA were found in INS-1 cells of 82.5 ± 10.1 min (n = 3) at 3 mM glucose and 84.9 ± 9.6 min (n = 4) at 15 mM glucose. Comparable T_{1/2} IRS-2 mRNA values in INS-1 cells and isolated rat islets indicate that these estimations were pertinent to primary pancreatic β-cells. There appeared to be no significant effect of glucose on the stability of IRS-2 mRNA in β-cells under...
these conditions (Figs. 1). Because there was ≥50% residual β-actin and preproinsulin mRNA after 8 h in islet β-cells in the presence of actinomycin-D (Fig. 1), the T1/2 values of these mRNAs in β-cells could not be accurately estimated other than to state that they were >8 h. There was no obvious effect of glucose on β-actin mRNA stability (Fig. 1), but preproinsulin mRNA tended to stabilize at stimulatory 15 mM glucose compared with that at basal 3 mM glucose (Fig. 1A) in agreement with previous observations in β-cell lines (30).

The T1/2 values of IRS-2 protein were also evaluated in rat islets and INS-1 cells incubated at basal 3 mM or stimulatory 15 mM glucose in the presence of either the transcriptional inhibitor actinomycin-D (1 μg/ml; Fig. 2, A and B) or the translational inhibitor cycloheximide (10 μg/ml; Fig. 2, C and D) over an 8-h incubation period at 37 °C. IRS-2 protein levels were assessed by immunoblot analysis at different time points. As for IRS-2 mRNA (Fig. 1), IRS-2 protein levels decayed relatively rapidly in islet β-cells compared with that for IRS-1, PI3′K(p85), PKB, or Erk-1/2 (Fig. 2, A and C). The estimated T1/2 for IRS-2 protein in the presence of actinomycin-D in islets at basal 3 mM glucose was 132.4 ± 12.8 min (n = 6) and at stimulatory 15 mM glucose 122.8 ± 11.1 min (n = 6) (Fig. 2B). Similar T1/2 values for IRS-2 protein in rat islets were observed in the presence of cyclohexamide 137.9 ± 11.6 min (n = 5) at 3 mM basal glucose and 128.3 ± 10.2 min (n = 5) at stimulatory 15 mM glucose (Fig. 2, C and D). As for IRS-2 mRNA, there appeared to be no significant effect of glucose on the stability of IRS-2 protein in rat islets. In comparison to other rat islet β-cell signaling proteins, IRS-2 protein was relatively unstable, with IRS-1, PI3′K(p85), PKB, and Erk-1/2 having protein half-lives in excess of 8 h (Fig. 2). Similar results for IRS-2 protein T1/2 was found in INS-1 cells (data not shown), indicating that the T1/2 estimations for IRS-2 protein were pertinent to primary β-cells of rat islets.

Glucose Specifically Increased IRS-2 Expression Levels in Rat Pancreatic Islet β-Cells—Isolated rat islets or INS-1 cells were also incubated at stimulatory 15 mM glucose over a time course between 0 and 24 h. At 1 h, there was a significant increase in IRS-2 mRNA expression in rat islets compared with “time 0” (p < 0.05) (Fig. 4A) that, by 4 h, had reached a maximal 3.7 ± 0.5-fold (n = 5; p ≤ 0.05) increase, which was mostly sustained up to 24 h. Perhaps not surprisingly, the time course of glucose-induced IRS-2 mRNA expression lagged behind that of IRS-2 mRNA levels (Fig. 4, B versus A). Significant glucose-induced increases in IRS-2 protein levels in rat islets were found at 2 h, reaching a maximal 5.3 ± 0.6-fold (n = 6; p ≤ 0.05) increase by 8 h, which was relatively sustained up to 24 h (Fig. 4B). A similar rapid increase in glucose-induced IRS-2 mRNA expression (Fig. 4C) and protein levels (Fig. 4D) were observed in INS-1 cells, although this tended to tail off at 24 h. Nonetheless, this reaffirmed that the rapid glucose-induced increase in IRS-2 expression occurred in the primary β-cells of rat islets.

Glucose-induced Control of IRS-2 Expression in Rat Islet β-Cells Was Predominately Mediated at the Transcriptional Level—The data presented so far indicate that IRS-2 mRNA and protein levels are regulated by glucose in parallel (Figs. 3 and 4). Moreover, there appears to be no effect of glucose on IRS-2 mRNA or protein stability (Figs. 1 and 2). In using titration of certain inhibitors of protein synthesis translation, no effect of glucose-induced translational control of IRS-2 expression in rat islets could be observed (data not shown). However, the inhibitor of gene transcription, actinomycin-D (1 μg/ml), completely prevented the glucose-induced increase in IRS-2 mRNA (p ≤ 0.01; Fig. 5A) and IRS-2 protein (p ≤ 0.01; Fig. 5B) expression levels in isolated rat islets over a 6-h incubation period. Collectively, these data indicate that glucose regulation of IRS-2 expression, over the relative short term (≤8 h) and physiological glucose concentration range (3–15 mM), in primary islet β-cells was predominately mediated at the transcriptional level.

Glucose-induced Control of IRS-2 Expression in Rat Islet β-Cells Required Glucose Metabolism and Was Not Mediated by Secreted Insulin—A stimulatory concentration of the non-metabolizable analog of glucose, 2-deoxyglucose (15 mM), unlike glucose itself, was unable to
promote a significant increase in islet IRS-2 mRNA or protein levels (Fig. 5, C and D, respectively). This was indicative that glucose metabolism was necessary for glucose-induced IRS-2 expression in islet β-cells. It was possible that the glucose-induced increase of β-cell IRS-2 expression was mediated by a positive feedback of secreted insulin on the β-cell (31). However, although glucose-stimulated insulin secretion was blocked ≥90% by the addition of somatostatin (data not shown) (25), a significant glucose-induced increase in IRS-2 mRNA and protein
Glucose Regulation of IRS-2 Expression in β-Cells

Glucose-regulated IRS-2 Expression in β-Cells Leads to Specific Increased IRS-2 Signaling via PKB Activation—Not only did glucose regulate IRS-2 expression levels in pancreatic β-cells but also IRS-2 intracellular localization (Fig. 7A). In quiescent INS-1 cells, IRS-2 was mostly localized in a cytosolic compartment and remained there when incubated for 8 h at basal 3 mM glucose (Fig. 7A). However, in INS-1 cells incubated at stimulatory 15 mM glucose for ≥4 h, IRS-2 was mostly localized to the β-cell plasma membrane, co-localizing with a β-cell plasma membrane marker, β-catenin (Fig. 7A). Co-immunoprecipitation analysis of INS-1 cells incubated at stimulatory 15 mM glucose for ≥4 h indicated that the increase in IRS-2 expression also resulted in a parallel increase in tyrosine phosphorylation of IRS-2 and subsequent association of the p85 regulatory subunit of PI3’K with IRS-2 (Fig. 7B). The tyrosine phosphorylation state of IRS-2 does not appreciably change over an 8-h incubation at basal 3 mM glucose (data not shown). Immunoblot analysis of the total amount of PI3’K(p85) in the INS-1 cell lysates used for co-immunoprecipitation analysis indicated that it remained constant, implying that the PI3’K(p85) IRS-2 association was specific and relative to the IRS-2 tyrosine phosphorylation state (Fig. 7B) (7, 8). In the same INS-1 cells, immunoblot analysis showed that the glucose-induced increase in IRS-2 expression also paralleled a phosphorylation activation of PKB at Thr308 and Ser473 of PKB without change in glucose-induced IRS-2 expression also paralleled a phosphorylation activation of Erk-1/2 that is independent of IRS-2 signaling (28, 33), whereas glucose-induced phosphorylation activation of Erk-1/2 did not correlate with glucose-stimulated IRS-2 expression in isolated rat islets (Fig. 7D), indicating that this occurred in the primary β-cells. However, in contrast, glucose-induced phosphorylation activation of Erk-1/2 did not correlate with glucose-stimulated IRS-2 expression in isolated rat islets following an earlier time course (Fig. 7D). These data also complement previous observations of an acute glucose-induced activation of Erk-1/2 that is independent of IRS-2 signaling (28, 33), whereas glucose-induced activation of PKB in β-cells takes much longer, having a lag period of ~2 h (11, 24, 28). This reafirms, in concept, that PI3’K/PKB signaling in β-cells is dependent on upstream activation of IRS-2 (11, 24, 28).

DISCUSSION

IRS-2 plays an important role in pancreatic β-cell growth and survival (3, 8). In this study, it was found that IRS-2 mRNA and protein are turning over at a relatively high rate compared with that of other signaling proteins, such as IRS-1, PI3’K(p85), Erk-1/2, and PKB, in rat primary islet β-cells. The rate of IRS-2 mRNA decay (T1/2 ~ 90 min) was slightly faster than that of IRS-2 protein (T1/2 ~ 2 h). Similar effects of relative IRS-2 protein instability in β-cells were found whether or not IRS-2 expression was inhibited at the transcriptional or translational levels.

expression was apparent (p ≤ 0.05) (Fig. 5, E and F, respectively). Thus, glucose-stimulated IRS-2 expression in islet β-cells was not mediated via secreted insulin.

Glucose-regulated IRS-2 Expression in Rat Islet β-Cells Is Ca2+-dependent—Investigation as to which particular secondary signals might evoke an increase in IRS-2 expression in islet β-cells downstream of glucose metabolism was conducted. Some effects of glucose on islet β-cells are mediated via a transient increase in [cAMP], and protein kinase A (PKA) activation (28, 32). However, the PKA inhibitors H-89 (10 μM) and KT5720 (1 μM) only decreased a glucose-induced increase in IRS-2 mRNA expression in rat islets by 25.0 ± 3.6 and 22.8 ± 3.1%, respectively (n = 5), which was not statistically significant compared with the controls (Fig. 6A). Likewise, H-89 (10 μM) and KT5720 (1 μM) only lessened the glucose-induced increase in rat islet IRS-2 protein expression by 30.1 ± 3.3 and 24.2 ± 2.1%, respectively (n = 6), and this was not statistically significant compared with the controls (Fig. 6B). In contrast, prevention of a glucose-induced increase in islet β-cell [Ca2+]i by excluding Ca2+ from the extracellular medium or the addition of the intracellular Ca2+ chelator BAPTA-AM (25 μM) significantly inhibited glucose-induced IRS-2 mRNA expression in rat islets by 96.4 ± 2.1 and 98.2 ± 3.5%, respectively (p ≤ 0.05; n = 5) (Fig. 6A). At the protein level, similar prevention of a glucose-induced increase in [Ca2+]i significantly inhibited islet IRS-2 protein expression at basal 3 mM glucose by 51.7 ± 3.2% (n = 12) as well as negated the glucose-induced increase in IRS-2 protein (p ≤ 0.05; Fig. 6B). These data indicate that the control of glucose-induced IRS-2 expression in islet β-cells is Ca2+-dependent and possibly partly mediated (~25%) via PKA.
Neither IRS-2 mRNA or protein stability was appreciably regulated by acute (≤8 h) exposure to glucose. However, it should be noted that a more chronic (≥24 h) exposure to elevated glucose levels (≥15 mM) can accelerate IRS-2 protein degradation via mammalian target of rapamycin-mediated Ser/Thr phosphorylation of IRS-2 and subsequent ubiquitination, which decreases IRS-2 expression levels, rendering $\beta$-cells more susceptible to apoptosis (20). For the moment, the mechanism behind the relative instability of IRS-2 mRNA and protein in $\beta$-cells under normal circumstances has yet to be determined. Nonetheless, this study indicates that there is a balance between IRS-2 production and disposal in $\beta$-cells, which if swayed toward IRS-2 degradation, could be detrimental to the $\beta$-cell population (12, 13, 20). This underlines the importance of maintaining IRS-2 expression in $\beta$-cells.

In this regard, it was found that variation in glucose within the physiologically relevant levels (3–15 mM), specifically regulated IRS-2 expression levels in rat primary islet $\beta$-cells in a dose-dependent manner. This was a relatively rapid effect with a maximal glucose-induced increase in IRS-2 mRNA and protein levels reached by 4 and 6 h, respectively. The glucose-induced increase in $\beta$-cell IRS-2 mRNA levels preceded that of IRS-2 protein, perhaps not surprisingly considering that the former is the template of the latter. This effect of glucose on rat islet $\beta$-cell IRS-2 expression appeared to be relatively specific, with no similar glucose effect seen on $\beta$-actin, PI3K(p85), Erk-1/2, or PKB expression levels. As previously mentioned, there appeared to be no effect of glucose on IRS-2 mRNA or protein stability in islet $\beta$-cells. Neither did there appear to be any regulatory effect of glucose on $\beta$-cell IRS-2 protein expression mediated at the translational level. Indeed, IRS-2 mRNA levels tended to parallel those of IRS-2 protein, although a 1–2-h lag of glucose-induced IRS-2 mRNA preceded that of IRS-2 protein. Moreover, the transcriptional inhibitor actinomycin-D prevented both glucose-induced increases in IRS-2 mRNA and protein levels. Collectively, these data indicate that glucose-induced expression of IRS-2 in pancreatic $\beta$-cells is predominately mediated at the transcriptional level.

Several genes are specifically up-regulated by glucose in the $\beta$-cell (34, 35), but prominent among these is the proinsulin gene (25, 36–39). In comparison to IRS-2 gene expression, glucose regulation of the proinsulin gene might appear to be relatively modest (only a 2-fold

**FIGURE 7.** Glucose induces translocation of IRS-2 to the plasma membrane in INS-1 cells and delayed PKB phosphorylation activation in rat pancreatic islets in parallel to increased IRS-2 expression. A, INS-1 cells were incubated at basal 3 mM or stimulatory 15 mM glucose for 0, 2, or 8 h as indicated and then immunofluorescence analysis conducted to examine the cellular localization of IRS-2 as described under “Experimental Procedures.” $\beta$-Catenin was used as a plasma membrane marker. Representative images are shown of one of three independent experiments. B, INS-1 cells were preincubated for 16 h at basal 3 mM glucose and then at a stimulatory 15 mM glucose for 0–8 h as indicated. IRS-2 was then immunoprecipitated (IP) from these cells and the immunoprecipitates subjected to immunoblot (IB) analysis for phosphotyrosine (Tyr-PO4) and PI3K(p85) as described under “Experimental Procedures.” Immunoblot analysis for total PI3K(p85) in an aliquot of each lysate used for immunoprecipitation was used as a loading control. C, immunoblot analysis for PKB phosphorylation activation of an aliquot of the same INS-1 cell lysates used for IRS-2 co-immunoprecipitation analysis shown in B. D, isolated rat pancreatic islets were preincubated at basal 3 mM glucose for 2 h and then for 0–12 h at stimulatory 15 mM glucose. IRS-2 protein levels and phosphorylation activation of PKB and Erk-1/2 were assessed by immunoblot analysis as described under “Experimental Procedures.” A representative immunoblot analysis is shown from one of at least four independent experiments.
increase of preproinsulin mRNA levels in $\geq 18$ h (25)). However, one must also consider that preproinsulin mRNA is much more stable in β-cells than that of IRS-2, and unlike IRS-2 mRNA, preproinsulin mRNA stability is also regulated by glucose in addition to that at the transcriptional level (30, 36). Notwithstanding, it is possible that glucose regulation of IRS-2 and preproinsulin gene expression in β-cells may share some mechanistic commonalities. In this regard, the observation that glucose-induced control of IRS-2 expression in primary β-cells is dependent on glucose metabolism and requires a downstream increase in cytosolic [Ca$^{2+}$], is similar to the mechanism of glucose-induced control of preproinsulin gene expression (37). The means by which increased glucose metabolism leads to an increase in cytosolic [Ca$^{2+}$] in islet β-cells is well established and is key to the glucose regulation of insulin secretion (40–42). This in turn raises the question as to whether glucose-induced increase in IRS-2 expression in β-cells is mediated by a positive feedback effect of secreted insulin on the β-cell, as some have suggested for glucose regulation of preproinsulin gene expression (31), although this remains controversial (25, 43). However, under circumstances where glucose-induced insulin secretion was inhibited $\sim 90\%$ by somatostatin, there was no significant effect on glucose-induced IRS-2 expression in the rat islet β-cells (Fig. 5). As such, it appears unlikely that glucose regulation of IRS-2 expression in β-cells is mediated by secreted insulin.

Another possible secondary signal for mediating glucose-induced IRS-2 expression is an increase in [cAMP], and subsequent activation of PKA (28, 32, 40). It has been previously shown that glucose can transiently elevate [cAMP], in primary islet β-cells leading to PKA activation in a Ca$^{2+}$-dependent manner (28, 32), most likely via Ca$^{2+}$/calmodulin-dependent activation of type 1 and 8 adenylate cyclases (32). However, inhibition of PKA in rat islets only inhibited glucose-induced IRS-2 expression by $\leq 25\%$ (Fig. 6) and was not statistically significant. Indeed, the majority of glucose-induced IRS-2 expression in β-cells was independent of cAMP/PKA regulation, despite being Ca$^{2+}$-dependent. Therefore, most of the glucose-induced IRS-2 expression is mediated via alternative pathways, likely via Ca$^{2+}$-dependent kinases and/or phosphoprotein phosphatases. However, it should be noted that cAMP/PKA signaling via phosphorylation activation of cAMP-responsive element-binding protein is involved in IRS-2 expression in β-cells when activated by incretins like GLP-1 (14). Considering that the action of GLP-1 on β-cells is glucose-dependent, essentially potentiating the effect of glucose (44), some mechanistic aspects of glucose-mediated signaling in β-cells will relate to GLP-1-mediated regulation of IRS-2 expression. In this regard, the regulation of TORC-1 (transducer of regulated AMP-responsive element-binding protein activity), via a phosphoprotein phosphatase-2B-mediated Ca$^{2+}$-dependent dephosphorylation, to enable cAMP-responsive element-binding protein import to the β-cell nucleus to undertake its transcriptional activity (45, 46) indicates a manner in which glucose and GLP-1 could work in concert to regulate IRS-2 expression. This may also be pertinent to the minor degree of the cAMP-dependent aspect of glucose-induced IRS-2 expression in β-cells. Nonetheless, the mechanism behind glucose-induced IRS-2 expression in β-cells requires further examination, particularly with a view to better defining the “glucose-regulatory cis-elements” in the IRS-2 promoter and transcription factors that bind to these. This may have some analogy to glucose regulation of the preproinsulin gene promoter in β-cells. However, it is unfortunate that glucose regulation of the preproinsulin gene promoter remains relatively undefined (36, 38, 47) and therefore analogies to glucose regulation of IRS-2 expression, at this moment, would be relatively unreliable.

IRS-2 has been shown to play a pivotal role in the maintenance of an optimal β-cell population (7, 8). Here, it is shown that physiological levels of glucose can up-regulate IRS-2 expression in islet β-cells under relatively normal conditions. This provides a mechanism whereby fluctuations in the normal range of glucose levels can promote β-cell well-being in a changing metabolic environment by adjusting IRS-2 expression levels. It has previously been shown that physiological concentrations of glucose promote β-cell survival (17, 18). Not only does glucose up-regulate IRS-2 expression in β-cells but also promotes its translocation to the β-cell plasma membrane where it can be tyrosine-phosphorylated by basal tyrosine kinase activities localized there (5, 7) (Fig. 7). This then leads to downstream activation of PI3′K and PKB, which are key factors for promoting β-cell survival (18, 48, 49). Glucose-induced activation of PKB has been shown to be relatively slow in β-cells, occurring after a lag period of $\sim 2$ h (11, 24). Complementary to this observation and that PI3′K and PKB activation in β-cells are dependent on IRS-2 expression (28, 49), we find that glucose-induced phosphorylation activation of PKB in islet β-cells parallels that of IRS-2 protein expression after an $\sim 2$-h lag. This is in contrast to glucose-induced activation of Erk-1/2, which is mediated via a Ca$^{2+}$/cAMP-dependent activation of PKA, independent of IRS-2 (28, 33).

Finally, it is now widely acknowledged that the onset of type-2 diabetes is marked by insulin secretory dysfunction and decreased β-cell mass, the latter caused by increased β-cell apoptosis. As such, a means of promoting β-cell survival has potential in delaying the onset of or perhaps even prevent type-2 diabetes (3). In this regard, the IRS-2/PI3′K/PKB branch of IRS-2 signaling pathways is a key factor in promoting β-cell survival (7, 48, 49). Increased IRS-2 expression can promote β-cell growth (11), survival (10), and in some model systems is protective against the onset of diabetes (9). In contrast, decreasing levels of IRS-2 expression leads to increased β-cell apoptosis and diabetes (12, 13). It has been speculated that activation of PKB in β-cells might also be a possible target in treating type-2 diabetes (48, 49). However, this is problematic because of the oncogenic potential of PKB (49–51), underlined by it being relatively stable in β-cells. In contrast to PKB, IRS-2 is rapidly turning over in β-cells, which would significantly lower the oncogenic risk. Thus, a therapeutic means of specifically increasing IRS-2 expression in β-cells, following the effects of glucose and/or GLP-1 (9, 14), holds a more viable strategy to preserving β-cell mass as a treatment for type-2 diabetes.

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