Glucose and Endoplasmic Reticulum Calcium Channels Regulate HIF-1β via Presenilin in Pancreatic β-Cells

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Pancreatic β-cell death is a critical event in type 1 diabetes, type 2 diabetes, and clinical islet transplantation. We have previously shown that prolonged block of ryanodine receptor (RyR)-gated release from intracellular Ca2+ stores activates calpain-10-dependent apoptosis in β-cells. In the present study, we further characterized intracellular Ca2+ channel expression and function in human islets and the MIN6 β-cell line. All three RyR isoforms were identified in human islets and MIN6 cells, and these endoplasmic reticulum channels were observed in close proximity to mitochondria. Blocking RyR channels, but not sarco/endoplasmic reticulum ATPase (SERCA) pumps, reduced the ATP/ADP ratio. Blocking Ca2+ flux through RyR or inositol trisphosphate receptor channels, but not SERCA pumps, increased the expression of hypoxia-inducible factor (HIF-1β). Moreover, inhibition of RyR or inositol trisphosphate receptor channels, but not SERCA pumps, increased the expression of presenilin-1. Both HIF-1β and presenilin-1 expression were also induced by low glucose. Overexpression of presenilin-1 increased HIF-1β, suggesting that HIF is downstream of presenilin. Our results provide the first evidence of a presenilin–HIF signaling network in β-cells. We demonstrate that this pathway is controlled by Ca2+ flux through intracellular channels, likely via changes in mitochondrial metabolism and ATP. These findings provide a mechanistic understanding of the signaling pathways activated when intracellular Ca2+ homeostasis and metabolic activity are suppressed in diabetestes and islet transplantation.

Inappropriate activation of pancreatic β-cell death pathways is involved in the pathogenesis of type 1 diabetes, type 2 diabetes, and several other forms of the disease (1–4). Increased programmed cell death also plays a critical role in the failure of clinical islet transplantation (5), an otherwise promising therapy for diabetes. We have recently shown that intracellular Ca2+ stores gated by the ryanodine receptor Ca2+ release channel (RyR)6 are essential for β-cell survival in vitro (6), but not for glucose-stimulated insulin release (7). Using islets from genetically engineered mice, we demonstrated that blocking RyR triggers a novel apoptosis pathway dependent on calpain-10 and resembling hypoglycemia-induced cell death (6). Despite the potential importance of this signaling network, other nodes in this pathway remain to be elucidated.

Multiple calcium-dependent modes of cell death have been described (6, 8–10), but the pathways activated after RyR blockade are incompletely understood. We have recently obtained evidence suggesting that apoptosis subsequent to RyR inhibition does not involve an endoplasmic reticulum (ER) stress response despite the localization of RyR in the ER. Excitotoxicity is unlikely to mediate ryanodine-induced apoptosis, as blocking RyR flux with a high dose of ryanodine suppressed, rather than enhanced, apoptosis caused by chronic hyperglycemia in β-cells (6). In neurons, blocking RyR with dantrolene also suppressed excitotoxicity and cell death caused by mutations in presenilin, a gene linked to familial Alzheimer disease (11, 12). On the other hand, because Ca2+ flux from the ER to the mitochondria in β-cells has been shown to contribute to the generation of ATP (13), chronic block of RyR might cause cell death by reducing metabolic activity.

Our previous experiments suggest that calpain-10 represents a common effector for hypoglycemia-induced β-cell death and ryanodine-induced apoptosis (6). Other calpains have also been implicated in the response to hypoglycemia and hypoxia (14, 15). In this regard, it is interesting that calpain-10 has recently been shown to localize to the mitochondria (16), where it would be strategically positioned to respond to changes in metabolic activity.

Changes in cellular metabolic activity induce the expression of hypoxia-inducible factors, transcription factors that can activate both pro-survival and pro-apoptotic programs. HIF-1β was reported to be the most significantly decreased gene in islets from type 2 diabetics, and mice lacking this gene had impaired glucose homeostasis (17, 18). Arntl2, a homologue of

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4 The abbreviations used are: RyR, ryanodine receptor; ER, endoplasmic reticulum; HIF, hypoxia-inducible factor; IP3-R, inositol trisphosphate receptor; SERCA, sarcoplasmic reticulum ATPase.
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HIF-1α, has been identified as a type 1 diabetes candidate gene in the Iddm6 locus of the NOD mouse (19). However, the signals that regulate hypoxia-inducible factors in β-cells are poorly characterized.

Another family of proteins that has been implicated in the response to reduced cellular metabolic activity is the presenilins. Presenilin mutations lead to familial Alzheimer disease (20). Presenilin is a critical component of the γ-secretase complex, which we have recently shown can regulate β-cell survival via the cleavage of Notch (21). Presenilins have also been implicated in intracellular Ca\(^{2+}\) homeostasis, apoptosis, and the response to hypoglycemia in neurons (12, 22, 23), but their functional role in pancreatic islet cells has not been studied in detail. Moreover, although the effects of mutant presenilins have been extensively researched, there are few studies on the endogenous regulators of these critical proteins. Interestingly, hypoxia has been shown to regulate the expression and the alternate splicing of presenilin-2 (24–26), possibly via binding sites in the presenilin-2 promoter specific for HIF-1 (25). Conversely, HIF-1α mRNA was identified as one of the most up-regulated targets of presenilin-1 by both microarray (27) and differential display (28). It is not known whether ryanodine-induced β-cell death or hypoglycemia (6) triggers this pathway.

In the present study, we sought to identify novel components of a β-cell survival gene network linked to RyR. We also sought to clarify the mechanisms associated with ryanodine-induced β-cell death and compare the effects of blocking RyR with inhibition of IP3R and SERCA. Analogies between ryanodine- and hypoxia-induced cell death caused us to focus our study on two key pathways that have been implicated in programmed cell death, namely the hypoxia-inducible pathway and the presenilins. We found that blocking RyR and/or IP3R, but not SERCA pumps, induced the expression of presenilins, as well as a hypoxia-inducible factor. We also determined that overexpression of presenilin-1 increased the levels of HIF-1α protein. Together these results provide the first evidence of a RyR-prensennilin-HIF genetic network controlling β-cell survival.

**EXPERIMENTAL PROCEDURES**

**Reagents and Cell Culture**—Ryanodine was purchased from Molecular Probes (Eugene, OR) or Tocris (Ellisville, MO) or Calbiochem (La Jolla, CA). Xestospongin C was purchased from AG Scientific (San Diego, CA). Thapsigargin was purchased from Calbiochem or Sigma. Carbonyl cyanide m-chlorophenyl-hydrazone (CCCP) was from Calbiochem. All drugs were kept as 1000stock solutions dissolved in Me2SO. Human islets were provided by Dr. Garth Warnock as part of the Centre for Human Islet Transplantation and Beta-cell Regeneration Core and cultured as described (29) in RPMI 1640 medium containing 5 mM glucose, 10% fetal bovine serum, penicillin/streptomycin (Invitrogen). Islets were kept in a 37 °C incubator with 5% CO2 and saturated humidity and were used within 3 days of their isolation from donors.

MIN6 cells were cultured in 5 or 25 mM glucose Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum and 2% penicillin and streptomycin (Invitrogen) as indicated. Cells were treated at a confluency of ~80%.

**Single Cell Imaging**—MIN6 cells were imaged through the ×100, 1.45 numerical aperture objective of a Zeiss 200M microscope. Images were deconvolved using Slidebook from Intelligent Imaging Innovations (Boulder, CO). For organelle studies, cells were transfected with fluorescent proteins targeted to ER using KDEL and calreticulin motifs or mitochondria using the pOCT sequence (gift from H. McBride, University of Ottawa). The ratiometric mitochondrial pericam was a gift from Tullio Pozzan, University of Padua. D1ER was a gift from Amy Palmer and Roger Tsien (University of California San Diego).

**Measurement of Apoptosis**—Apoptosis was measured in single dispersed human islet cells, cultured overnight as indicated, using the ApoPercentage kit (Biocolor). This kit uses a dye that stains cells as they undergo the membrane “flip-flop” event that mediates phosphatidylserine translocation to the outer leaflet (28). This event is considered diagnostic of apoptosis, but not necrosis. Apoptotic cells appeared bright pink against the white background of phenol red-free RPMI 1640 medium. Apoptosis in MIN6 cells was assayed using terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling (TUNEL) (Roche Applied Science) according to the manufacturer’s instructions. MIN6 cells were counterstained with hematoxylin.

**ATP/ADP Ratio Assay**—MIN6 cells were grown on tissue culture-treated 96-well luminometer assay plates and exposed to drugs as indicated. Following culture, cells were lysed and ATP/ADP ratios were measured using a commercial kit (Alexis, Axxora, San Diego, CA) according to the manufacturer’s instructions. A luminometer (Molecular Devices, Sunnyvale, CA) was used to read the plates.

**Immunoblot and Reverse Transcriptase PCR**—Western blots were performed as described (21). The following primary antibodies were used: monoclonal pan-RyR clone 34C (Affinity Bioreagents), rabbit polyclonal calnexin (Sigma), calpain-10 (Abcam), HIF-1β (BD Biosciences), presenilin-1 (Cell Signaling), presenilin-2 (Cell Signaling), β-actin (Novus).

Reverse transcriptase PCR was carried out as described (21) for 35 cycles. Primer sequences are available upon request.

**Gene Overexpression in MIN6 Cells**—A full-length presenilin-1 clone in a CMV promoter vector (pCMV6-XL5-PSEN1) was purchased from Origene (Rockville, MD). MIN6 cells were transfected with 1 μg of pCDNA3 or green fluorescent protein (as controls) or pCMV6-XL5-PSEN1 using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Cells were incubated for 48 h in a 37 °C incubator with 5% CO2 and were subsequently harvested and subjected to Western blot analysis as described above.

**RESULTS**

**Relationship between β-Cell ER and Mitochondria**—A close physical and functional relationship between the ER and mitochondria has been demonstrated in many cell types but has received less attention in β-cells. To assess the subcellular distribution of both organelles simultaneously, MIN6 cells were co-transfected with fluorescent proteins targeted to each organelle. ER was distributed widely throughout the cytoplasm, and mitochondria were typically found nearby (Fig. 1A). A sim-
ilar pattern was observed when mitochondria were labeled with a Ca\(^{2+}\)-sensitive probe, the ratiometric pericam. Apparent mitochondrial Ca\(^{2+}\) hotspots were observed near to ER (Fig. 1B). Together, these data suggest that ER and mitochondria have many sites of close proximity to each other in -cells and illustrate the physical possibility of ER-to-mitochondria communication in these cells.

In other cell types, ER calcium can be mobilized through both IP\(_3\)-R and RyR Ca\(^{2+}\) release channels. We employed the Ca\(^{2+}\)-sensitive fluorescent protein-based probe D1ER cameleon (30) to determine whether these channels are functional on β-cell ER. Indeed, both a stimulatory dose of ryanodine and the IP\(_3\)-generating agonist carbachol could release Ca\(^{2+}\) from the ER lumen (Fig. 1C). This result provided further functional evidence that both RyRs and IP\(_3\)-Rs can be present in MIN6 β-cells.

Identification of RyR Channel Isoforms in -Cells—The presence of RyR mRNA in pancreatic β-cells remains controversial (31). With the knowledge that RyRs can be subject to alternate splicing (32), we used PCR primers targeting multiple regions of the mRNA for each of the three RyR genes. These experiments demonstrated that all three RyR genes are expressed in human islets and MIN6 cells (Fig. 2, A and B). Exons 6 and 45 of the RyR2 were also detected using additional primers (not shown).
**β-Cell Metabolic Signaling**

**FIGURE 3. Blocking RyR induces programmed cell death in human islet cells and MIN6 cells.** A and B, phosphatidylserine translocation in apoptotic human islet cells treated with 10 μM ryanodine for 72 h. C, quantification of A and B (n = 3). Star, significant difference from control. p < 0.05. Error bars, ± S.E. D and E, increased numbers of TUNEL-positive MIN6 cells in cultures treated with 10 μM ryanodine for 48 h (n = 4). F, Western blot analysis of calpain-10 in human islets treated as indicated. The 25-kDa isoform of calpain-10 is shown (n = 3).

Overall, the expression of RyR2 appeared to be stronger and more consistent than that of RyR1 or RyR3. Together, these studies suggest RyRs are present in islet cells.

Next we employed pan-RyR antibodies to study the subcellular localization of these channels in MIN6 β-cells. Consistent with our previous studies in human β-cells (7), RyR immunoreactivity was not observed in insulin secretory granules but was found in endosomes (Fig. 2C). As expected, we also observed RyR staining in ER, especially in regions close to mitochondria (Fig. 2C). Together, these studies further point to a role for RyR in β-cell function.

**Blocking ER Ca**2+** Channels Induces Apoptosis—**We demonstrated in a previous study that chronically blocking RyR Ca**2+** release channels led to a calpain-10-dependent form of programmed cell death in mouse islets (6). In the present study, we sought to confirm and extend these findings using other assays and β-cell models. Treatment of dispersed human islet cells for 48 h with 10 μM ryanodine increased the percentage of cells with exposure of phosphatidylserine on the outer plasma membrane to ~50% from ~14% in control cells (Fig. 3, A–C). Previous studies in primary T lymphocytes have demonstrated that this event can occur in caspase-3-independent modes of apoptosis (33). We also confirmed that MIN6 cells underwent programmed cell death in response to 48-h treatment with ryanodine, as indicated by increased terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling (TUNEL) staining (Fig. 3, D and E).

Our previous investigation also pointed to increased calpain-10 mRNA in ryanodine-treated primary mouse islets (6). In the present study, we examined calpain-10 protein levels in human islets. We found that a blocking dose of ryanodine increased calpain-10 protein, predominantly a lower molecular weight splice variant preferentially expressed in islets (6, 34) (Fig. 3F). Similar results were observed in MIN6 cells (not shown). Blocking ER IP3R Ca**2+** release channels with 1 μM xestospongin C increased calpain-10 levels in human islets (Fig. 3F), but not MIN6 cells (not shown). Together, these results show that calpain-10 protein levels are increased when intracellular Ca**2+** release is blocked in human islets.

**Effects of Intracellular Ca**2+** Channels and Pumps on ATP/ADP Ratio—**Ca**2+** flux through intracellular release channels has been implicated in the control of mitochondrial function in many cell types, including β-cells (13). Our previous studies suggested the possibility that Ca**2+** flux from RyR-gated intracellular Ca**2+** stores was required to prevent a form of cell death that resembled hypoglycemia (6). To directly test the hypothesis that RyRs control β-cell metabolism, we examined the effects of Ca**2+** channel inhibitors on the ATP to ADP ratio using a luciferase-based assay in MIN6 β-cells. At both high and low glucose, 24-h treatment with a blocking dose of ryanodine significantly decreased the ATP to ADP ratio (Fig. 4). Thapsigargin increased the ATP to ADP ratio, suggesting the possibility that ATP was being spared from hydrolysis in the absence of SERCA activity. The mitochondrial uncoupler carbonyl cyanide m-chlorophenyl-hydrazone was used as a positive control, and its effect likely marks the lower limit of the ATP to ADP ratio. Thus, blocking Ca**2+** flux via RyR reduced the ATP to
ADP ratio by approximately half of what was seen with carbonyl cyanide m-chlorophenyl-hydrazone. In the low glucose condition, the ATP to ADP ratio was increased by opening RyR with a stimulatory low dose of ryanodine (6, 7) (Fig. 5). Activating RyR did not induce an additional increase in the ATP to ADP ratio in the presence of high glucose. Together, these studies indicate that normal Ca\textsuperscript{2+} flux through RyR is critical for the control of cellular metabolism in β-cells.

**Role of Specific ER Ca\textsuperscript{2+} Channels in Hypoxia-inducible Factor Expression**—The observation that intracellular Ca\textsuperscript{2+} release channels played a role in β-cell energy metabolism prompted us to examine the expression of hypoxia-inducible factors, which are also activated by non-hypoxic stresses, including hypoglycemia, in other cell types. HIF-1β was recently shown to be the most significantly altered mRNA in islets from type 2 diabetic patients and was necessary for β-cell function in mice (17). In human islets, blocking RyR with 100 μM ryanodine markedly increased HIF-1β protein levels (Fig. 6A). We also compared the role of IP\textsubscript{3} receptors using the inhibitor xestospongin C and found that it did not alter HIF-1β levels in human islets. In MIN6 cells, blocking IP\textsubscript{3}R caused an increase in HIF-1β protein expression, whereas xestospongin had no consistent effect at this time point (Fig. 6B). Blocking SERCA with thapsigargin reduced HIF-1β expression in all cases. Together, these results indicate that when intracellular Ca\textsuperscript{2+} flux is compromised in β-cells a hypoxia-associated pathway is affected. We also directly examined the effects of different glucose concentrations on HIF-1β protein levels. The expression of HIF-1β was markedly induced at low glucose and suppressed at high glucose in MIN6 cells (Fig. 6C).

**ER Ca\textsuperscript{2+} Flux and Glucose Regulate in Presenilin**—Studies in other cell types have implicated presenilins in the control of hypoxia-inducible factors and vice versa (25, 27). Microarray analysis of MIN6 cells treated with 100 μM ryanodine\textsuperscript{6} pointed to the possibility that presenilin mRNA was increased when RyRs were blocked. To confirm the presence of presenilin proteins in β-cells, we performed immunofluorescent staining on MIN6 cells. Using deconvolution microscopy we observed that presenilin-1 was found in a punctate cytoplasmic and nuclear pattern in MIN6 cells (Fig. 7A) consistent with the observation that, in β-cells, presenilins are predominately found in the form of C-terminal fragments (see Fig. 8A below). Presenilin-2 was observed in a cytoplasmic pattern reminiscent of the Golgi apparatus (Fig. 7B). These findings are in agreement with the known subcellular location of presenilins in other cells types (35, 36). In human islets, reducing the glucose or blocking RyR dramatically increased presenilin-1 levels, whereas blocking IP\textsubscript{3}R or SERCA did not (Fig. 7, C and E). Presenilin-2 levels were not consistently altered in human islets by hypoglycemia or Ca\textsuperscript{2+} manipulation. In the MIN6 β-cell model, blocking either RyR or IP\textsubscript{3}R led to an increase in both presenilin-1 and presenilin-2 levels (Fig. 7F). This was observed at either 5 or 25 mM glucose (data not shown) and suggests that both channels play an important role in the regulation of this pathway in MIN6 cells. Interestingly, blocking SERCA pumps with thapsigargin decreased presenilin levels, especially in MIN6 cells cultured in low glucose. This response to thapsigargin was in line with its effects on HIF-1β. These findings also correlate with the opposing effects of channel inhibitors and pump inhibitors on the ATP to ADP ratio. Together, these studies show that presenilin levels can be affected by Ca\textsuperscript{2+} flux through intracellular Ca\textsuperscript{2+} channels in pancreatic β-cells.

**Effect of Presenilin Overexpression on HIF-1β Levels**—The experiments reported above demonstrated that blocking intracellular Ca\textsuperscript{2+} release channels or hypoglycemia leads to the induction of presenilin-1 and HIF-1β. To test whether the increase in presenilin-1 could be responsible for the rise in HIF-1β protein, we overexpressed presenilin-1 in the MIN6

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\textsuperscript{6} J. D. Johnson, unpublished data.
expression in the context of programmed β-cell death pathways. Our study has three major findings. First, we demonstrated that RyRs are present in MIN6 cells and human islets and that blocking these channels reduced the bulk ATP/ADP ratio. This provides evidence that RyRs regulate cellular metabolism to influence β-cell function and survival. These effects on metabolism led to the second major finding, that blocking intracellular Ca2⁺ channels or exposing β-cells to hypoglycemia induced the expression of HIF-1β, an essential gene implicated in β-cell function (17). To our knowledge, this is the first evidence that intracellular Ca2⁺ stores can alter HIF-1β levels in any cell type. The third key observation was that intracellular Ca2⁺ channels and glucose controlled the expression of presenilins and that an increase in presenilin-1 was sufficient to up-regulate HIF-1β. Presenilins have previously been shown to alter Ca2⁺ flux through RyR and IP₃R, but this is the first evidence of ER Ca2⁺ feedback on presenilins. Together, these studies elucidate a previously undescribed signaling network controlled by ER Ca2⁺ in human islets and MIN6 β-cells. Differences in the relative roles of RyR and IP₃R in human islets and MIN6 cells likely reflect distinct expression profiles of these channels between each cell type (6, 7).

Altered intracellular Ca2⁺ homeostasis and perturbed β-cell metabolism are hallmarks of diabetes (37, 38). In addition, islet transplant failure is associated with hypoxia and related stresses during isolation, culture, and the post-transplantation period (39, 40). In the present study, we show that blocking Ca2⁺ flux...
from intracellular Ca\(^{2+}\) channels induced the expression of HIF-1\(\beta\), a key member of the hypoxia-inducible factor family. The finding that HIF-1\(\beta\) levels could be acutely modulated in \(\beta\)-cells is particularly interesting because HIF-1\(\beta\) was recently shown to be dramatically reduced in islets from type 2 diabetic patients (17). \(\beta\)-Cell-specific knock out of HIF-1\(\beta\) led to diabetes in the mouse (17). Interestingly, inhibition of SERCA pumps with thapsigargin, which leads to ER stress-mediated apoptosis, down-regulated HIF-1\(\beta\) expression in our experiments. Although both thapsigargin and high ryanodine induce programmed \(\beta\)-cell death, we have found that these agents act via distinct mechanisms (6, 41). It is possible that HIF-1\(\beta\) is up-regulated in an attempt to protect \(\beta\)-cells from ryanodine-induced death. It is also possible that an excess of HIF-1\(\beta\) may be deleterious in the \(\beta\)-cell. Several studies in other cell types have demonstrated pro-apoptotic effects of HIF-1\(\beta\), in addition to its anti-apoptotic actions (42, 43). Similarly, although up-regulation of the related protein, HIF-1\(\alpha\), is associated with the expression of protective genes, HIF-1\(\alpha\) is also required for hypoxia- and hypoglycemia-induced apoptosis (44). Together, these studies point to a dual role for HIFs in the control of apoptosis.

A number of interesting parallels between neurons and \(\beta\)-cells have been established, including shared transcription factor networks during embryonic development (45). Diabetes and Alzheimer disease are risk factors for each other, and it is possible that these two diseases may share common molecular defects (46, 47). The Alzheimer disease genes, presenilin-1 and presenilin-2, are key mediators in neurodegeneration (20, 47) but their role in \(\beta\)-cell degeneration remains unclear. Previous studies have shown that presenilin-1 and -2 are present in human and rodent pancreas and that they localize to \(\beta\)-cells (48, 49), but nothing was known about stimuli that might increase or decrease their expression or the signaling pathways they control in the endocrine pancreas. Our results confirm the presence of presenilin-1 and presenilin-2 in \(\beta\)-cells. In the case of presenilin-1, the bulk of this protein exists as nuclear and cytosolic C-terminal fragments. We also show that presenilin-1 levels can be controlled by glucose and Ca\(^{2+}\) signals and that presenilin-1 can regulate HIF-1\(\beta\) expression. Like HIF-1\(\beta\), both an excess of presenilin activity and the loss of presenilin have been shown to increase cell death in other cell types (12, 20, 50).

Although it is not clear whether presenilins are involved in the generation of amyloid plaques seen in type 2 diabetes (51), it is known that presenilins can regulate cell differentiation and survival in both \(\gamma\)-secreta-dependent and \(\gamma\)-secreta-independent ways (52). We have recently shown that \(\gamma\)-secreta activity is essential for adult \(\beta\)-cell survival (21). Multiple studies have linked the \(\gamma\)-secreta-independent presenilin activity to the control of ER Ca\(^{2+}\) release under basal conditions and during caspase-3-mediated apoptosis (53–55). In general, the presenilin-1 gain-of-function mutations in Alzheimer disease lead to an overload of releasable ER Ca\(^{2+}\), while presenilin-2 mutations have been reported to decrease Ca\(^{2+}\) stores (56). Considerable evidence points to a direct link between presenilin and RyR (57–59). For example, presenilin-2 mutations have been shown to increase apoptosis and decrease differentiation by enhancing RyR-mediated Ca\(^{2+}\) release (59, 60). The hearts of presenilin-2 knock-out mice demonstrated greater amplitude of Ca\(^{2+}\) transients and increased contractility, specifically under conditions of low Ca\(^{2+}\) (57). In humans, presenilin mutations have been linked to cardiomypathy and altered Ca\(^{2+}\) homeostasis (61). Although previous studies have demonstrated that presenilins can have significant effects on intracellular Ca\(^{2+}\) signaling, we show for the first time in any cell type a reciprocal effect, blocking intracellular Ca\(^{2+}\) channels significantly increases presenilin levels. In the \(\beta\)-cell, presenilins appear to participate in a signaling network implicated in mitochondrial function and cell fate. Interestingly, presenilin-1 has been localized to the mitochondria in other cell types (62, 63) and mitochondrial respiration was decreased in knock-in mice with a presenilin-1 gain-of-function mutation (64). Studies also point to the up-regulation of presenilins in hypoxic conditions (12, 24, 27, 65), similar to our observation that hypoglycemia increases presenilin-1 expression. Together, these findings indicate that presenilins interact with intracellular Ca\(^{2+}\) homeostasis and metabolism on many levels. Our studies mark an important step toward understanding the role of presenilins in pancreatic \(\beta\)-cells. The regulation of presenilin proteins by \(\beta\)-cell Ca\(^{2+}\) flux may also shed light on the regulation of these proteins in other cell types.

In conclusion, we have shown that blocking intracellular Ca\(^{2+}\) release channels can reduce \(\beta\)-cell metabolism and induce expression of HIF-1\(\beta\) via presenilin up-regulation (Fig. 8C). This novel \(\beta\)-cell signaling network is likely to play a key role in the process of islet transplantation, during which \(\beta\)-cells are subjected to considerable stress. The implication of HIF-1\(\beta\) in glucose homeostasis and human type 2 diabetes suggests possible roles for this pathway in vivo.

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