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Protein histidine [de]phosphorylation in insulin secretion: abnormalities in models of impaired insulin secretion

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Abstract

In the majority of cell types, including the islet β -cell, transduction of extracellular signals involves ligand binding to a receptor, often followed by the activation G proteins and their effector modules. The islet β -cell is unusual in that glucose lacks an extracellular receptor. Instead, events consequent to glucose metabolism promote insulin secretion *via* the generation of diffusible second messengers and mobilization of calcium. A selective increase in intracellular calcium has been shown to regulate the phosphorylation status key islet proteins thereby facilitating insulin secretion. In addition to classical protein kinases [e.g., protein kinases A and C], recent studies from our laboratory have focused on the expression and function of various forms of NDPK/nm23-like histidine kinases in clonal β -cells, normal rodent, and human islets. Further, we recently reported localization of a cytosolic protein histidine phosphatase [PHP] in INS 832/13 cells, normal rat islets, and human islets. siRNA-mediated knock down of nm23-H1 and PHP in insulin-secreting INS 832/13 cells significantly attenuated glucose-induced insulin secretion. We also observed significant alterations in the expression and function of nm23-H1/PHP in β -cells chronically exposed to elevated levels of glucose and saturated fatty acids, such as palmitate (i.e., glucolipotoxicity). Similar changes were also noted in islets from the Goto-Kakizaki and Zucker Diabetic Fatty rats, two known models for type 2 diabetes. It is concluded that protein histidine phosphorylation–dephosphorylation cycles play novel regulatory roles in G protein-mediated physiological insulin secretion and that abnormalities in this signaling axis lead to impaired insulin secretion in glucolipo-toxicity and type 2 diabetes.

Keywords

Pancreatic islet; Insulin secretion; Histidine kinases; Histidine phosphatases; nm23

Introduction

Insulin secretion from the pancreatic β -cell is regulated principally by the circulating concentrations of glucose. However, the molecular and cellular mechanisms underlying the stimulus-secretion coupling of glucose-stimulated insulin secretion [GSIS] remain only partially understood. It is widely felt that GSIS is mediated largely *via* the generation of soluble second messengers, such as adenine [e.g., ATP] and guanine [e.g., GTP] nucleotides, cyclic nucleotides [e.g., cAMP], hydrolytic-products of phospholipases [e.g., arachidonic acid, lysophospholipids] and increase in intracellular calcium concentrations (Prentki and Matschinsky 1987; MacDonald 1990; Metz 1991; Newgard and McGarry 1995). It is also well established that intracellular generation of specific soluble second messengers leads to functional activation of various protein kinases [e.g., protein kinase-A, protein kinase-C, and calcium-calmodulin-dependent protein kinases] and phosphatases (e.g., protein phosphatase 2A, calcineurin etc.; Jones and Persaud 1998; Nesher et al. 2002; Kowluru 2005). While the above-mentioned protein kinases are known to phosphorylate their respective phosphoprotein substrates at serine [P-Ser], threonine [P-Thr], or tyrosine [P-Tyr] residues, available evidence also suggests expression and functional regulation of protein histidine kinases, which phosphorylate proteins at a histidine [P-His] residue in multiple cell types (Alex and Simon 1994; Matthews 1995; Klumpp and Krieglstein 2002; Steeg et al. 2003; Besant and Attwood 2005). In this context, our own studies have demonstrated expression of histidine kinases in a variety of insulin-secreting cells, including clonal β -cells and rodent and human islets (Kowluru 2003a, b, 2008 for reviews). Furthermore, emerging evidence demonstrates expression of protein histidine phosphatases (PHP) of insulin secretion in the islet β -cell, suggesting potential roles of PHP in physiological insulin secretion (Kamath et al. 2010). The following is a brief overview of the available evidence on the regulatory roles of protein histidine phosphorylation and dephosphorylation in the stimulus secretion of the pancreatic islet β -cell. Specifically, it describes the existing evidence on the identification, subcellular distribution and characterization of protein histidine kinases and protein histidine phosphatases, and on potential alterations in the protein histidine phosphorylation-dephosphorylation signaling axis in *in vitro* and *in vivo* models of glucolipotoxicity and diabetes.

Protein histidine kinases and phosphatases in insulin-secreting cells

Protein histidine kinases in insulin-secreting cells

As described above, in addition to adenine nucleotides [e.g., ATP], guanine nucleotides [e.g., GTP] have been shown to play key regulatory roles in GSIS. Along these lines, original observations from Metz's laboratory have suggested permissive roles for intracellular GTP in GSIS. For example, using mycophenolic acid [MPA], a selective inhibitor of inosine monophosphate dehydrogenase, a committed step in the *de novo* biosynthesis of GTP, these investigators have shown marked inhibition of GSIS in clonal β -cells and rodent islets (Metz et al. 1992, 1993). In addition to GTP, MPA treatment modestly, but significantly, reduced ATP and increased uridine triphosphate [UTP] levels. Interestingly, however, co-provision of adenine to MPA-treated cells normalized ATP and UTP levels, while leaving unaltered the decrements in intracellular GTP and GSIS, suggesting an essential role for GTP in GSIS. This formulation was further supported by their findings indicating a restoration of GTP levels and GSIS in MPA-treated cells following co-provision of guanine, which fuels the "salvage" pathway for purine synthesis.

Based on these observations, it was concluded that GTP is a permissive factor for exocytotic insulin release (Metz et al. 1992, 1993). Despite this compelling evidence for a regulatory role for GTP in GSIS, potential mechanisms, whereby it regulates GSIS, remains less understood even though it was speculated that it might be serving as a potential highenergy phosphate substrate for one [or more] G proteins endogenous to the islet (β -cell (Metz et al. 1992, 1993). In further support of regulatory roles for intracellular GTP in G protein activation, we demonstrated that glucose-induced carboxymethylation [and activation] of small G proteins (e.g., Cdc42; Kowluru et al. 1996a, b) and the γ -subunits of trimeric G proteins (Kowluru et al. 1997) was markedly attenuated in clonal HIT-T15 β -cells and normal rat islets following depletion of intracellular GTP by MPA. Together, these findings appear to implicate critical roles for intracellular GTP in glucose-induced activation of monomeric and trimeric G proteins and subsequent exocytotic secretion of insulin.

Shortly after the demonstration that GTP is required for GSIS, we reported expression of nucleoside diphosphate kinase [NDPK] in clonal β -cells, normal rat islets, and human islets and provided evidence to suggest that NDPK might regulate intracellular generation of GTP from ATP and GDP *via* transphosphorylation reactions [Kowluru and Metz 1994]. We have been able to quantitate NDPK activity in subcellular fractions isolated from normal rat islets and clonal β -cells. Western blot analyses and functional assays of NDPK activity [i.e., histidine autophosphorylation, NDPK activity] have demonstrated that nm23-H1 [NDPK-A] is predominantly localized in the soluble compartment, whereas nm23-H2 [NDPK-B] is found to be distributed in cytosolic as well as membranous compartments. Using purified subcellular fractions from isolated β -cells, we have also demonstrated that nm23-H4 remains associated with the mitochondrial fraction (Kowluru et al. 2002; Table 1). In addition to these, we characterized a novel protein histidine kinase in pancreatic β -cells, which exhibited sensitivity to acid and temperature for its ability to phosphorylate histone 4 [Kowluru 2002]. Such histone 4 phosphorylating histidine kinase [H4-HK] was detected in normal rat islets, human islets, and clonal β -[HIT-T15 and INS-1] cells. We demonstrated that H4-HK utilizes either ATP or GTP as a phosphoryl donor. On a size exclusion column, H4-HK's molecular mass was estimated to be in the range of 60–70 kDa. Like other islet NDPKs, H4-HK was inhibited by known *in vitro* inhibitors of protein histidine phosphorylation (e.g. UDP or cromoglycate; Kowluru 2002). Interestingly, Mastoparan [Mas], a global activator of G proteins (Higashijima et al. 1988, 1990) and insulin secretion from isolated β -cells (Kowluru 2003a, b and references therein), stimulated H4-HK activity. Mas-17, an inactive analog of Mas, failed to exert stimulatory effects on H4-HK and insulin secretion, suggesting a critical involvement of this activity in a G protein-mediated signaling pathway (Kowluru 2002). In summary, available evidence, thus far, suggests localization of at least four proteins with histidine kinase activity in insulin-secreting cells (Table 1).

Despite the aforestated evidence that nm23/H4-HK-like enzymes are localized in the islet β -cell, potential roles of these kinases in the stimulus-secretion coupling of GSIS remains poorly understood. Three lines of evidence in β -cells appear to implicate these proteins in GSIS. In the first, it has been shown that Mas, a global activator of G proteins and insulin secretion, but not its inactive analog, significantly stimulated the H4-HK activity and histidine phosphorylation of the β -subunit of trimeric G proteins (G β -subunit; Kowluru 2002). In the second, overexpression of an inactive mutant of nm23-H1 [H118F], has been shown to markedly reduce GSIS in INS 832/13 cells (Kowluru et al. 2006). In the third approach, we selectively knocked down nm23-H1 using siRNA-nm23-H1 [see footnote 1 for sequence of duplexes] and quantitated the effects of nm23-H1 depletion on GSIS in INS 832/13 cells. Our preliminary findings suggested a significant knock down [~40%] of nm23-H1 expression under these conditions and resulted in a marked attenuation [~50%] of GSIS in nm23-H1-depleted cells compared to scrambled siRNA-transfected cells. Together, these findings implicate nm23-H1 in physiological insulin secretion. The reader is referred to a

recent review article for additional descriptions of putative regulatory roles of protein histidine kinases in islet function and insulin secretion (Kowluru 2008).

Protein histidine phosphatases in insulin-secreting cells

Emerging evidence also suggests the existence of PHPs in many cell types (Klumpp and Krieglstein 2002, 2009; Pia et al. 2002; Wieland et al. 2010). Original observations from by Klumpp and Krieglstein have provided evidence on the identification and functional characterization of PHP in rat liver soluble extracts. Subsequent studies by these researchers, including amino acid analysis and measurements of inhibitor sensitivity, conclusively demonstrated that PHP [~ 16 kDa] is distinct from other known phosphatases acting on P-Ser, P-Thr, and P-Tyr (Klumpp and Krieglstein 2002). In a logical extension to our studies on protein histidine phosphorylation in islet function, we recently examined the expression and functional roles of PHPs physiological insulin secretion. Data from these studies demonstrated localization of a cytosolic PHP [an apparent molecular weight of 16 kDa] in INS 832/13 cells, normal rat islets, and human islets (Kamath et al. 2010). Furthermore, siRNA-mediated knock down of the expression of endogenous PHP markedly reduced glucose or mitochondrial fuel-induced, but not KCl-induced, insulin secretion in INS 832/13 cells. siRNA-mediated silencing of PHP expression also attenuated Mas-induced insulin secretion, suggesting its potential participation of PHP in a G protein-sensitive signaling step[s] leading to insulin secretion. In further support of our immunological and nm23-H1 knock down data, we have been able to quantitate PHP activity in lysates derived from INS 832/13 cells and normal rat islets by determining the rates of dephosphorylation of ^{32}P -labeled ATP-citrate lyase [ACL] in soluble liver extracts (Kamath et al. 2010). Even though our studies identified ACL as one of the potential substrates for PHP in insulin-secreting cells, the physiological significance of our findings in the context of GSIS is uncertain since considerable debate still exists in the context of putative roles of ACL in GSIS. For example, published evidence from MacDonald's laboratory suggested no effects of its shRNA-mediated knock down of ACL on GSIS in INS 832/13 cells (MacDonald et al. 2007). These findings are compatible with observations by Joseph and associates demonstrating no significant effects of ACL silencing on GSIS in rat islets (Joseph et al. 2007). On the contrary, Guay and coworkers reported a marked attenuation in GSIS in INS 832/13 cells following shRNA-mediated knock down of ACL (Guay et al. 2007). It should be noted that such differential effects of ACL knock down on GSIS were not attributable to differences in cell types since all the three studies utilized INS 832/13 cells. Further, percent reduction in the expression [or knock down] was comparable in these studies [~ 70 – 80%]. It should also be noted that Guay et al. have further confirmed their molecular biological findings using a pharmacological inhibitor of ACL [e.g., radicicol], which also reduced GSIS significantly in INS 832/13 cells. Together, these studies rule out the possibility that the contradictory findings on regulatory roles of ACL in GSIS may be due to cell type, method, or degree of knock down of ACL [i.e., mRNA, protein, or enzyme activity]. Lastly, our recent data from co-immunoprecipitation and confocal microscopy studies revealed minimal effects of stimulatory glucose concentrations in promoting interaction between ACL and PHP in INS 832/13 cells (Kamath et al. 2010). Therefore, it remains to be verified if PHP exerts its modulatory effects on GSIS *via* dephosphorylation of ACL. Taken together, findings described above on the expression and regulatory roles of protein histidine kinases and phosphatases in insulin secretion raise an important question on potential identity of

¹In siRNA-mediated knock down of nm23-H1 studies, we used a pool of three duplexes. The sequences for those duplexes are duplex A: sense, GGAAAGAAGUGAUCACAAAtt and antisense, UUUGU GAUCACUUCUUUCtt; duplex B: sense, GAACAAUUCUC CAACCUAUtt and antisense, AUAGGUUGGAGAAUUGUUCtt; and duplex C: sense, GUAGCUAAUCUCUU-GUGUUtt and antisense, AACACAAGAGAU-UAGCUACtt [all sequences are provided in 5'→3' orientation; purchased from Santa Cruz Biotechnology, Santa Cruz, CA].

candidate proteins, which are subjected to protein histidine phosphorylation and dephosphorylation. Furthermore, the signaling cascade underlying histidine phosphorylation–dephosphorylation-mediated insulin secretion appear more complex since, as described above, inhibition of histidine kinases as well as histidine phosphatases leads to inhibition of insulin secretion. This raises an interesting possibility that phosphorylation–dephosphorylation cycles of specific phosphoproteins might underlie insulin secretion. Additional studies are needed to identify the candidate histidine phosphorylated proteins, which are involved in various aspects of islet function, including facilitation of GSIS.

Potential defects in the expression and function of protein histidine kinases and phosphatases in islet models of impaired insulin secretion

The lesion intrinsic to the pancreatic β -cell in human type 2 diabetes remains only partially understood. In the search to identify such a defect, animal models of diabetes have frequently been used. We have utilized islets derived from the Goto-Kakizaki [GK] rat and the Zucker Diabetic Fatty [ZDF] rat in our studies to examine potential defects, if any, in the expression and activity of protein histidine kinases and phosphatase (Metz et al. 1999; Kowluru 2003a, b; Kamath et al. 2010). In addition, we studied potential defects in this signaling cascade in INS 832/13 cells or rodent islets chronically exposed to high glucose [i.e., glucotoxicity], saturated fatty acids, such as palmitate [i.e., lipotoxicity], or both (i.e., glucolipotoxicity; Veluthakal et al. 2009; Kamath et al. 2010). Data accrued from these studies are summarized in Table 2. It is evident that glucolipotoxic conditions significantly reduce the expression and function of protein histidine kinases. Interestingly, these observations held true for islets isolated from animal models of type 2 diabetes. It should also be noted that exposure of INS 832/13 cells to ceramide [CER], a sphingolipid generated from palmitate *via* the *de novo* pathway, also results in similar reduction in the expression and NDPK activity (Table 2). These data suggest that palmitate-mediated effects on nm23 expression and activity may, in part, be due to intracellularly generated CER (Veluthakal et al. 2009). Taken together, these data support the hypothesis that protein histidine phosphorylation and NDPK functions are significantly impaired in models of impaired insulin secretion. Furthermore, data on PHP in these model systems, albeit limited, appear to suggest modest to significant increase in the expression of PHP (Table 2). If such an increase in PHP expression translates into increased PHP activity, it would implicate significant defects in the histidine phosphorylation status of candidate proteins as a consequence of decreased kinase and increased phosphatase activities.

Lastly, it may be pertinent to ask the question: What is the significance of these findings, specifically at the level of impaired insulin secretion seen in the abovedescribed *in vitro* and *in vivo* models? Along these lines, previous studies by Metz and coworkers (Metz et al. 1999) have demonstrated that Mas, but not Mas-17, its inactive analog, completely restored insulin secretory defects in GK rat islets. Based on these observations and our findings described in Table 2, we propose that insulin secretory defects might be due to defects in Mas-sensitive signaling steps leading to insulin secretion. As stated above, these signaling steps may be more complex since Mas-induced insulin secretion is also reduced in cells in which PHP functions are negated *via* siRNA-PHP approaches (Kamath et al. 2010). Thus, it is likely that protein histidine phosphorylation–dephosphorylation cycles of specific proteins involved in insulin exocytosis is necessary for GSIS to occur. Additional studies are needed to further substantiate this hypothesis.

Future directions

Based on the above discussion, it is likely that defects in histidine kinase–phosphatase signaling cascade could lead to insulin secretory abnormalities and metabolic dysfunction of

the islet β -cell. We will highlight four potential avenues to further examine roles of nm23-like enzymes and PHPs in islet function and insulin secretion.

First, emerging evidence clearly implicates novel regulatory roles of protein histidine kinase–phosphatase signaling axis in islet β -cell function. Despite these advances, a significant gap still exists in regard to potential regulation of G protein [i.e., trimeric as well as monomeric] activation by nm23-H1-like enzymes. For example, several earlier studies have provided convincing evidence to suggest direct activation of trimeric G proteins by NDP kinase B (Wieland et al. 1993; Cuello et al. 2003; Hippe et al. 2003; Hippe et al. 2009). They also demonstrated novel roles for NDP kinase B in basal and β -adrenoreceptor-mediated activation of adenylate cyclase and associated intracellular generation of cAMP (Hippe et al. 2011). However, very little is known of such roles for nm23-like enzymes in the activation of trimeric G proteins in the islet β -cell. In this context, we reported earlier (Kowluru et al. 1996a, b) that the G β -subunit undergoes histidine phosphorylation in the membrane and secretory granule fractions purified from normal rat islets, human islets, and clonal (β -cell preparations. In reconstitution protocols involving the purified $\alpha\beta\gamma$ -subunits and islet subcellular fractions, we also demonstrated that a membrane-associated factor [nm23-like kinase?] is required for G β -subunit phosphorylation to occur. Lastly, in the same studies, we were able to demonstrate that incubation of the phosphorylated G β -subunit with purified Ga.GDP resulted in rapid dephosphorylation of G β -subunit followed by emergence of Ga.GTP. These data do not provide adequate information with regard to the identity of the putative kinase; it is likely that the membrane-associated nm23-like enzyme might be involved in the phosphorylation of G β -subunit. Such a working model is compelling to test since glucose, the major physiological insulin secretagogue elicits insulin secretory effects in a non-receptor-dependent fashion (Kowluru 2003a, b, 2008 for reviews).

Second, data overviewed herein suggest significant reduction in the expression and activity of nm23-like enzymes *in vitro* and *in vivo* models of glucolipotoxicity, impaired insulin secretion, and diabetes. Further, we recently reported a significant increase in the expression [at the protein level] of PHP in islet β -cells exposed to high glucose or saturated fatty acids *in vitro* and in islets from the ZDF rat, a model for obesity and type 2 diabetes (Kamath et al. 2010). Still a considerable amount of work is needed to precisely identify the phosphoprotein substrates, the phosphorylation status of which is altered significantly in β -cells under the duress of glucolipotoxicity and diabetes. Further, studies from multiple laboratories have utilized nm23/NDP kinase knockout animals to assess the regulatory roles of this class of proteins in cellular function (Arnaud-Dabernat et al. 2003; Di et al. 2010). Indeed, an examination of such knockout animal models might provide fresh insights in further confirmation of roles of nm23/NDPK-like proteins in physiological insulin secretion.

Third, our recent findings suggested that exposure of INS 832/13 cells to CER results in a dose-dependent inhibition of the expression of nm23-H1 and nm23-H2 and the associated NDP kinase activity (Veluthakal et al. 2009). Under these conditions, CER has been shown to induce a significant reduction in the mitochondrial membrane potential, cytochrome-C release, caspase-3 activation, and metabolic dysfunction of the (β -cell (Veluthakal et al. 2005). Interestingly, several recent studies (Becker et al. 2010a, b, c) have demonstrated damaging roles of sphingolipids, including CER in inflammation seen in cystic fibrosis, a condition in which significant defects in insulin secretion, impaired glucose tolerance, and the onset of diabetes have been widely reported (Tofe et al. 2005; Alves Cde et al. 2007; Laguna et al. 2010). Indeed, recent studies have also shown that small molecule inhibitors of acid sphingomyelinases [e.g., amitriptyline] normalized CER levels in cystic fibrosis (Becker et al. 2010b). Furthermore, using proteomics approach Gomes-Alves and coworkers (Gomes-Alves et al. 2010) have recently documented a link between NDPK B and cystic fibrosis. Indeed, these studies could form the basis for the hypothesis that abnormal

sphingolipid/ CER metabolism seen in cystic fibrosis could contribute to the β -cell failure, specifically by negatively modulating the expression and functional regulation of nm23-like enzymes. Indeed, these hypotheses can be tested in animal models of cystic fibrosis (Becker et al. 2010a, b, c; Gulbins 2010).

Lastly, recent studies from multiple laboratories, including ours clearly suggested that a tonic increase in the generation of reactive oxygen species [ROS] may be necessary for insulin secretion elicited by glucose and mitochondrial fuels. Further, these studies have implicated phagocyte-like NADPH oxidase [Nox] as one of the potential sources for the increased ROS in the stimulated β -cell (see Kowluru 2011 for a recent review). Interestingly, however, chronic activation of Nox in β -cells following exposure to high glucose, high saturated fatty acids [e.g., palmitate; as above], CER or a mixture of cytokines [i.e., IL-1 β , TNF- α , and IFN- γ] has been shown to create increased oxidative stress in the β -cell, which may, in part, be due to excessive ROS generated via the Nox signaling pathway (Syed et al. 2010; Subasinghe et al. 2011). Although not demonstrated in the islet β -cell, recent data in other cell types appear to suggest antioxidant roles for nm23-H1. For example, it has been shown that overexpression of nm23-H1 in HeLa cells afforded a higher protection against oxidative stress potentially involving crosstalk between nm23-H1 and p53 signaling pathways (An et al. 2008). It has also been demonstrated nm23-H1 undergoes functional inactivation under a variety of oxidation conditions via disulfide cross-linking. These studies have also demonstrated that glutathionylation and oxidation of Cys-109 markedly reduced the NDP kinase activity of nm23-H1. Additional studies revealed that oxidation of nm23-H1 also inhibited its ability as a metastasis suppressor. Potential roles of Cys-109 in the retention of metastasis suppressor and enzyme activities was further supported by the observations indicating that overexpression of nm23-H1-C109A mutant retained the enzymatic and metastasis suppressor activities under the duress of oxidative stress (Le et al. 2009). Therefore, although it remains to be verified experimentally, it is plausible that the generation of excessive ROS and associated oxidative stress seen under conditions of gluco-, lipo-, or glucolipotoxicity and cytokine or CER exposure could potentially lead to functional inactivation of nm23-H1 in the islet β -cell leading to defects in the downstream signaling events. These aspects are being investigated currently.

Abbreviations

ACL	ATP-citrate lyase
CER	Ceramide
GSIS	Glucose-stimulated insulin secretion
GK rat	Goto-Kakizaki rat
H4-HK	Histone 4 phosphorylating histidine kinase
IMPDH	Inosine monophosphate dehydrogenase
MPA	Mycophenolic acid
NDPK	Nucleoside diphosphate kinase
Nox	Phagocyte-like NADPH oxidase
PHP	Protein histidine phosphatase
ROS	Reactive oxygen species
ZDF rat	Zucker diabetic fatty rat

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References

- Alex LA, Simon MI. Protein histidine kinases and signal transduction in prokaryotes and eukaryotes. *Trends Genet.* 1994; 10:133–138. [PubMed: 8029829]
- Alves Cde A, Aguiar RA, Alves AC, Santana MA. Diabetes mellitus in patients with cystic fibrosis. *J Bras Pneumol.* 2007; 33:213–221. [PubMed: 17724542]
- An R, Chu YL, Tian C, Dai XX, Chen JH, Shi Q, Han J, Dong XP. Over-expression of nm23-H1 in HeLa cells provides cells with higher resistance to oxidative stress possibly due to raising intracellular p53 and GPX1. *Acta Pharmacol Sin.* 2008; 29:1451–1458. [PubMed: 19026164]
- Arnaud-Dabernat S, Bourbon PM, Dierich A, Le Meur M, Daniel JY. Knockout mice as a model systems for studying nm23/ NDP kinase gene functions. Application to the nm23-M1 gene. *J Bioenerg Biomembranes.* 2003; 35:19–30.
- Becker KA, Tummaller B, Gulbins E, Grassme H. Accumulation of ceramide in the trachea and intestine of cystic fibrosis mice causes inflammation and cell death. *Biochem Biophys Res Commun.* 2010a; 403:368–374. [PubMed: 21078296]
- Becker KA, Riethmuller J, Luth A, Doring G, Kleuser B, Gulbins E. Acid sphingomyelinase inhibitors normalize pulmonary ceramide and inflammation in cystic fibrosis. *Am J Respir Cell Mol Biol.* 2010b; 42:716–724. [PubMed: 19635928]
- Becker KA, Riethmuller J, Zhang Y, Gulbins E. The role of sphingolipids and ceramide in pulmonary inflammation in cystic fibrosis. *Open Respir Med J.* 2010c; 4:39–47. [PubMed: 20556203]
- Besant PG, Attwood PV. Mammalian histidine kinases. *Biochim Biophys Acta.* 2005; 1754:281–290. [PubMed: 16188507]
- Cuello F, Schulze RA, Heemeyer F, Meyer HE, Lutz S, Jakobs KH, Niroomand F, Wieland T. Activation of heterotrimeric G proteins by a high energy phosphate transfer via nucleoside diphosphate kinase (NDPK) and G β subunits. Complex formation of NDPK B with G $\beta\gamma$ dimers and phosphorylation of His-266 in G β . *J Biol Chem.* 2003; 278:7220–7226. [PubMed: 12486123]
- Di L, Srivastava S, Zhdanova O, Sun Y, Li Z, Skolnik EY. Nucleoside diphosphate kinase B knock-out mice have impaired activation of the K⁺ channel Kca3.1, resulting in defective T cell activation. *J Biol Chem.* 2010; 285:38765–38771. [PubMed: 20884616]
- Gomes-Alves P, Couto F, Pesquita C, Coelho AV, Penque D. Rescue of F508del-CFTR by RXR motif inactivation triggers proteome modulation associated with unfolded protein response. *Biochim Biophys Acta.* 2010; 1804:856–865. [PubMed: 20044041]
- Guay C, Madiraju SR, Aumais A, Joly E, Prentki M. A role for ATP citrate lyase, malic enzyme, and pyruvate/citrate cycling in glucose-induced insulin secretion. *J Biol Chem.* 2007; 282:35657–35665. [PubMed: 17928289]
- Gulbins E. Lipids control mucus production in cystic fibrosis. *Nat Med.* 2010; 16:267–268. [PubMed: 20208510]
- Higashijima T, Uzu S, Nakajima T, Ross EM. Mastoparan, a peptide toxin from wasp venom mimics receptors by activating GTP-binding regulatory proteins (G-proteins). *J Biol Chem.* 1988; 263:6491–6494. [PubMed: 3129426]
- Higashijima T, Burnier J, Ross EM. Regulation of G_i and G_o by mastoparan, related amphiphilic peptides, and hydrophobic amines. *J Biol Chem.* 1990; 265:14176–14181. [PubMed: 2117607]
- Hippe HJ, Lutz S, Cuello F, Knorr K, Vogt A, Jakobs KH, Wieland T, Niroomand F. Activation of heterotrimeric G proteins by a high energy phosphate transfer via nucleoside diphosphate kinase (NDPK) and G β subunits. Specific activation of G α by an NDPK B-G $\beta\gamma$ complex in H10 cells. *J Biol Chem.* 2003; 278:7227–7233. [PubMed: 12486122]
- Hippe HJ, Wolf NM, Abu-Taha I, Mehninger R, Just S, Lutz S, Niroomand F, Postel EH, Katus HA, Rottbauer W, Wieland T. The interaction of nucleoside diphosphate kinase B with G $\beta\gamma$ dimers

- controls heterotrimeric G protein activation. *Proc Natl Acad Sci USA*. 2009; 106:16269–16274. [PubMed: 19805292]
- Hippe HJ, Abu-Taha I, Wolf NM, Katus HA, Wieland T. Through scaffolding and catalytic actions nucleoside diphosphate kinase B differentially regulates basal and β -adrenoreceptor-stimulated cAMP synthesis. *Cell Signal*. 2011; 23:579–585. [PubMed: 21111809]
- Jones PM, Persaud SJ. Protein kinases, protein phosphorylation, and the regulation of insulin secretion from pancreatic β -cells. *Endocr Rev*. 1998; 19:429–461. [PubMed: 9715374]
- Joseph JW, Odegaard ML, Ronnebaum SM, Burgess SC, Muehlbauer J, Sherry AD, Newgard CB. Normal flux through ATP-citrate lyase or fatty acid synthase is not required for glucose-stimulated insulin secretion. *J Biol Chem*. 2007; 282:31592–31600. [PubMed: 17823126]
- Kamath V, Kyathanahalli CN, Jayaram B, Syed I, Olson K, Ludwig K, Klumpp S, Krieglstein J, Kowluru A. Regulation of glucose- and mitochondrial fuel-induced insulin secretion by a cytosolic protein histidine phosphatase in pancreatic beta-cells. *Am J Physiol Endocrinol Metab*. 2010; 299:E276–E286. [PubMed: 20501872]
- Klumpp S, Krieglstein J. Phosphorylation and dephosphorylation of histidine residues in proteins. *Eur J Biochem*. 2002; 269:1067–1071. [PubMed: 11856347]
- Klumpp S, Krieglstein J. Reversible phosphorylation of histidine residues in proteins from vertebrates. *Sci Signal*. 2009; 2:pe13. [PubMed: 19278958]
- Kowluru A. Identification and characterization of a novel protein histidine kinase in the islet β -cell: evidence for its regulation by mastoparan, an activator of G-proteins and insulin secretion. *Biochem Pharmacol*. 2002; 63:2091–2100. [PubMed: 12110368]
- Kowluru A. Defective protein histidine phosphorylation in islets from the Goto-Kakizaki diabetic rat. *Am J Physiol Endocrinol Metab*. 2003a; 285:E498–E503. [PubMed: 12799314]
- Kowluru A. Regulatory roles for small G-proteins in the pancreatic beta cell: lessons from models of impaired insulin secretion. *Am J Physiol Endocrinol Metab*. 2003b; 285:E669–E684. [PubMed: 12959934]
- Kowluru A. Novel regulatory roles for protein phosphatase-2A in the islet beta cell. *Biochem Pharmacol*. 2005; 69:1681–1691. [PubMed: 15935144]
- Kowluru A. Emerging roles for protein histidine phosphorylation in cellular signal transduction: lessons from the islet beta-cell. *J Cell Mol Med*. 2008; 12:1885–1908. [PubMed: 18400053]
- Kowluru A. Friendly, and not so friendly, roles of Rac1 in islet β -cell function: lessons learnt from pharmacological and molecular biological approaches. *Biochem Pharmacol*. 2011 doi: 10.1016/j.bcp.2011.01.013.
- Kowluru A, Metz SA. Characterization of nucleoside diphosphokinase activity in human and rodent pancreatic β cells: evidence for its role in the formation of guanosine triphosphate, a permissive factor for nutrient-induced insulin secretion. *Biochemistry*. 1994; 33:12495–12503. [PubMed: 7918472]
- Kowluru A, Seavey SE, Rhodes CJ, Metz SA. A novel regulatory mechanism for trimeric GTP-binding proteins in the membrane and secretory granule fractions of human and rodent beta-cells. *Biochem J*. 1996a; 313:97–108. [PubMed: 8546716]
- Kowluru S, Seavey SE, Li G, Sorenson RL, Weinhaus AJ, Nesher R, Rabaglia ME, Vadakekalam J, Metz SA. Glucose- and GTP-dependent stimulation of the carboxyl methylation of Cdc42 in rodent and human pancreatic islets and pure beta cells. Evidence for an essential role of GTP-binding proteins in nutrient-induced insulin secretion. *J Clin Invest*. 1996b; 98:540–555. [PubMed: 8755667]
- Kowluru A, Li G, Metz SA. Glucose activates the carboxyl-methylation of gamma subunits of trimeric GTP-binding proteins in pancreatic beta cells. Modulation in vivo by calcium, GTP, and pertussis toxin. *J Clin Invest*. 1997; 100:1596–1610. [PubMed: 9294129]
- Kowluru A, Tannous M, Chen HQ. Localization and characterization of the mitochondrial isoform of the nucleoside diphosphate kinase in the pancreatic β -cell: evidence for its complexation with mitochondrial succinyl-CoA synthetase. *Arch Biochem Biophys*. 2002; 398:160–169. [PubMed: 11831846]

- Kowluru A, Veluthakal R, Kaetzel DM. Regulatory roles for nm23/nucleoside diphosphate kinase-like enzymes in insulin secretion from the pancreatic islet beta cell. *J Bioenerg Biomembr*. 2006; 38:227–232. [PubMed: 16957985]
- Laguna TA, Nathan BM, Moran A. Managing diabetes in cystic fibrosis. *Diab Obes Metab*. 2010; 12:858–864.
- Le E, Jeong J, Kim SE, Song EJ, Kang SW, Lee K-J. Multiple functions of nm23-H1 are regulated by oxido-reduction system. *PlosOne*. 2009; 4:1–14.
- MacDonald MJ. Elusive proximal signals of beta-cells for insulin secretion. *Diabetes*. 1990; 39:1461–1466. [PubMed: 2245873]
- MacDonald MJ, Smith AD III, Hasan NM, Sabat G, Fahein LA. Feasibility of pathways for transfer of acyl groups from mitochondria to the cytosol to form short chain acyl-CoAs in the pancreatic beta cell. *J Biol Chem*. 2007; 282:30596–30606. [PubMed: 17724028]
- Matthews HR. Protein kinases and phosphatases that act on histidine, lysine, or arginine residues in eukaryotic proteins: a possible regulator of the mitogen activated protein kinase cascade. *Pharmacol Ther*. 1995; 67:323–350. [PubMed: 8577821]
- Metz SA. The pancreatic islet as a Rubik's cube. Is phospholipid hydrolysis a piece of the puzzle. *Diabetes*. 1991; 40:1565–1573. [PubMed: 1756897]
- Metz SA, Rabaglia ME, Pintar. Selective inhibitors of GTP synthesis impede exocytotic insulin release from intact rat islets. *J Biol Chem*. 1992; 267:12517–12527. [PubMed: 1352288]
- Metz SA, Meredith M, Rabaglia ME, Kowluru A. Small elevations of glucose concentration redirect and amplify the synthesis of guanosine 5'-triphosphate in rat islets. *J Clin Invest*. 1993; 92:872–882. [PubMed: 8349822]
- Metz SA, Meredith ME, Vadakekalam J, Rabaglia ME, Kowluru A. A defect late in stimulus-secretion coupling impairs insulin secretion in Goto Kakizaki diabetic rats. *Diabetes*. 1999; 48:1754–1762. [PubMed: 10480605]
- Nesher R, Anteby E, Yedovizky M, Warwar N, Cerasi E. Beta-cell protein kinases and the dynamics of the insulin response to glucose. *Diabetes*. 2002; 51 suppl 1:S63–S73.
- Newgard CB, McGarry JD. Metabolic coupling factors in pancreatic β -cell signal transduction. *Annu Rev Biochem*. 1995; 64:689–719. [PubMed: 7574498]
- Pia EK, Pettersson G, Bo EK, Gong F, Li JP, Zetterqvist O. Identification and characterization of mammalian 14-kDa phosphohistidine phosphatase. *Eur J Biochem*. 2002; 269:5016–5023. [PubMed: 12383260]
- Prentki M, Matschinsky FM. Calcium, cAMP, and phospholipid-derived messengers in coupling mechanisms of insulin secretion. *Physiol Rev*. 1987; 67:1223–1226.
- Steeg PS, Palmieri D, Ouatas T, Salerno M. Histidine kinases and histidine phosphorylated proteins in mammalian cell biology, signal transduction and cancer. *Can Lett*. 2003; 190:1–12.
- Subasinghe I, Syed I, Kowluru A. Phagocyte-like NADPH oxidase promotes cytokine mitochondrial dysfunction in pancreatic beta-cells: evidence for regulation by Rac1. *Am J Physiol Regul Integr Comp Physiol*. 2011; 300 R21–20.
- Syed I, Jayaram B, Subasinghe W, Kowluru A. Tiam1/Rac1 signaling pathway mediates palmitate-induced, ceramide-sensitive generation of superoxides and lipid hydroperoxides and the loss in mitochondrial membrane potential in pancreatic beta-cells. *Biochem Pharmacol*. 2010; 80:874–883. [PubMed: 20493824]
- Tofe S, Moreno JC, Maiz L, Alonso M, Escobar H, Barrio R. Insulin-secretion abnormalities and clinical deterioration related to impaired glucose tolerance in cystic fibrosis. *Eur J Endocrinol*. 2005; 152:241–247. [PubMed: 15745932]
- Veluthakal R, Palanivel R, Zhao Y, McDonald P, Gruber S, Kowluru A. Ceramide induces mitochondrial abnormalities in insulin-secreting INS-1 cells: potential mechanisms underlying ceramide-mediated metabolic dysfunction of the beta-cell. *Apoptosis*. 2005; 10:841–850. [PubMed: 16133874]
- Veluthakal R, Suresh MV, Kowluru A. Down-regulation of expression and function of nucleoside diphosphate kinase in insulin-secreting beta-cells under in vitro conditions of glucolipotoxicity. *Mol Cell Biochem*. 2009; 329:121–129. [PubMed: 19367376]

- Wieland T, Nunberg B, Ulibarri I, Kaldenberg-Stasch S, Schultz G, Jakobs KH. Guanine nucleotide-specific phosphate transfer by guanine-binding regulatory protein beta-subunits. Characterization of phosphorylated amino acids. *J Biol Chem.* 1993; 268:18111–18118. [PubMed: 8349688]
- Wieland T, Hippe HJ, Ludwig K, Zhou XB, Korth M, Klumpp S. Reversible histidine phosphorylation in mammalian cells: a teeter-totter formed by nucleoside diphosphate kinase and protein histidine phosphatase 1. *Meth Enzymol.* 2010; 471:379–402. [PubMed: 20946858]

Table 1

Subcellular distribution of histidine kinases in insulin-secreting cells

Type of histidine kinase[s]	Subcellular localization	Reference
nm23-H1	Predominantly cytosolic	Kowluru et al. 2002
nm23-H2	Cytosolic as well as membranous	
nm23-H4	Mitochondrial	
Histone H4 phosphorylating histidine kinase [H4-HK]	Cytosolic	Kowluru 2002

Table 2

Summary of evidence suggesting significant defects in protein histidine kinases and PHP in in vitro and in vivo models of gluco-, lipo-, glucolipotoxicity, and diabetes

Condition[s] and sources of β -cells	Effects on protein histidine kinases	Effects on PHP
In vitro exposure of INS 832/13 cells to high glucose	Significant reduction in nm23-H1 and nm23-H2 expression and associated NDP kinase activity (Veluthakal et al. 2009)	Modest, but significant increase in the expression of PHP (Kamath et al. 2010)
In vitro exposure of INS 832/13 cells to palmitate	Marked reduction in nm23-H1 and nm23-H2 expression and associated NDP kinase activity (Veluthakal et al. 2009)	Not determined
In vitro exposure of INS 832/13 cells to cell-permeable ceramide	Significant reduction in the nm23-H1 and nm23-H2 expression and associated NDP kinase activity (Veluthakal et al. 2009)	Not determined
In vitro exposure of normal rat islets to high glucose	Not determined	Significant increase in the expression of PHP (Kamath et al. 2010)
Isolated islets from Goto-Kakizaki rat	Marked reduction in NDPK expression, autophosphorylation, and activity of NDPK. Significant reduction in histidine phosphorylation of histone 4 phosphorylation (Metz et al. 1999; Kowluru 2003a, b)	Not determined
Isolated islets from ZDF rat	Not determined	Significant increase in the expression of PHP (Kamath et al. 2010)