NAD kinase regulates the size of the NADPH pool and insulin secretion in pancreatic β-cells

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Gray JP, Alavian KN, Jonas EA, Heart EA. NAD kinase regulates the size of the NADPH pool and insulin secretion in pancreatic β-cells. Am J Physiol Endocrinol Metab 303: E191–E199, 2012. First published May 1, 2012; doi:10.1152/ajpendo.00465.2011; doi:10.1152/ajpendo.00465.2011.—NADPH is an important component of the antioxidant defense system and a proposed mediator in glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells. An increase in the NADPH/NADP+ ratio has been reported to occur within minutes following the rise in glucose concentration in β-cells. However, 30 min following the increase in glucose, the total NADPH pool also increases through a mechanism not yet characterized. NAD kinase (NADK) catalyzes the de novo formation of NADP+ by phosphorylation of NAD+. NAD kinases have been shown to be essential for redox regulation, oxidative stress defense, and survival in bacteria and yeast. However, studies on NADK in eukaryotic cells are scarce, and the function of this enzyme has not been described in β-cells. We employed INS-1 832/13 cells, an insulin-secreting rat β-cell line, and isolated rodent islets to investigate the role of NADK in β-cell metabolic pathways. Adenoviral-mediated overexpression of NADK resulted in a two- to threefold increase in the total NADPH pool and NADPH/NADP+ ratio, suggesting that NADP+ formed by the NADK-catalyzed reaction is rapidly reduced to NADPH via cytosolic reductases. This increase in the NADPH pool was accompanied by an increase in GSIS in NADK-overexpressing cells. Furthermore, NADK overexpression protected β-cells against oxidative damage by the redox cycling agent menadione and reversed menadione-mediated inhibition of GSIS. Knockdown of NADK via shRNA exerted the opposite effect on all these parameters. These data suggest that NADK kinase regulates intracellular redox and affects insulin secretion and oxidative defense in the β-cell.

In the β-cell cytosol, NADPH can be formed by the reduction of its oxidized counterpart NADP+ via pyruvate cycling pathways mediated by cytosolic malic enzyme (ME1) and cytosolic isocitrate dehydrogenase (ICDe) (reviewed in Ref. 17) as well as via glucose-6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the pentose phosphate shunt (7). Inside the mitochondria, NADPH is regenerated via NADP+-dependent reduction mediated by ME3 and mitochondrial isocitrate dehydrogenase (18, 40), as well as NADH-dependent reduction of NADP+ via nicotinamide nucleotide transhydrogenase (NNT) (16). However, the operation of these pathways changes only the proportion of the reduced/oxidized form of NADPH, without the size of the total (NADPH + NADP+) nicotinamide adenine dinucleotide phosphate pool being affected.

NAD kinase [NADK; ATP: NAD(H)2 phosphotransferase] is the only known mammalian enzyme that catalyzes the conversion of NAD+ to NADP+ (reviewed in Ref. 38) and thus regulates the size of the (NADPH + NADP+) pool. NADKs play a crucial role in cell metabolism, survival, and oxidative defense in a variety of organisms, including bacteria, yeasts, and plants (reviewed in Ref. 50). Whereas several isoforms of NADK have been described in yeasts and plants (50), only one single cytosolic isoform exists in mammals (23). Despite the importance of this enzyme for intracellular redox regulation, the existence and function of this enzyme in insulin-secreting cells has not been investigated to date.

In the current study, we demonstrate for the first time the presence of NADK in β-cells and show that this enzyme regulates the size of the NADPH pool, insulin secretion, and β-cell survival. Together, these data suggest that NADK is an integral part of the β-cell redox and metabolic network.

MATERIALS AND METHODS

Materials

Collagenase was from Roche, and fetal calf serum was from Hyclone. All other chemicals were from Sigma-Aldrich unless otherwise specified.

Cell and Islet Preparation and Culture

Clonal INS-1 832/13 cells, provided by Dr. Christopher Newgard (Duke University), were maintained and cultured as described previously (10). Male CD-1 mice and Sprague-Dawley rats (Charles River) were euthanized by halothane. All procedures were performed in accordance with the Institutional Guidelines for Animal Care in compliance with US Public Health Service regulations and were approved by the Institutional Animal Care and Use Committee at the Marine Biological Laboratory. Pancreatic islets were isolated by collagenase digestion (Roche, Indianapolis, IN), as described previously (12). Islets were used after an overnight culture in RPMI

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supplemented with 10% fetal calf serum (HyClone), penicillin-streptomycin, and 5 mM glucose.

Construction of Short Hairpin RNA Plasmids, Adenovirus, and Lentivirus

Plasmids containing the green fluorescent protein (GFP) sequence and short hairpin RNA (shRNA)-encoding sequences (cat. no. TG708143) targeted against rat NADK (GenBank accession no. NM_001109678) or GFP and noncoding sequence (scrambled control, cat. no. TR30013) were custom-designed and constructed by Origene (Rockville, MD). The 29mer shRNA constructs against rat NADk were GAATCTGACTACGGCTTACCAACTGT and TGACATTTCCACGAGTACCTTCATCA.

NADK-overexpressing adenovirus. Recombinant, replication-deficient type 5 adenovirus (Ad-NADK)-expressing human NADK (Origene, Rockville, MD) was custom-constructed by Vector BioLabs (Philadelphia, PA). The expression of NADK is under the control of the cytomegalovirus promoter, which also directs the expression of GFP from an internal ribosome entry site. A control virus containing GFP sequence only (Ad-control) was constructed in parallel. Viral titers were determined by the plaque formation assay.

NADK-silencing lentivirus. The lentiviral plasmid expressing shRNA was from Open Biosystems (cat. no. RMM4431-101290079). The shRNA sequence against mouse/rat NADK was AGATCGAGATGCCAGCTT. For control, the scrambled, nonsilencing shRNA sequence (Open Biosystems, cat. no. RHS4346) was used. The lentiviruses were produced according to published methods (20). The pGIPZ vectors containing either shRNA sequence against NADK or the scrambled sequence were cotransfected with the packaging Δ8.9 and the vesicular stomatitis virus G protein vectors into human embryonic kidney (HEK)-293T cells. Seventy-two hours after transfection, the supernatant was centrifuged for 2 h at 100,000 g. The viral pellet was dissolved in PBS and used as a source of lentivirus.

Overexpression and Knockdown of NADK in INS-1832/13

For NADK overexpression, cells (at 60–70% confluence) were transduced with Ad-NADK or Ad-control at 50 MOI for 12 h; then, viral medium was replaced with the appropriate growth medium. Functional assays were performed 36–48 h posttransduction. For NADK knockdown, cells were infected with lentivirus-carrying NADK shRNA sequence or control lentivirus-carrying scrambled sequence for 12 h; then, viral medium was replaced with the appropriate growth medium. Functional assays were performed 72–96 h posttransduction. Transduction and transfection efficiencies, as determined by GFP fluorescence, reached >80% under these conditions.

Overexpression and Knockdown of NADK in Islets

For NADK overexpression and knockdown, islets were used immediately following their isolation. Islets were transduced with the adenovirus-carrying NADK sequence or control adenovirus at 50 MOI for 12 h; then, viral medium was replaced with the appropriate growth medium. Functional assays were performed 36–48 h posttransduction. For NADK knockdown, islets were infected with lentivirus-carrying shRNA sequence and control lentivirus-carrying scrambled sequence for 12 h; then, viral medium was replaced with the appropriate growth medium. Functional assays were performed 72–96 h posttransduction. Transduction and transfection efficiencies, as determined by GFP fluorescence, reached >80% under these conditions.

Quantitative RT-PCR

Total RNA was extracted using TriReagent (Sigma, St. Louis, MO) or the RNeasy Micro Kit (Qiagen, Valencia, CA), and RNA was reverse-transcribed using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems) according to the manufacturers’ protocols. Standard curves were generated using serial twofold dilutions from pooled cDNA samples to confirm ≥90% reaction efficiency for each primer set. Real-time PCR was performed using SYBR Green PCR Master Mix (Bio-Rad, Hercules, CA) on a MyiQ Real-Time PCR Detection System (Bio-Rad). All PCR primer sequences were generated using PrimerQuest (Integrated DNA Technologies, Coralville, IA). A minimum of three samples was analyzed for each experimental group. Primer sequences are listed in Table 1.

INS-1 832/13 Autofluorescence by Two-Photon Excitation of NAD(P)H

Cells cultured on poly-d-lysine-coated cover slips of 35-mm confocal dishes (MatTek) were imaged on a Zeiss LSM510 confocal microscope equipped with a heated stage, using two-photon excitation of NAD(P)H as described previously (12). NAD(P)H was excited by 150-fs pulses of 710 nm light from a Mira laser focused through a ×40 objective. Autofluorescence was collected through a 380-550-nm band pass filter (Chroma) and images were analyzed using Zeiss imaging software.

Determination of Nucleotides

Following alkali extraction, INS-1 832/13 cells (confluent 35-mm dish/experiment) and islets (200 islets/experiment) were vortex-mixed and sonicated for 10 s on ice. Aliquots were heated at 60°C for 20 min to destroy NAD+ and NADP+. Nonheated aliquots were used for determination of total NADH + NAD+ and

Table 1. Real-time PCR primer sequences

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward (5′ → 3′)</th>
<th>Reverse (5′ → 3′)</th>
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</thead>
<tbody>
<tr>
<td>Rat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-2MG</td>
<td>ACAGTGAATCTTCAACCAAGAGA</td>
<td>TGTAGTACTAGTCTGCTGCAAGGT</td>
</tr>
<tr>
<td>ME1</td>
<td>GGTGGTTGATTGTCGCCAACAAATA</td>
<td>TTTGCTCTCCATCATGCACAgCAAGA</td>
</tr>
<tr>
<td>ME2</td>
<td>AAGATTTGCACCAGAGAGGGTTTT</td>
<td>CATGACATCTGCGAGCAGGGA</td>
</tr>
<tr>
<td>ME3</td>
<td>AACAAGCAACCATGGCTCCTTACT</td>
<td>ATGCTGTCAGACGCTTTCGACACT</td>
</tr>
<tr>
<td>PC</td>
<td>ATGATTGTTGCTGCTGCAAGGTTG</td>
<td>ATGTCTGAGATTGCGACATGAG</td>
</tr>
<tr>
<td>ICD1</td>
<td>TGGCTGCAAGAACGACTACCT</td>
<td>AGCATATGGTGTGCTTTCGAGATCT</td>
</tr>
<tr>
<td>ICD2</td>
<td>AGAGCTCTCATCGGAGTTCAGAGA</td>
<td>AGCTTGTGCTGCACAGTTCGAC</td>
</tr>
<tr>
<td>NNT</td>
<td>CAACGCTGCTGCTGCTGCTTGCTACT</td>
<td>AAGATGTTGACTGCGAGTAAAG</td>
</tr>
<tr>
<td>NADK</td>
<td>TATGTGCTGAGGCGAGCAGGAAA</td>
<td>TGGATGCTGCTGCTGCTGCTCTCAT</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NADK</td>
<td>ACGCCAGACTTTCCACTCAAGGTTACTT</td>
<td>TGAGGACAGGGCTTCTGAGAACAT</td>
</tr>
</tbody>
</table>

β-2MG, β-2-microglobulin; ME1, -2, and -3, malic enzyme 1, 2, and 3, respectively; PC, pyruvate carboxylase; ICD1 and -2, isocitrate dehydrogenase 1 and 2, respectively; NNT, nicotinamide nucleotide transhydrogenase; NADK, NAD kinase. All sequences are designed for Rattus norvegicus, except as indicated.
NADPH + NADP+. ATP/ADP ratio was determined using bioluminescent detection method. All adenine nucleotides were determined using NAD+/NADH, NADP+/NADPH, and ATP/ADP kits (Abcam, Cambridge, MA) according to the manufacturer’s protocols.

**Determination of Intracellular Oxidative Stress**

Cells were preloaded with 10 μM 2′,7′-dichlorodihydrofluorescein-diacetate (DCFH-DA) for 60 min and treated with hydrogen peroxide (10 μM) for 3 h. Fluorescence (485 nm excitation, 520 nm emission) was quantified using a SpectraMax M5 plate reader.

**Determination of NADK Activity**

NADK activity in cell lysates was assayed as described previously (39) in reaction mixture containing 50 mM Tris·HCl, pH 7.8, 10 mM MgCl2, 5 mM NAD+, and 10 mM ATP. The amount of NADP+ produced by NADK-dependent phosphorylation was then determined by a cycling assay in the presence of 5 mM of glucose 6-phosphate, NADP-specific yeast G6PD, and 0.5 mM MTT/mPMS. Reduction of MTT was measured at 600 nm. Calibration curve was generated using known amounts of NADP+ standards in the cycling reaction. Reduction of MTT was monitored at 600 nm using a Sunrise spectrophotometer (Tecan). One unit was defined as the amount of enzyme producing 1 μmol of NADP in 1 min at 37°C.

**Insulin Secretion**

INS-1 832/13 (48-well plates) and isolated islets (15 islets/tube) were preincubated for 2 h in the presence of 2 mM (INS-1 832/13 cells) or 4 mM (islets) glucose in Krebs-Ringer bicarbonate buffer. The amount of released insulin was determined after 60 min of static incubation, using an ELISA kit (Alpco Diagnostics, Salem, NH). Data were normalized for protein content determined by the Micro-BCA Protein Assay Kit (Pierce, Rockford, IL).

**Statistical Analysis**

Data are expressed as means ± SE. Significance was determined for multiple comparisons using one-way analysis of variance. A P value of <0.05 was considered significant.

**RESULTS**

**NADK Expression in β-Cells and Rodent Islets**

Relative expression of mRNA in INS-1 832/13 cells and rat and mouse islets was determined using quantitative real-time PCR (Fig. 1). Real-time-PCR primer sequences are listed in Table 1. Cell and islet mRNA expression levels were normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase. The mRNA levels of NADK were found to be similar to the mRNA levels of ME2 and ME3, mitochondrial NAD+ and NADP+-dependent malic enzymes in both INS-1 832/13 cells and mouse islets (Fig. 1). Expression of NADK in INS-1 832/13 cells was not different following 48 h treatment with basal or stimulatory glucose levels (data not shown).

**NADK Overexpression and its Effect on NADPH levels, the NADPH/NADP+ Ratio, and Insulin Secretion**

Adenoviral-mediated overexpression of NADK resulted in a >10-fold increase in NADK mRNA (Fig. 2A) and NADK enzymatic activity (Table 2). The total NADPH pool (NADPH and NADP+) and the NADPH/NADP+ ratio were measured following 10, 30, and 60 min of 2 or 4 and 16 mM glucose exposure. In control (control virus treated) and untreated cells and islets, an increase in the total NADPH pool, in addition to an increase in the ratio, was noted following 30 and 60 min of exposure to 16 mM glucose (Figs. 2B and C, and 5A), and these effects were enhanced in the NADK-overexpressing cells (Figs. 2B and 5A). Upon analysis, it was found that the increase in the size of the total NADPH pool was due mainly to the increase in NADPH, since NADK overexpression did not cause significant changes in the NADH/NAD+ pool or in the NADH/NAD+ ratio (Fig. 2E). This is not surprising considering that the size of the total (NADH +
NAD\(^{+}\) pool is far greater than the size of the (NADPH + NADP\(^{+}\)) pool (12) and that alternate synthetic pathways might serve to replenish any NAD\(^{+}\) phosphorylated by NADK (43).

In INS-1 832/13, NADK overexpression caused a \(~30\%\) increase in glucose-stimulated insulin secretion (GSIS), whereas insulin secretion initiated by the depolarizing agent KCl was not affected significantly (Fig. 2D). However, in rodent islets, NADK overexpression elicited only a modest increase in GSIS, which was not statistically significant (Fig. 5B).

NADK Knockdown and its Effect on NADPH, the NADPH/NADP\(^{+}\) Ratio, and Insulin Secretion

Transfection of INS-1 832/13 cells with NADK-shRNA plasmids decreased NADK mRNA expression (Fig. 4A) and NADK activity (Table 2). Similarly, NADK activity was decreased in isolated islets infected with shRNA lentivirus (Table 2). Nonspecific shRNA (scrambled) plasmid and control lentivirus had no significant effect on NADK activity, as determined by comparison of NADK activity in untreated native cells vs. cells and islets treated with scrambled shRNA and control shRNA lentivirus (Table 2).

NADK knockdown caused decreases in both the total (NADPH + NADP\(^{+}\)) pool (Figs. 4B and 5A) and the NADPH/NADP\(^{+}\) ratio (Fig. 4C). The incremental increase in the total (NADPH + NADP\(^{+}\)) pool observed following 60 min of exposure to 16 mM glucose in control cells and islets was virtually abolished by NADK knockdown (Figs. 4B and 5A), suggesting that NADK kinase is indeed responsible for de novo synthesis of NADPH and NADP\(^{+}\) following prolonged exposure to 16 mM glucose.

Similarly, GSIS was inhibited significantly by NADK knockdown in both INS-1 832/13 cells and mouse islets (Fig. 4D and 5B), without significant effect on KCl-mediated secretion (Fig. 4D), suggesting that NADK manipulation affects intracellular metabolic pathways proximal to plasma membrane depolarization.

Effect of NADK on the \(\beta\)-Cell Oxidative Defense

Compared with other tissues, \(\beta\)-cells have unusually low levels of classical antioxidant enzymes such as superoxide...
dismutase, catalase, and glutathione peroxidase (reviewed in Ref. 37), leaving the possibility that NADPH-dependent systems, such as the thioredoxin or glutaredoxin systems, might play a more important role in the antioxidant defense of these cells. Since we have demonstrated that NADK regulates NADPH levels in β-cells, we tested whether NADK overexpression affected their capacity to resist oxidative stress. Menadione, a redox cycling agent that produces hydrogen peroxide (H₂O₂), was shown at a dose of 10 µM to inhibit GSIS in INS-1 832/13 cells, and this inhibitory action of menadione on GSIS was reversed by the overexpression of NADK (Fig. 6A). Menadione redox cycling produces H₂O₂ (3), and the application of high and toxic doses of H₂O₂ (50 µM) was previously shown to inhibit insulin secretion (47). To determine whether NADK-dependent removal of H₂O₂ is the mechanism behind its protection against toxic menadione doses, H₂O₂ levels following the application of 50 µM H₂O₂ were measured in control and NADK-overexpressing cells (Fig. 6B). NADK overexpression decreased H₂O₂ levels, consistent with the NADPH-dependent maintenance of the glutaredoxin system and the role of glutathione reductase in destruction/removal of H₂O₂ (5).

DISCUSSION

The glucose-dependent increase in the β-cell NADPH/NADP⁺ ratio has been reported to occur as early as a few minutes after the elevation of glucose concentration from basal to stimulatory levels (12). However, the effect of stimulatory

Fig. 3. Effect of NADK overexpression on autofluorescence of NAD(P)H. NAD(P)H autofluorescence was monitored in live control (A and B) or NADK(+) (C and D) INS-1 832/13 cells in the presence of 2 or 16 mM glucose (2G or 16G) by 2-photon excitation of NAD(P)H, as described previously (12).

Fig. 4. Effect of NADK knockdown on NADK mRNA (A), total (NADPH + NADP⁺) levels (B), the NADPH/(NADPH + NADP⁺) ratio (C), insulin secretion (D), and NADH/(NADH + NAD⁺) and ATP/ADP ratio (E) in INS-1 832/13 cells. Nucleotide determination and insulin secretion were performed as described in the legend to Fig. 2. Data are means ± SE from 3–5 independent experiments performed in duplicate or triplicate measurements. *P < 0.05 NADK(−) vs. NADK control; #P < 0.05 2G vs. 16G.
Thus, the glucose-stimulated increase in Ca\textsuperscript{2+} reported that an increase in the NADPH pool (a net rise in NADPH and a corresponding decrease in the NADP\textsuperscript{-} pool) was compensated for completely by the glucose exposure (14, 19), where the glucose-dependent increase in NADPH was found to remain roughly the same following 2 h of stimulatory glucose exposure (39). The glucose-dependent increase in the NADPH pool (a net rise in NADPH and a corresponding decrease in the NADP\textsuperscript{-} pool) was found to remain roughly the same following 2 h of stimulatory glucose exposure (39).

Fig. 5. Effect of NADK overexpression knockdown on total (NADPH + NADP\textsuperscript{+}) levels (A) and insulin secretion (B) in mouse islets. Nucleotide determination and insulin secretion were performed as described in the legend to Fig. 2. Data are means ± SE from 2–3 independent experiments performed in duplicate or triplicate measurements. *P < 0.05 NADK(+) or NADK(−) vs. NADK control; #P < 0.05 4G vs. 16G.

Although mammalian NADK kinase can utilize either NADH or NAD\textsuperscript{+} as a substrate, it has a strong preference for NAD\textsuperscript{+} (39), the existing level of these enzymes has sufficient capacity to efficiently convert glucose to NADPH. This suggests that the NADP\textsuperscript{+}, formed by NADK, is rapidly converted to NADPH via NADP\textsuperscript{+}-dependent enzymes. Our findings are in agreement with reported data in HEK-293 cells, where overexpression of NADK resulted in a similar increase in the NADPH rather than NADP\textsuperscript{+} pool (39). Indeed, mammalian cells typically maintain an elevated NADPH/NADP\textsuperscript{+} ratio to support reductive biosynthesis and to protect the cells from oxidative stress (56). Because mammalian NADK is located in the cytosol (31, 39), several NADP\textsuperscript{+}-dependent, NADPH generating cytosolic enzymes are potential acceptors of NADP\textsuperscript{+} formed via NADK in β-cells. These include enzymes of the pyruvate-cycling pathways: ME1, ICDe, and the pentose phosphate pathway. Although the pentose phosphate pathway has been suggested to not be significantly active in β-cells (reviewed in Ref. 26), its rate-limiting enzyme G6PD has received recent attention as a critical determinant of antioxidant defense in pancreatic β-cells (reviewed in Ref. 58). However, mRNA expression of these enzymes was not affected by NADK overexpression (data not shown). This suggests that, similar to findings reported in HEK-293 cells (39), the existing level of these enzymes has sufficient capacity to accommodate the reduction of additional NADP\textsuperscript{+} generated following NADK overexpression.

NADPH has been suggested to be a coupling mediator for GSIS (15). Overexpression and knockdown of NADK increased and decreased, respectively, the level of NADPH. Whereas NADK knockdown inhibited GSIS significantly in both clonal INS-1 832/13 cells and isolated islets, overexpression of NADK...

Fig. 6. NADK overexpression in INS-1 832/13 protects against menadione (MEN)-dependent inhibition of glucose-stimulated insulin secretion (A) and decreases H2O2 levels (B) in INS-1 832/13 cells. MEN and H2O2 were applied at 10 and 50 μM, respectively. Insulin secretion was performed as described in the legend to Fig. 2. H2O2 was determined by 2’,7’-dichlorodihydrofluorescein (DCFH) fluorescence. Data are means ± SE from 2–3 independent experiments performed in duplicate or triplicate measurements. *P < 0.05 NADK(+) vs. NADK control; #P < 0.05 2G vs. 16G.
had only a modest effect on GSIS. This suggests that the existing level of NADPH is sufficient to support GSIS-dependent pathways. The defect in GSIS following NADK knockdown supports the role of NADPH as a coupling mediator, or it may suggest that a decrease in NADPH can, in general, negatively affect pathways involved in the regulation of GSIS. Further studies are needed to fully elucidate this topic.

NADPH serves as a reducing cofactor for glutaredoxin and thioredoxin, cytosolic defense systems involved in protection from oxidative stress that reduce inappropriate disulfide bonds, restoring cysteinyll sulfhydryl residues and eliminating hydroperoxides (13). NADPH-dependent oxidative defense might be particularly important in β-cells since they have unusually low levels of classical antioxidant enzymes (reviewed in Ref. 37) and might rely on other defense systems to combat oxidant challenge. Depending on their dose, reactive oxygen intermediates (ROI) can either be detrimental to β-cell function or serve a positive role as signaling messengers (reviewed in Ref. 11). This is in agreement with the notion that low and physiological levels of ROI occur as a natural part of metabolism under physiological conditions and serve a signaling function in various cell types (8, 9, 27, 28, 44–46, 52). In agreement with the latter, several studies reported glucose-dependent elevation of ROI content in β-cells (2, 22, 36, 53), whereas others demonstrated the opposite effect (21, 30, 41). Since careful time course analysis of the rise and fall of ROI has not been performed in any of these studies, the timing of ROI measurement may be of the essence and explain these contradictory results. We hypothesize that glucose-dependent activation of NADK via activation of the glutaredoxin/thioredoxin system in the cytosol and other cellular membranes to support glucose-mediated signaling and provide low physiological levels of ROI at the plasma and endoplasmic reticulum (6, 51), and NADPH oxidase activity in endothelial cells was associated with the cytoskeletal fractions (24). Recently, NADPH oxidase has been shown to be present in islets (54) and was implicated in the regulation of insulin secretion (33, 34, 53). Therefore, NADK, by regulating the de novo formation of NADPH, might play a dual role in β-cells by supporting functionally and compartmentally distinct systems, i.e., glutaredoxin and thioredoxin systems in the cytosol by increasing the reduced/oxidized ratio of glutathione and thioredoxin while simultaneously providing NADPH that would activate NADPH oxidase and provide low physiological levels of ROI at the plasma and other cellular membranes to support glucose-mediated signaling under stimulatory glucose levels. Thus, NADK-dependent activation of the glutaredoxin/thioredoxin system in the cytosol and NADPH oxidase in the membranes might result in complementary functions (antioxidant vs. pro-oxidant) enabled by the compartmentalization of these two components (reviewed in Ref. 55).

Future studies are underway to evaluate role of NADK in these pathways. Altogether, we have demonstrated that via its control of the level of NADPH, NADK regulates insulin secretion and protects β-cells from oxidative stress.
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GRANTS

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DISCLOSURES

The authors report no competing interests, financial or otherwise.

AUTHOR CONTRIBUTIONS

J.P.G., K.N.A., E.A.J., and E.A.H. performed the experiments; J.P.G. and E.A.H. analyzed the data; J.P.G. and E.A.H. interpreted the results of the experiments; J.P.G. edited and revised the manuscript; K.N.A. and E.A.H. did the conception and design of the research; J.P.G. prepared the figures; J.P.G. and E.A.H. approved the final version of the manuscript; E.A.H. drafted the manuscript.

REFERENCES