Increased Phagocyte-Like NADPH Oxidase and ROS Generation in Type 2 Diabetic ZDF Rat and Human Islets

Role of Rac1–JNK1/2 Signaling Pathway in Mitochondrial Dysregulation in the Diabetic Islet

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OBJECTIVE—To determine the subunit expression and functional activation of phagocyte-like NADPH oxidase (Nox), reactive oxygen species (ROS) generation and caspase-3 activation in the Zucker diabetic fatty (ZDF) rat and diabetic human islets.

RESEARCH DESIGN AND METHODS—Expression of core components of Nox was quantitated by Western blotting and densitometry. ROS levels were quantitated by the 2',7'-dichlorodihydrofluorescein diacetate method. Rac1 activation was quantitated using the gold-labeled immunosorbent assay kit.

RESULTS—Levels of phosphorylated p47^phox, active Rac1, Nox activity, ROS generation, Jun NH2-terminal kinase (JNK) 1/2 phosphorylation, and caspase-3 activity were significantly higher in the ZDF islets than the lean control rat islets. Chronic exposure of INS 832/13 cells to glucolipotoxic conditions resulted in increased JNK1/2 phosphorylation and caspase-3 activity; such effects were largely reversed by SP600125, a selective inhibitor of JNK. Incubation of normal human islets with high glucose also increased the activation of Rac1 and Nox. Lastly, in a manner akin to the ZDF diabetic rat islets, Rac1 expression, JNK1/2, and caspase-3 activation were also significantly increased in diabetic human islets.

CONCLUSIONS—We provide the first in vitro and in vivo evidence in support of an accelerated Rac1–Nox–ROS-JNK1/2 signaling pathway in the islet β-cell leading to the onset of mitochondrial dysregulation in diabetes. Diabetes 60:2843–2852, 2011

Glucose-stimulated insulin secretion (GSIS) involves a series of metabolic and cationic events leading to translocation of insulin granules toward the plasma membrane for fusion and release of insulin into circulation (1–3). Insulin granule transport and fusion involve interplay between vesicle-associated membrane proteins on the insulin granules and docking proteins on the plasma membrane. In addition, a significant cross talk among multiple small G-proteins, including Arf6, Cdc42, and Rac1, was shown to be critical for GSIS (4–6). Several effector proteins for these G-proteins have been identified in the islet β-cell (4,7,8). We recently reported regulatory roles for Rac1 in the activation of phagocyte-like NADPH oxidase (Nox) and generation of reactive oxygen species (ROS) leading to GSIS (9).

Excessive ROS generation is considered central to the development of diabetes complications. The generation of free radicals is relatively low under physiologic conditions; however, increased levels of circulating glucose promote intracellular accumulation of superoxides, leading to cellular dysfunction. Although mitochondria remain the primary source for free radicals, emerging evidence implicates Nox as a major source of extra-mitochondrial ROS. Nox is a highly regulated membrane-associated protein complex that promotes a one-electron reduction of oxygen to superoxide anion involving oxidation of cytosolic NADPH. The Nox holoenzyme consists of membrane and cytosolic components (Fig. 1). The membrane-associated catalytic core consists of gp91^phox and p22^phox, and the cytosolic regulatory core includes p47^phox, p67^phox, p40^phox, and Rac1. After stimulation, the cytosolic core translocates to the membrane for association with the catalytic core for functional activation of Nox. Immunologic localization and functional regulation of Nox have been described in clonal β-cells and in rat and human islets (10–13).

Recent findings from studies of pharmacologic and molecular biologic approaches suggest that ROS derived from Nox play regulatory “second-messenger” roles in GSIS (9–11,13,14). In addition to the positive modulatory roles for ROS in islet function, recent evidence also implicates negative modulatory roles for ROS in the induction of oxidative stress and metabolic dysregulation of the islet β-cell under the duress of glucolipotoxicity, cytokines, and ceramide (15). The generation of ROS in these experimental conditions is largely due to the activation of Nox, because inhibition of Rac1 or Nox activation markedly attenuated deleterious effects of these stimuli (15–17). Despite this compelling evidence, potential roles of Nox in islet dysfunction in animal models of type 2 diabetes remain unexplored. We therefore undertook the current study to examine the functional status of Nox in islets from the ZDF rat, which develops obesity, hyperinsulinemia, hyperglycemia, and a decline in β-cell function. We present evidence to suggest significant activation of Nox, ROS generation, and caspase-3 activation in the...
defects in islets from type 2 diabetic human islets.

Materials. SP600125 and 2'-dichlorofluorescein diacetate (DCHFDA) were from Sigma (St. Louis, MO). Antibodies for p47phox and phospho-p47phox were from Santa Cruz Biotechnology (Santa Cruz, CA) and Abcam (Cambridge, MA), respectively. Antibodies for caspase-3, JNK1/2, and extracellular signal–related kinase (ERK) 1/2 were from Cell Signaling Technology (Boston, MA). The gold-labeled immunosorbent assay (GLISA) Rac1 activation kit was from Cytoskeleton (Denver, CO).

Rac1, guanosine-5′-diphosphate (GDP) was converted to Rac1 guanosine-5′-triphosphate (GTP), which binds to p67phox, and the complex translocates to the membrane. Existing evidence suggests that phosphorylation of p47phox also triggers its translocation to the membrane to form the Nox holoenzyme complex that culminates in the enzyme activation and associated increase in ROS.

Nox activity in diabetic rodent and human islets

ZDF islets. Our findings also suggest similar metabolic defects in islets from type 2 diabetic human islets.

RESEARCH DESIGN AND METHODS

Materials. SP600125 and 2′,7′-dichlorofluorescein diacetate (DCHFDA) were from Sigma (St. Louis, MO). Antibodies for p47phox and phospho-p47phox were from Santa Cruz Biotechnology (Santa Cruz, CA) and Abcam (Cambridge, MA), respectively. Antibodies for caspase-3, JNK1/2, and extracellular signal–related kinase (ERK) 1/2 were from Cell Signaling Technology (Boston, MA). The gold-labeled immunosorbent assay (GLISA) Rac1 activation kit was from Cytoskeleton (Denver, CO).

Rodent and human pancreatic islets and INS 832/13 β-cells. Male (9–11 weeks) ZDF and ZLC rats (Charles River Laboratories, Wilmington, MA) were maintained in a 12-h light/dark cycle with free access to water and food (Purina Diet 5008, Charles River Laboratories). All animal protocols were reviewed and approved by our Institutional Animal Care and Use Committee.

Hyperglycemia in diabetic rats was confirmed by tail vein puncture using Glucoseometer Elite (Bayer, Germany). Body weights of the ZLC and the ZDF rats were 300 ± 6 and 396 ± 12 g, respectively (n = 11; P < 0.05).

Islets were isolated by collagenase digestion method (18). Human islets from normal and diabetic donors were obtained from Prodo Laboratories, Inc. (Irvine, CA). Control islets from a 54-year-old male donor (85–90% purity) and diabetic islets from a 45-year-old male donor (~60% purity) and a 56-year-old male donor (~85–90% purity) were homogenized in Tris-HCl (50 mmol/L, pH 7.4) containing sucrose (250 mmol/L), EDTA (1 mmol/L), dithiothreitol (1 mmol/L), and protease inhibitor cocktail and used in this study. INS 832/13 cells (provided by Dr. Chris Newgard) were cultured and processed using previously described protocols (17).

Quantitation of ROS. Control and diabetic (rodent and human) islets and INS 832/13 cells were incubated with DCHFDA (10 μmol/L) for 30 min in RPMI-1640 media without serum and glucose (9). After incubation, islets were washed with ice-cold phosphate-buffered saline and sonicated. Equal amounts of protein were used for fluorescence measurements (λex 485 nm and λem 535 nm) using a luminescence spectrophotometer (PerkinElmer, Waltham, MA).

Rac1 activation assay. Activated Rac1 was quantitated using the GLISA activation assay kit according to the manufacturer’s instructions. Briefly, lysates were clarified by centrifugation at 14,000 rpm for 2 min. Equal amounts of islet lysate protein were incubated in the Rac1-GTP affinity plate for 30 min at 4°C. The wells were washed twice with washing buffer and incubated with anti-Rac1 primary antibody and secondary antibody, followed by additional incubation with horseradish peroxidase–detection reagent. Horseradish peroxidase–stop buffer was added to stop the reaction, and the absorbance was measured at 490 nm using a microplate reader.

Other assays and statistical analysis of data. Western blot protein bands were visualized from Kodak Imaging System (Rochester, NY) and analyzed densitometrically using UN-SCAN-It software (Orem, UT). Statistical significance of differences between control and experimental groups was determined by the Student t test and ANOVA analysis. P < 0.05 was considered significant.

RESULTS

ROS levels and expression and phosphorylation of p47phox are significantly increased in ZDF islets. The ZDF rats presented a fourfold increase in blood glucose levels compared with age-matched ZLC rats (323 ± 15 vs. 85 ± 1 mg/dL). A significant increase (~60%) in ROS generation was observed in the ZDF rat islets compared with the ZLC islets (Fig. 2A). Because recent evidence indicated a significant increase in Nox-derived ROS generation in isolated β-cells after exposure to high glucose, palmitate, or cytokines (15–17), we next examined Nox as a potential source of increased ROS in the ZDF rat islets.

The Nox holoenzyme consists of cytosolic and membranous components (Fig. 1). Recent evidence also suggests that the cytosolic components require post-translational modifications, including phosphorylation of p47phox and prenylation of Rac1 for optimal holoenzyme assembly (9,19).

The expression of p47phox was also significantly increased in isolated β-cells after exposure to high glucose, palmitate, or cytokines (15–17). Therefore, we determined the expression levels and the degree of phosphorylation of p47phox in islets from the control and diabetic rats. Pooled data acquired from multiple islet preparations, as determined by Western blotting and densitometry (Fig. 2B and C), indicated a significant increase (~40%) in the expression of p47phox in the ZDF islets compared with the ZLC islets. Levels of the phosphorylated p47phox were also significantly higher (~50%) in the ZDF islets (Fig. 2D and E).

Rac1, a cytosolic component of Nox, is activated in the ZDF islets. We next quantitated Rac1 expression and activation in the ZLC and the ZDF islets. The underlying premise here is that an increase in the Nox-derived ROS generation in the diabetic islets (Fig. 2A) requires activation of Rac1. Data showed a marked increase (>60%) in the expression of Rac1 in the diabetic islets compared with the control islets (Fig. 3A). Further, the abundance of the activated Rac1 is significantly higher (~2.25-fold) in the
diabetic islets than in the control islets (Fig. 3B). The observed increase in Rac1 activation (Fig. 3C) may not be a reflection of increased Rac1 expression in the ZDF islets (Fig. 3A) because the ratio of activated to total Rac1 also indicated a significant increase (>40%) in the diabetic rats compared with the control islets (Fig. 3D). Together the data (Figs. 2 and 3) indicate an increase in the phosphorylation status of p47\textsuperscript{phox} and activation of Rac1 in the ZDF islets, which are required for holoenzyme assembly and activation of Nox and subsequent increase in ROS generation (Fig. 2A).

**Increased expression of gp91\textsuperscript{phox} in the ZDF islets.** Numerous studies have focused on potential alterations in the expression of the cytosolic components of Nox in β-cells under the duress of glucolipotoxicity and cytokines (16,17,19,20); however, relatively little is known about alterations in the expression of the membrane components of Nox under such conditions. We therefore quantitated expression levels of gp91\textsuperscript{phox} in islets from the ZLC or the ZDF rats and noticed an increase in the expression of the gp91\textsuperscript{phox} subunit in the ZDF islets (Fig. 4A). Densitometric quantitation of protein bands indicated an increase of >40% in gp91\textsuperscript{phox} expression in the ZDF islets (Fig. 4B), thus supporting the overall hypothesis that an increase in the intracellular ROS in diabetic islet may be partly due to increased activation of Nox via an increase in the expression and phosphorylation of individual subunits.

**Assessment of mitochondrial dysregulation in the ZDF islets.** Using in vitro models systems of glucolipotoxicity or cytokine exposure, we have recently proposed that Nox activation leads to loss of mitochondrial membrane potential and caspase-3 activation (16,17). We also reported that inhibition of Rac1 activation by using NSC23776 to attenuate the function of T-cell lymphoma invasion and metastasis–inducing protein 1 (Tiam1), a guanine nucleotide exchange factor for Rac1, or using GGTI-2147 to inhibit prenylation of Rac1, leads to partial restoration of mitochondrial dysfunction induced by a mixture of cytokines (16). We therefore quantitated caspase-3 activation in the control and diabetic rat islets. Our findings (Fig. 4C and D) indicated a significant activation of caspase-3 in islets from the ZDF but not from the ZLC islets. These data are suggestive of mitochondrial defects in the ZDF islets at an age where significant changes in Nox activation are observed (see above).
Differential regulation of JNK1/2 and ERK1/2 in the ZDF islets. Stress-activated JNK activation lies upstream to caspase-3 activation (21). Further, constitutive activation of Rac1 promotes JNK phosphorylation and activation (22,23). Existing evidence also implicates significant cross talk between ROS and JNK1/2 (24). Therefore, we quantitated the phosphorylation status of JNK1/2 in islets from the ZLC and ZDF rats. Western blot analysis of lysates from the control and diabetic rats indicated consistently higher levels of phosphorylated JNK1 and JNK2 in ZDF rat islets (Fig. 5A). The ratios of phosphorylated to total JNK1 and JNK2, determined by densitometric quantitation of the protein bands (Fig. 5B) indicated a significant increase (60%) in the phosphorylation of JNK1/2 in the diabetic islets.

We next quantitated ERK1/2 phosphorylation in the ZLC and the ZDF islets to further determine if diabetic conditions elicit regulatory effects on activation of this enzyme because it has been implicated in islet β-cell function at multiple levels, including insulin gene expression, GSIS, and β-cell proliferation (25,26). ERK1/2 phosphorylation in the ZDF islets was significantly attenuated compared with the control islets (Fig. 5C and D). Together, these findings (Fig. 5) suggest differential regulation of JNK1/2 and ERK1/2 in diabetic islets, conditions that might favor proapoptotic and non-proliferative events in the diabetic islets. Our recently published observations on increased Nox activity in β-cells under the duress of glucolipotoxic conditions (17) and our current observations in the ZDF islets led us to hypothesize that glucolipotoxic distress may elicit such dual regulatory effects on JNK1/2 and ERK1/2 phosphorylation and activation. This hypothesis was further tested in clonal β-cells via studies described in the next section.

In vitro exposure to high glucose or palmitate exerts differential effects on JNK1/2 and ERK1/2. To assess if glucotoxicity or lipotoxicity are responsible for the differential regulatory effects on JNK1/2 and ERK1/2 seen in the ZDF islets, INS 832/13 cells were incubated for 48 h with high glucose (20 mmol/L) or palmitate (400 μmol/L),
and the relative abundance of total and phospho JNK1/2 and ERK1/2 was determined by Western blotting, followed by densitometry. Pooled data (Fig. 6A) indicated a marked increase (40–87%) in JNK1 and JNK2 phosphorylation in β-cells treated with high glucose (lanes 3 and 4) or palmitate (30–34%; lanes 5 and 6) compared with their levels under basal conditions (lanes 1 and 2). However, total levels of JNK1/2 remained unchanged under these conditions. We also observed a significant reduction in ERK1/2 phosphorylation in INS 832/13 cells treated with high glucose (22–48%) or palmitate (60%); but these conditions did not affect the abundance of total ERK1/2 (Fig. 6B).

Together, these in vitro findings in INS 832/13 cells are compatible to those observed in the ZDF islets (Fig. 5) and suggest differential regulation of JNK1/2 and ERK1/2 under the duress of glucotoxic or lipotoxic conditions.

To verify if inhibition of JNK1/2 phosphorylation would restore high glucose-induced caspase-3 activation, INS 832/13 cells were cultured under basal or high glucose (30 mmol/L) conditions in the absence or presence of SP600125 (20 μmol/L; 24 h), a selective inhibitor of JNK1/2. Degrees of JNK1/2 and caspase-3 activation were determined by Western blotting, followed by densitometry. There was a marked attenuation, by SP600125, of high glucose-induced JNK1/2 phosphorylation (Fig. 6C), and caspase-3 activation (Fig. 6D). These data implicate JNK1/2 activation is upstream to caspase-3 activation seen under the duress of glucotoxicity.

**Regulation of Nox in human islets.** We next studied regulation of Nox under glucotoxic conditions in human islets. At the outset, ROS generation and Rac1 activation were quantitated in normal human islets incubated with glucose (5.8 or 30 mmol/L) for 48 h. The data indicated a 2.2-fold increase in ROS generation in human islets after incubation with high glucose (Fig. 7A). These data are compatible with our observations in INS 832/13 cells and normal rat islets (9) and ZDF rat islets (current studies). Incubation of human islets with high glucose resulted in a significant (1.5-fold) activation of Rac1 (Fig. 7B). These data were compatible with our observations in INS 832/13 cells and normal rat islets (9) and ZDF rat islets (current studies). Incubation of human islets with high glucose resulted in a significant (1.5-fold) activation of Rac1 (Fig. 7B). A consistent increase in Rac1 expression, JNK1/2 phosphorylation and caspase-3 degradation were also demonstrated in type 2 diabetic human islets (Fig. 7C, D, and E, respectively), findings compatible with data in the ZDF islets. However, the levels of phosphorylated or total p47phox and gp91phox (Fig. 7C and E, respectively) were comparable between normal and diabetic patients.
diabetic human islets. Limited availability of diabetic human islets precluded us from quantitation of Nox and Rac1 activities. Nonetheless, our preliminary findings in human islets support our current findings in the diabetic ZDF islets or in INS 832/13 cells after exposure to glucolipotoxic conditions.

**DISCUSSION**

Existing evidence in multiple cell types, including the pancreatic β-cell, implicates post-translational phosphorylation and prenylation of individual components as a requisite for the optimal activation of Nox (9,19). The main objective of the current study was to determine the functional status of Nox in islets derived from the ZDF rat, a well-studied model for obesity and type 2 diabetes, and to determine potential regulation of Nox components in human islets under the duress of glucolipotoxicity and diabetes. Our data suggested a significant activation of Nox and associated ROS generation in the ZDF islets compared with those derived from the ZLC islets. Lastly, our data in diabetic human islets corroborated our findings in the ZDF islets.

Several recent studies have demonstrated activation of Nox after exposure to physiologic concentrations of glucose in a variety of insulin-secreting cells (10–13). Data from studies of pharmacologic and molecular biologic inhibition of Nox revealed that a tonic increase in Nox-derived ROS is necessary for GSIS (10,20) and that prenylation of Rac1 appears to be necessary for glucose-induced Nox activation and ROS generation in isolated β-cells (9). Emerging

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**FIG. 5.** Phosphorylation of JNK1/2 and ERK1/2 in the ZLC or the ZDF rat islets. Islet lysates from control and diabetic rats were prepared in RIPA buffer. A: Total and phospho-JNK1/2 were determined by Western blotting and analyzed densitometrically. B: Data are expressed as fold change in phosphorylation over total JNK1/2. Data are mean ± SEM (error bars) from islet lysates derived from six rats in each group. *P < 0.05 vs. the ZLC islets. Lysates of islets from control and diabetic rats were prepared in radioimmunoprecipitation assay buffer. An equal amount of lysate protein was resolved by SDS-PAGE. Relative abundance of total and phospho-ERK1/2 were determined by Western blotting (C), followed by densitometry (D). Data are expressed as fold change in phosphorylation over total ERK1/2 and are mean ± SEM (error bars) from islets from six rats in each group. *P < 0.05 vs. ZLC islets.
evidence also implicates Nox in metabolic dysfunction of the islet β-cell under conditions of glucolipotoxicity and exposure to cytokines (16,17). These studies demonstrated an increase in the expression and phosphorylation of Nox subunits (i.e., p47phox), together with significant activation of Rac1. In addition, the activation status of Rac1 was under the precise control of Tiam1, a guanine nucleotide exchange factor for Rac1 in β-cells (27). In further support of this, we reported a marked reduction in high glucose-, high palmitate-, and cytokine-induced Rac1 and Nox activation and ROS generation in isolated β-cells after exposure to NSC23766, a selective inhibitor of the Tiam1/Rac1 signaling axis (16,17). Taken together, previous in vitro findings implicated participatory roles of Nox in exerting effects at the mitochondrial level, including loss in membrane potential, cytochrome C release, and activation of caspase-3 culminating in islet β-cell dysfunction (16).

In addition to an increase in p47phox and gp91phox expression, Rac1 activation, and ROS generation, we observed a significant increase in the phosphorylation of JNK1/2 in the ZDF islets compared with the control islets. Similar changes in the activation of JNK1/2 were demonstrable in INS 832/13 cells after incubation with high glucose or palmitate. Selective inhibition of JNK1/2 using SP600125 markedly attenuated caspase-3 activation under glucotoxic conditions, suggesting that JNK1/2 activation lies upstream to mitochondrial dysfunction and caspase-3 activation. These data are in accord with findings of Cunha et al. (28) demonstrating significant inhibition of palmitate-induced JNK activation and cell apoptosis in INS-1E cells by SP600125 and L-TAT-JNKi, a small peptide inhibitor of JNK. Along these lines, several recent studies have also demonstrated inhibition of caspase-3 activation after inhibition of JNK1/2 activation in models of cellular apoptosis (21,29–31).

The observed reduction of ERK1/2 activation under glucolipotoxic conditions in the ZDF rat islets in vivo and in the INS 832/13 cells in vitro are indicative of impaired metabolic function and β-cell proliferation. Our current findings on reduction in ERK1/2 phosphorylation in INS 832/13 cells are in accord with studies of Costes et al. (32), who demonstrated a significant reduction in

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**FIG. 6.** Glucotoxic or lipotoxic conditions differentially regulate JNK1/2 and ERK1/2 and mitochondrial dysfunction in INS 832/13 pancreatic β-cells. INS 832/13 cells were cultured in the presence of low glucose (LG; 2.5 mmol/L), high glucose (HG; 20 mmol/L), or palmitate (PA; 400 μmol/L) for 48 h. At the end of incubation, cells were lysed and the expression of total and phosphorylated JNK1/2 (A) and ERK1/2 (B) was determined by Western blotting. In a separate set of experiments, INS 832/13 cells were incubated with glucose (30 mmol/L) with or without SP600125 (20 μmol/L) for 24 h. Cell lysates were prepared in radioimmunoprecipitation assay buffer for Western blot analysis to determine the degree of JNK1/2 (C) and caspase-3 activation (D). Data were quantitated densitometrically and are expressed as mean ± SEM (error bars) from three independent experiments. *P < 0.05 vs. 2.5 mmol/L glucose; **P < 0.05 vs. 30 mmol/L glucose alone.
ERK1/2 phosphorylation in MIN6 cells after exposure to 25 mmol/L glucose for 24 h. From the results of further studies, these investigators concluded that glucotoxic conditions downregulate the ERK1/2-cAMP-responsive element–binding protein-signaling pathway, leading to the apoptotic demise of the β-cell.

Recent studies by Zhang et al. (33) demonstrated a significant increase in JNK1/2 phosphorylation and reduction in ERK1/2 phosphorylation during mevastatin-induced apoptosis of salivary adenoid carcinoma cells, suggesting a potential inverse relationship between JNK1/2 and ERK1/2 phosphorylation in the induction of cellular apoptosis.

Together, our observations in INS 832/13 cells, ZDF islets, and diabetic human islets support involvement of the Nox–ROS stress-activated signaling axis in the metabolic dysfunction; however, additional studies are needed to substantiate this formulation. Recent studies by Nakayama et al. (34) demonstrated the functional activation of Nox in islets of db/db mice and in Otsuka Long-Evans Tokushima Fatty rats (34), and ZDF rat (current study), islets from animals fed a high-fat diet exhibited markedly lower expression levels of p47phox and gp91phox subunits and ROS production compared with control rat islets. These investigators attributed this toward increased glucose oxidation and GSIS seen in islets from animals fed a high-fat diet in response to glucose (35).

On the basis of the existing information and our current findings, we propose the following model for Nox-mediated induction of β-cell dysfunction in diabetes (Fig. 8): Exposure of isolated β-cells to glucolipotoxic conditions or islets derived from the diabetic condition in ZDF rats or humans results in increased activation of Rac1 and Nox. Consequential generation of ROS and the associated oxidative stress, in turn, promote activation of JNK1/2 and mitochondrial dysregulation. Alternatively, activation of the cytosolic Nox–ROS–JNK1/2 signaling pathway increases superoxide generation that impairs the functional efficiency of mitochondria. This proposal is supported by findings of Valle et al. (35) recently examined potential changes in Nox in islets derived from obese animals fed a high-fat diet. In contrast to islets from db/db mice, Otsuka Long-Evans Tokushima Fatty rats (34), and ZDF rat (current study), islets from animals fed a high-fat diet exhibited markedly lower expression levels of p47phox and gp91phox subunits and ROS production compared with control rat islets. These investigators attributed this toward increased glucose oxidation and GSIS seen in islets from animals fed a high-fat diet in response to glucose (35).
Hyperglycemia & Hyperlipidemia

[in vitro, ZDF, T2DM Human islets]

Rac1 Activation

Apocynin, DPI, p47phox siRNA

ROS generation

Stress Activated Kinases [JNK1/2]

SP600125

Mitochondrial Function

Caspase - 3 Activity

β - Cell Dysfunction

FIG. 8. Proposed model for Nox-induced ROS-mediated mitochondrial dysregulation in diabetes. Based on the data accrued from the current studies, we propose a model for the Nox–ROS–JNK signaling in the metabolic dysfunction of the pancreatic β-cell under the duress of hyperglycemia and hyperlipidemia. Glucotoxicity or lipotoxicity induces Nox activation by promoting the phosphorylation of p47phox and Rac1 activation. We have recently demonstrated that inhibition of Rac1 activation by NSC23766, or prenylation inhibitors, attenuates high glucose- or palmitate-induced Nox activation and ROS generation (15, 17). Likewise, inhibition of Nox action by apocynin, diphenylene iodonium, or siRNA-p47phox alleviates ROS generation and oxidative stress under the duress of high glucose, high palmitate, or cytokines (15–17). Nox activation and excessive ROS generation leads to the activation of stress-activated kinases (JNK1/2), culminating in mitochondrial dysfunction and caspase-3 activation. In support of this formulation, our current studies using SP600125 demonstrated significant inhibition in glucose-induced JNK1/2 phosphorylation and caspase-3 activation. On the basis of these data, we propose that the collective effects of Tiam1-mediated Rac1 activation, p47phox phosphorylation, Nox holoenzyme assembly, and associated ROS generation, followed by inhibition of ERK1/2 and activation of JNK1/2, result in mitochondrial dysregulation and caspase-3 activation leading to the islet β-cell dysfunction and demise in diabetes. DPI, diphenylene iodonium; siRNA, short interfering RNA; T2DM, type 2 diabetes mellitus.

Bindokas et al. (36) that demonstrated excessive superoxide levels in islet mitochondria from the ZDF rat. In summary, our current findings implicate Nox as one of the sources of oxidative stress in the diabetic islet. It will be interesting to determine if pharmacologic intervention of Nox activation seen in islets under diabetic conditions can be restored to its normal function. Such intervention modalities include NSC23766, a selective inhibitor of Tiam1/Rac1, which we have used in in vitro experiments to restore mitochondrial function in β-cells exposed to elevated glucose, lipids, and cytokines (16,17). In this context, recent investigations have successfully used NSC23766, a selective inhibitor of the Tiam1–Rac1 signaling axis, to correct Nox-mediated effects on cellular function in vitro and in vivo (15). Using the streptozotocin diabetic mouse model, Shen et al. (37) demonstrated a regulatory role for Rac1 in hyperglycemia-induced apoptosis in cardiomyocytes. They demonstrated that upregulation of Rac1, Nox activity, and increased ROS generation led to apoptosis of cardiomyocytes under the duress of hyperglycemia. Treatment of diabetic db/db mice with NSC23766 significantly inhibited Nox activity and cell apoptosis (37). Additional studies are needed to pinpoint the regulatory roles of Tiam1–Rac1–Nox–ROS signaling in the metabolic dysfunction in the diabetic islet.

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I.S., C.N.K., B.J., and S.G. conducted the experimental work. C.J.R. and R.A.K. planned the studies and reviewed the manuscript. A.K. reviewed the literature, planned the studies, supervised experimental work, and wrote and revised the manuscript.

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