Current Perspective

Dual Role of Nitric Oxide in Pancreatic β-Cells

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Abstract. An involvement of inducible nitric oxide (NO) synthase (NOS) in pancreatic β-cell degeneration during the process of type 1 diabetes has been well discussed. Recently, there is growing evidence for pivotal roles of constitutive NOS (cNOS) in β-cells; the presence of NOS1 and NOS3 in pancreatic β-cells and the effects of low-concentration NO, which is assumed to be derived from cNOS, on β-cell functions have been reported. However, the roles of cNOS-derived NO in β-cells are still under debate. One of the reasons seems to be that NO has multiple biological activities, which are dependent on its concentration. In β-cells, NO has been shown to exert positive and negative regulation of insulin secretion and anti- and pro-apoptotic activities, which is likely to be dependent on concentrations. In this review article, we will describe the current understanding of the roles of NO in pancreatic β-cells, especially focusing on cNOS-derived NO and its differential roles depending on concentrations.

Keywords: nitric oxide, pancreatic islet, β-cell, insulin secretion, apoptosis

1. Introduction

Pancreatic β-cells play a critical role in controlling glucose homeostasis by secreting the hormone insulin. In type 2 diabetes mellitus (DM), both a progressive defect in β-cell insulin secretion and insulin sensitivity are involved, and the former depends on β-cell function and mass. Elevated levels of glucose and free fatty acids (FFAs) in plasma are assumed to further cause impaired insulin secretion and β-cell apoptosis (1, 2). Thus, glucotoxicity and lipotoxicity are likely contributors to impairments during the development of type 2 DM.

Nitric oxide (NO) is a key mediator in various physiological and pathophysiological processes, which is produced by diverse sources including pancreatic β-cells. NO synthase (NOS) synthesizes NO through an NADPH-dependent conversion of L-arginine to citrulline. There are three NOS isoforms: NOS1 (neuronal NOS, nNOS), NOS2 (inducible NOS, iNOS), and NOS3 (endothelial NOS, eNOS); NOS1 and NOS3 are constitutively expressed and Ca2+/calmodulin-dependently activated, while NOS2 is induced by various stimuli, including inflammatory cytokines and hypoxia, and produces large amounts of NO (3). A small amount of NO, which is produced by constitutive NOS (cNOS), i.e., NOS1 and NOS3, plays a crucial role in a number of physiological functions. NO is well known to bind to the regulatory heme in soluble guanylate cyclase, producing cGMP. Most of the physiological effects of cNOS-derived NO are primarily mediated by the cGMP-dependent mechanism, although a small amount of NO may also regulate the activity of various proteins by post-translational modifications such as S-nitrosylation, independently of cGMP. In contrast, a large amount of NO produced by inducible NOS2 mediates the antimicrobial activity of macrophages (4), but is often attributed to pathophysiological changes through indirect as well as direct effects. The indirect effects involve the reaction of NO with superoxide anion (O2−) to produce peroxynitrite (ONOO−), with H2O2 to produce hydroxyl radical, or with O2 to produce dinitrogen trioxide (N2O3), a nitrosative stress intermediary, to generate highly toxic reactive species.

A large number of studies have provided evidence for an involvement of NOS2 in β-cell degeneration during the process of type 1 DM (1). In addition, there is growing evidence for pivotal roles of cNOS-derived NO in pancreatic β-cells; the presence of constitutive NOS1...
and NOS3 in pancreatic β-cells and physiological roles of cNOS-derived NO, such as modulation of insulin secretion and protection of β-cells from apoptosis, are suggested. It is highly possible that the deregulation of cNOS-derived NO production or metabolism is involved in the onset and development of type 2 DM. In this review article, we will describe the current understanding of the roles of NO in pancreatic β-cells, especially focusing on cNOS-derived NO and its differential roles depending on concentrations.

2. NO production in pancreatic islets

Several studies have demonstrated the presence of NOS1 in pancreatic β-cells (5–7). Moreover, the amino acid sequence of NOS1 expressed in rat pancreatic islets and the β-cell line INS-1 was determined, which presents a 99.8% homology of amino acid sequence with the NOS1 cloned in the cerebellum (5). In contrast to NOS1, there is less information about NOS3 in pancreatic islets. We have shown positive immunostaining for NOS3 in β- and δ-cells, but not in α-cells, in adult male rats (6). The expression of NOS3 was also confirmed by western blotting in the β-cell line INS-1 (Kaneko, unpublished data) and RINm5F (8). We further demonstrated the production of NO in β-cells by image analysis with the NO-reactive fluorescence dye DAF-2: glucose increased the DAF-2 fluorescence in β-cells, which was inhibited by a blocker of voltage-dependent Ca2+ channels (VDCC) or an NOS inhibitor, suggesting that NO is produced by glucose via the activation of cNOS in β-cells in a Ca2+-dependent manner (6).

3. Dual effects of NO on insulin secretion

Glucose-stimulated insulin secretion (GSIS) from pancreatic β-cells is attributed to a sequence of events; acceleration of glucose metabolism, ATP/ADP ratio elevation, closure of ATP-sensitive K+ (KATP) channels, membrane depolarization, VDCC activation, and exocytosis triggered by Ca2+ influx (9). This KATP channel–dependent mechanism is the primary pathway for GSIS. GSIS consists of two phases: a transient first phase and a sustained second phase. The first phase is currently ascribed to a KATP channel–dependent increase in Ca2+ influx through VDCC that triggers exocytosis of a readily releasable pool of insulin granules. The second phase requires both the triggering elevation of intracellular Ca2+ concentration ([Ca2+]i) and the augmentation of exocytosis via KATP channel–independent mechanisms. Although several possible mechanisms have been suggested for the KATP channel–independent mechanisms (10), it still remains to be established.

The roles of endogenous NO in the regulation of insulin secretion from β-cells have been a matter of controversy. Nonselective NOS inhibitors, such as Nω-nitro-l-arginine methyl ester (l-NAME) and Nε-nitro-l-arginine (l-NNA), were shown to inhibit insulin secretion induced by l-arginine, a substrate for NOS, in the β-cell line HIT-T15 (11) and in isolated rat islets (12). NOS inhibition also decreased insulin secretion from isolated human pancreas (13) and plasma insulin level in healthy humans (14). These findings suggest a stimulatory effect of NO on insulin secretion. Conversely, NOS inhibitors were shown to increase l-arginine- or glucose-induced insulin secretion from isolated islets of mice (15) and rats (16). Moreover, controversial data have also been presented that aqueous NO solution or some NO donors stimulated (17, 18) or inhibited (15, 19) insulin secretion. We have provided evidence for concentration-dependent dual effects of NO on insulin secretion, which could explain at least partly the discrepancies (20). In most studies showing the inhibitory effects of NO on insulin secretion, relatively high concentrations of NO donors or aqueous NO solution were used, whereas relatively low concentrations of NO donors were used in most studies showing the stimulatory effects. We clearly demonstrated that NO at tens of nanomolar concentrations facilitates glucose-induced [Ca2+]i oscillations of β-cells and insulin secretion in a cGMP-dependent manner, whereas NO at sub-micromolar concentrations inhibits them in a cGMP-independent manner (20). To reveal the mechanisms underlying the dual role of NO, we have investigated the effects of the NO donor NOC7 on KATP channel activity in isolated rat β-cells using patch clamp analysis (21). In accordance with the dual effects on insulin secretion, low-concentration NO exerted an inhibitory effect on KATP channels, which would lead to facilitation of insulin secretion, whereas high-concentration NO activated KATP channels. Moreover, we showed that the inhibitory effect of low-concentration NO on KATP channel activity is mediated by cGMP, while the stimulation by high-concentration NO is independent of cGMP. These findings indicate that NO has dual effects on insulin secretion depending on its concentration and through different mechanisms. The inhibition of phosphofructokinase activity may be the site responsible for the impairment of glucose metabolism and KATP-channel activation induced by NO in β-cells (16). At further higher concentrations, NO seems to attenuate insulin secretion by inhibiting the oxidation of glucose to CO2 and the activity of mitochondria iron-sulfur center–containing enzymes such as aconitase and complexes of the electron transporter system (22, 23).

In addition, intriguing studies about NO function on insulin secretion have been reported in association with
glucokinase (GK). The phosphorylation of glucose by GK is the rate-limiting step for insulin secretion from β-cells and thus net GK activity is a critical determinant of glucose sensitivity in β-cells. In the β-cell line βTC3 expressing fluorescent proteins fused to GK, the localization of GK and the association of GK with insulin secretory granules were modulated through NO generated by NOS1 (7). Moreover, mutation of cysteine-371 to serine, which blocks S-nitrosylation of GK, caused GK to remain tightly bound to insulin secretory granules. On the other hand, naturally occurring, diabetes-associated point mutations in GK have been shown to negatively affect S-nitrosylation of GK (24). Thus, S-nitrosylation of GK by NOS1-derived NO is suggested to promote the dissociation of GK from insulin secretory granules, thereby facilitating insulin secretion. This GK activation by S-nitrosylation may contribute to beneficial effects of glucagon-like peptide 1 (GLP-1) on β-cell functions (25, 26).

Conversely, there are reports supporting that GK is negatively regulated by tyrosine nitration by NO. Chronic ethanol consumption, which is known as a risk factor in type 2 DM, induced the down-regulation and inactivation of GK by inducing tyrosine nitration of GK, resulting in pancreatic β-cell apoptosis and dysfunction (27).

Recently, S-nitrosylation of Syntaxin 4 by NO has been reported to be involved in exocytosis of insulin granules in the β-cell line MIN6 and human islets (28). S-nitrosylation of Syntaxin 4 occurred in response to acute glucose stimulation within 5 min and resulted in conformation changes of SNARE proteins and facilitation of insulin granule docking and fusion with the plasma membrane. Moreover, since other candidate proteins in β-cells targeted to S-nitrosylation by NO are proposed (29), novel mechanisms for the regulation of insulin secretion by NO may be provided in future studies.

4. Dual effects of NO on β-cell mass

Pancreatic β-cell mass is regulated by at least four independent ways, i.e., β-cell replication, β-cell size, β-cell neogenesis, and β-cell apoptosis (30). Among them, increased β-cell apoptosis is considered to be the most crucial factor contributing to the onset and/or development of type 1 and type 2 DM (1, 2). Similarly to the regulation of insulin secretion, NO exhibits dual effects on the regulation of β-cell apoptosis in a concentration-dependent manner: anti-apoptotic and pro-apoptotic effects at low and high concentrations, respectively (31).

In type 1 DM, β-cells are destroyed by autoimmunity through the action of pro-inflammatory cytokines: interleukin (IL)-1β per se or in combination with interferon (IFN)-γ activates the transcription factor nuclear factor (NF)-κB, which regulates numerous genes in β-cells including that encoding NOS2 (32). The large amount of NO produced by NOS2 is one of the mediators involved in β-cell apoptosis in type 1 DM (33). The NO-induced β-cell apoptosis has been reported in several β-cell lines, INS-1 (31, 34), RINm5F (35), and MIN6 (36). NO induces β-cell apoptosis in a cGMP-independent manner: NO or RNS formed from NO increases the stability and transcriptional activity of p53, which induces the expression of pro-apoptotic proteins such as Bax and PUMA (37). On the other hand, a p53-independent mechanism has also been suggested in which NO inhibits Ca2+-ATPase of the endoplasmic reticulum (ER), thereby inducing ER stress (38). A reduction in β-cell mass is also observed in type 2 DM. However, hyperglycemia or hyperlipidemia in type 2 DM seems not to induce IL-1β expression or NF-κB activation in β-cells (1, 39). Thus, the mechanisms of β-cell death in type 1 and type 2 DM are suggested to be different and NO is unlikely to be involved in the onset of type 2 DM.

Conversely, low-concentration NO, which is assumed to be produced by eNOS, has been shown to exhibit an anti-apoptotic effect in β-cells. Low concentrations of an NO donor inhibited apoptotic signals induced by serum deprivation, such as cytochrome c release from mitochondria, caspase-3 activation, and Bel-2 downregulation in the β-cell line RINm5F (40, 41). This protective pathway seems to involve the activation of c-Src, phosphatidylinositol 3-kinase, and Akt1. We also demonstrated that the NO donor DETA/NO at low concentrations protected β-cells from apoptosis induced by ER stress (31), which is involved in glucotoxicity and lipotoxicity during the development of type 2 DM (1,2). Anti-apoptotic effects of low-concentration NO have been reported in several systems, the mechanisms of which are divided into two, i.e., cGMP-dependent and -independent, and likely cell-type specific (42). In β-cells, the anti-apoptotic effect of NO is likely to be mediated by a cGMP-independent mechanism (31).

5. NO in type 2 DM

Several studies have implied the involvement of cNOS in β-cell dysfunction during the development of type 2 DM. The abnormal insulin responsiveness to l-arginine observed in type 2 diabetic model rats induced by streptozotocin and nicotinamide is suggested to be due to an impairment of NOS1 expression and/or activity in β-cells (43). Such an alteration might develop as a consequence of the diabetic state. In contrast, NOS1 activity and NO production were increased by the overnight exposure to high glucose and palmitate, while the inhibition of NOS1 by siRNA or l-NAME increased JNK
phosphorylation and β-cell apoptosis (44). Thus, NOS1-derived NO seems to have an essential role in protecting β-cell function under conditions of glucolipotoxicity.

It is of interest that insulin resistance and type 2 DM are associated with an elevation of plasma levels of asymmetrical dimethylarginine (ADMA) (45). ADMA is an endogenous inhibitor of NOS, which is produced by methylation of arginine residues in intracellular proteins via protein arginine N-methyltransferases (PRMT) and degraded by dimethylarginine dimethylaminohydrolase (DDAH). There are two DDAH isoforms, DDAH1, and DDAH2 (46). The mRNA expression level of DDAH2 has been shown to be decreased in high-fat diet-induced type 2 DM model mice (47; Kaneko, unpublished data). Thus, the elevation of ADMA due to the decreased DDAH2 may result in a decrease in NO production by cNOS, thereby impairing the insulinotropic and anti-apoptotic effects of low-concentration NO. DDAH2 has also been suggested to NO-independently increase GSIS by increasing the transcription of secretagogin, an insulin vesicle docking protein (47).

Conversely, abnormally increased NO production by NOS2 has been reported in pancreatic islets of type 2 DM model GK rats, resulting in impaired GSIS (48). In addition, hyperglycemia and hyperlipidemia have been shown to induce expression of NOS2 in β-cells concomitant with decreased GSIS (49). These results favor the notion that excessive NO production mediated by NOS2 induction also occurs in β-cells of nonimmunogenic type 2 DM, which induces the impaired insulin secretion and β-cell dysfunction. Further studies will be required to define more fully the role of NO in type 2 DM.

6. Conclusion

NO is recognized as one of the mediators involved in the onset and development of type 1 DM. Inflammatory cytokines produced during the process of type 1 DM induce the expression of NOS2. Micromolar levels of NO produced by NOS2 lead to impaired insulin secretion and β-cell apoptosis. In contrast, low-concentration NO produced by cNOS in β-cells exhibits differential activity. cNOS produces homeostatic levels of NO necessary for physiological functions. NO at tens of nanomolar concentrations exhibits insulinotropic activity in a cGMP-dependent manner. Conversely, sub-micromolar NO, which is also assumed to be produced by cNOS, decreases GSIS in a cGMP-independent manner. A cGMP-independent, S-nitrosylation-mediated mechanism is also involved in the facilitator effect of cNOS-derived NO on GSIS. In addition, cNOS-derived NO exerts a protective effect against apoptosis in a cGMP-independent manner. Although differential roles of NOS1 and NOS3 in the regulation of β-cell functions have not been determined, NOS1 and NOS3 may primarily participate in the regulation of insulin secretion and the protection against apoptosis, respectively. This hypothesis is supported by the findings that NOS1 is primarily distributed on the insulin granule in β-cells (7) and NOS3 overexpression has a protective effect against diabetes induced by streptozotocin and alloxan (50).

NO has multiple biological activities, which makes NO a fascinating target for research. It should be noted, however, that the biological responses to NO are highly dependent on its concentration, and further that NO is a reactive molecule and easily adsorbed and decomposed (51). The concentration of NO will determine the mode...
of action, direct or indirect, the distance it diffuses, and the target molecules. Other radical species are also a critical determinant of NO concentration. The combination of these factors will determine the resultant response to NO, which would alter depending on cell types. In pancreatic \( \beta \)-cells, NO exerts positive and negative regulation of insulin secretion and anti- and pro-apoptotic activity at low and high concentrations, respectively (Fig. 1). It is thus easy to anticipate that the deregulation of NO production or metabolism in \( \beta \)-cells participates in the pathogenesis and natural course of type 2 DM.

References


