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

Joshua P. Gray,1,2 Kambiz N. Alavian,3 Elizabeth A. Jonas,3 and Emma A. Heart2

1United States Coast Guard Academy, New London, Connecticut; 2Cellular Dynamics Program, Marine Biological Laboratory, Woods Hole, Massachusetts; 3Yale University, New Haven, Connecticut

Submitted 7 September 2011; accepted in final form 28 April 2012

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.

nicotinamide adenine dinucleotide; reduced nicotinamide adenine dinucleotide phosphate/nicotinamide adenine dinucleotide phosphate+, insulin secretion; β-cells; cytosolic oxidoreductases

INSULIN SECRETION, A HALLMARK FEATURE of pancreatic β-cells, occurs in response to the increase in glucose concentration from basal, nonstimulatory levels (4–7 mM) to stimulatory levels (8–16 mM). Glucose metabolism inside the β-cell leads to an increase in the reduced/oxidized ratio of both NADH and NADPH (4, 29, 49) occurring within minutes following an increase in glucose concentration. NADPH has been suggested to be a coupling factor for insulin secretion (15, 42) based on a study showing that injection of NADPH triggered insulin granule exocytosis from pancreatic β-cells (15). In addition to its putative role as a metabolic mediator of insulin secretion, NADPH is a cofactor for reductive biosynthetic pathways and is essential for oxidative defense, since it replenishes the glutaredoxin and thioredoxin systems (13).

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 (ICDc) (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 a 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
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 GAATCTGACTCAGCTCTTACAAGTGT and TGACATTTCAACACAGATAGACTTCA.

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 AGATC-GGAGATGCCAGCTT. 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 (Open Biosystems, cat. no. RHS4346) targeted against rat NADK (GenBank accession no. NM_001109678) or the scrambled sequence were cotransfected with the packaging vectors 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.

Table 1. Real-time PCR primer sequences

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward (5′ → 3′)</th>
<th>Reverse (5′ → 3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-2MG</td>
<td>ACAGCTGAGTTCAACACCCACCGAGA</td>
<td>TGGATTACATGGTTCTCGGTCACAGGT</td>
</tr>
<tr>
<td>ME1</td>
<td>GTGGGCGATTGATGACGCACAAATA</td>
<td>TTGCGTCTTCATGCTGACACAGA</td>
</tr>
<tr>
<td>ME3</td>
<td>AAGATGTCGCAAGTGGGACGAGTT</td>
<td>TCTGGAGCTCGAGCGAGGAA</td>
</tr>
<tr>
<td>PC</td>
<td>ATGAGTGGCTGGCAGACAGTT</td>
<td>ATGTTGCGCTGAGGCAAGAA</td>
</tr>
<tr>
<td>ICD1</td>
<td>TGCGGATCGAAGGACGAGTT</td>
<td>ACCCGATTTGAGGCAAGTA</td>
</tr>
<tr>
<td>ICD2</td>
<td>AGACGTCTCATGAGTGAGAGAAA</td>
<td>AGTTGCGCTGAGGCAAGAA</td>
</tr>
<tr>
<td>NNT</td>
<td>CAACCTGGTGGTGGTGGAT</td>
<td>AAAGGGTACCGCCCGAGTAA</td>
</tr>
<tr>
<td>NADK</td>
<td>TGGTATGCTGAGGCAAAGAA</td>
<td>TGGTTGAGGCAAGGCAAAGAA</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NADK</td>
<td>ACCCGAAGTATTCTCAAGGTAAGT</td>
<td>TGGAGAACGCGGCTTCAAGGA</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.

Overexpression and Knockdown of NADK in Islets

For NADK overexpression and knockdown, islets were used immediately following their isolation. Islets were transfected 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 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.

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 manufacturer’s 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 coverslips of 35-mm confluent 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 custom-made Chroma filter (Bennett BD 1996 JBC), 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 NADH and NADPH. Nonheated aliquots were used for determination of total NADH + NADPH contents.
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 mMTris·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. 2, B 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 but not NADP+. This was illustrated further by the two-photon imaging of live NAD(P)H autofluorescence, demonstrating a 245 ± 35% increase in NADPH overexpression (Fig. 3).

Since only the reduced form of adenine nucleotides (NADPH and NADH) displays autofluorescence, this increase in signal was due 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 an \sim 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 NAD 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.

---

E195
glucose on the total (reduced plus oxidized) pool of NADPH (NADPH + NADP+) in β-cells has not been addressed directly. Data in the literature regarding changes in the total pool of NADPH are byproducts of studies related to different aspects of β-cell metabolism and yield conflicting results. In some studies, the total NADPH pool (NADPH + NADP+) was found to remain roughly the same following 2 h of stimulatory glucose exposure (14, 19), where the glucose-dependent increase in NADPH was compensated for completely by the corresponding decrease in the NADP+. However, other studies reported that an increase in the NADPH pool (a net rise in NADPH with no converse decrease in the pool of NADP+) occurred after 40 min and longer of stimulatory glucose exposure (1, 35). In line with these latter observations, we also found an increase in the total NADPH pool following 1-h exposure of INS-1 832/13 cells to stimulatory glucose (Figs. 2B and 5A). Our data support the hypothesis that NADK is responsible for this increase in the total NADPH pool, since knockdown of NADK blunted this increase (Fig. 4B), and further suggest that NADK kinase activity increases under elevated glucose levels. NADK activity has been shown to be sensitive to free Ca2+ levels, with half-maximal activity observed at ~400 nM (57), and an increase in glucose from basal (2–5 mM) to stimulatory levels (10–20 mM) is known to elicit a rise in the free cytosolic Ca2+ from <100 to ~500 nM (32, 48). Thus, the glucose-stimulated increase in Ca2+ may activate NADK in β-cells (Fig. 7).

Although mammalian NADK kinase can utilize either NADH or NAD+ as a substrate, it has a strong preference for the latter (39). Despite this preference, our data demonstrate that the NADK-dependent increase in the (NADPH + NADP+) pool is due mainly to the increase in NADPH and not so much NADP+. This suggests that the NADP+, formed by NADK, is rapidly converted to NADPH via NADP+-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+ pool (39). Indeed, mammalian cells typically maintain an elevated NADPH/NADP+ 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+-dependent, NADPH generating cytosolic enzymes are potential acceptors of NADP+ 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+ 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...
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 cysteinyI 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 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 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.

Fig. 7. Role of NADK in the context of β-cell metabolic pathways. The glucose-dependent increase in pyruvate decarboxylation (1) leads to the rise in the ATP/ADP ratio (3), closure of the K\textsubscript{ATP} channels (4), plasma membrane depolarization, and Ca\textsuperscript{2+} influx (5). The rise in cytosolic Ca\textsuperscript{2+} activates NADK (6), which phosphorylates NADPH to produce NADPH\textsuperscript{+} (7). The net increase in the NADPH\textsuperscript{+} pool serves as a substrate for pyruvate cycling pathways (activated by the rise in glucose via an increase in pyruvate cycling; 2) and leads to the NADK-dependent net increase in the NADPH pool (8). PDH, pyruvate dehydrogenase; PC, pyruvate carboxylase.
ACKNOWLEDGMENTS

We thank M. Meow for helpful comments and support in the preparation of the manuscript.

GRANTS

This work was supported by American Diabetes Association Grant 7-08-JF-18, National Institute of Diabetes and Digestive and Kidney Diseases Grant R56-NIDDK-088093 (E. A. Heart), the Alexander Trust Fund, the James A. and Faith Miller Memorial Fund, and the Elisabeth Samuelsson Fund (J. P. Gray). J. P. Gray is a professor at the US Coast Guard Academy. The views presented here are those of J. P. Gray and not necessarily those of the US Coast Guard Academy or other branches of the US government.

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. approved the final version of the manuscript; E.A.H. drafted 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. edited and revised the manuscript; K.N.A., E.A.J., and E.A.H. approved the final version of the manuscript; E.A.H. drafted the manuscript.

REFERENCES


