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Review

Aspects of the involvement of interleukin-1 and nitric oxide in the pathogenesis of insulin-dependent diabetes mellitus

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Abstract

The possible involvement of the cytokine interleukin-1 (IL-1) and nitric oxide (NO) in the pathogenesis of insulin-dependent diabetes mellitus (IDDM) is reviewed and current and potential therapies are discussed. IDDM is a common disorder in the Western world and it is rising in incidence. In IDDM, islet-infiltrating macrophages produce IL-1 which is cytotoxic specifically to β-cells *in vitro*. IL-1 increases β-cell formation of NO, ceramide, prostaglandins, heat-shock proteins, and activates a protease. Additionally, IL-1 depresses β-cell energy production, insulin gene expression and cyclic AMP synthesis, and impacts negatively on different parts of the insulin stimulus-secretion coupling, actions mimicked by NO. Conversely, blocking NO formation prevented many of these effects in most reports published. Also, changes in cyclic AMP and prostaglandins seem unlikely events in mediating the cytotoxicity of IL-1, while the role of ceramide remains less clear. Peptides capable of blocking β-cell IL-1 receptors, and agents blocking NO synthesis may prove valuable in preserving β-cell function in IDDM. Although IDDM causes immense morbidity and expense, uniformly effective preventive or β-cell protective therapy is not currently available. If IL-1 is causing β-cell dysfunction in human IDDM through NO production, several processes in the IL-1-NO connection are appropriate targets for agents protecting β-cells from destruction and functional inhibition in IDDM.

Keywords: nitric oxide; interleukin-1; pancreatic islet; insulin secretion; diabetes mellitus

Abbreviations: AP, association protein; ATF, activated transcription factor; ATP, adenosine trisphosphate; cAMP, adenosine 3′, 5′-cyclic monophosphate; cGMP, guanosine 3′, 5′-cyclic monophosphate; COX, cyclooxygenase; DAG, 1,2-diacylglycerol; DNA, deoxyribonucleic acid; DTT, dithiothreitol; GTP, guanosine trisphosphate; IDDM, insulin-dependent diabetes mellitus; IL-1, interleukin-1; iNOS, inducible nitric oxide synthase; IRAP, IL-1 receptor antagonistic protein; JNK, janus kinase; NAD, nicotinamide dinucleotide; NF-KB, nuclear factor kappa B; NO, nitric oxide; NOD, non-obese diabetic; PCR, polymerase chain reaction; PG, prostaglandin; PKC, protein kinase C; PPase, protein phosphatase; RNA, ribonucleic acid; SIN-1, 3-morpholino-sydnonimine; SOD, superoxide dismutase; Sp-cAMP[S], Sp-diastereomer of cyclic adenosine-3′,5′-monophosphothioate; TLCK, Na-p-tosyl-L-lysine chloromethyl ketone; TPA, 12-O-tetradecanoylphorbol-13-acetate

Introduction

Recognized since the time of Aristotle, diabetes mellitus remains a major health care problem in the Western world, afflicting 2–5% of the population. The disease is characterized by an absolute or relative deficiency of insulin, leading to vast derangements in glucose and lipid homeostasis with ravaging vascular complications. Insulin-dependent diabetes mellitus (IDDM) accounts for $\approx$10% of diabetes in Western societies, and is still rising in incidence among children and adolescents. IDDM is a chronic disease characterized by progressive destruction of the insulin-producing pancreatic β-cells in the islets of Langerhans (Tisch and McDevitt, 1996). Although the causes of this disease still remain largely elusive, evidence favors a role for autoimmune assault on the β-cells in genetically predisposed individuals, possibly triggered by viral infection (Tisch and McDevitt, 1996). Histologically, the endocrine pancreas shows heavy infiltration of immune cells, an event preceding the clinical outbreak of diabetes (Mandrup-Poulsen, 1996). It is now generally agreed that the pathogenesis of IDDM is primarily a cell-mediated immune event, involving $CD4^+$ (helper/inducer) and $CD8^+$ (suppressor/cytotoxic) T cell subsets, as well as monocytes and macrophages (Mandrup-Poulsen, 1996).

In this paper, I will review extant data implicating the cytokine interleukin-1 (IL-1) and the free radical gas nitric oxide (NO) as key effectors in the pathogenesis of IDDM. Because of space limitations imposed, the reader will frequently be referred to more extensive reviews. A cap on references precluded citing key sources, and I apologize to the colleagues whose work could not be cited. Additionally, I will restrict myself to events occurring in islets once the immune system has been activated; however, excellent reviews on the autoimmune etiology of IDDM have been published (Tisch and McDevitt, 1996; Vyse and Todd, 1996).

Interleukin-1: actions on islet β-cells and role in IDDM

Some 10 years ago it was shown, by the pioneering work of Mandrup-Poulsen and associates, that supernatants from activated macrophages were cytotoxic towards β-cells *in vitro*

(Mandrup-Poulsen, 1996). These islet-infiltrating leukocytes have been shown to produce a number of cytokines (Jiang and Woda, 1991), a group of soluble peptides with important messenger functions within the immune system. Interleukin-1β (IL-1) is the best characterized of these and has been extensively studied for the past 10 years with regard to effects on the pancreatic islet cells *in vitro* (reviewed by Mandrup-Poulsen, 1996; Sandler *et al*, 1991). Extant data indicate that IL-1 is able to exert both inhibitory and cytotoxic actions on islet β-cells *in vitro* (Mandrup-Poulsen, 1996; Sandler *et al*, 1991). Early investigators showed that IL-1 inhibits insulin secretion at least in part by selectively interfering with β-cell mitochondrial substrate oxidation, leading to NAD^+ depletion and a reduced energy production (Sandler *et al*, 1991). Glucose-sensitive insulin release seems to be preferentially affected, resembling the situation in pre-IDDM (Sandler *et al*, 1991). Additionally, insulin gene expression, and (pro)insulin biosynthesis is selectively affected by IL-1 (Sandler *et al*, 1991), supposedly resulting in the insulinopenia that characterizes IDDM.

These combined findings gave rise to the notion that IL-1 may be involved in the pathogenesis of IDDM (Mandrup-Poulsen, 1996). A brief (60 min) exposure of islets to IL-1 is sufficient to impose a long-lasting functional impairment by the β-cell (Eizirik *et al*, 1996). Because of repair systems activated, however, some β-cells are able to recover and if these cells are cultured for several days in the absence of the cytokine they regain their secretory activity (Eizirik *et al*, 1996).

On both normal and clonal β-cells, high-affinity cell surface receptors binding IL-1 have been identified and partially characterized. It appears that these, like in macrophages, are of type-1 and of approximately 80 kDa is size (Sandler *et al*, 1991). Additionally, an IL-1 receptor antagonistic protein, named IRAP, has been shown to be co-secreted by macrophages (Mandrup-Poulsen, 1996). IRAP is 22 kDa in size and shows 72% sequence homology to IL-1 (Mandrup-Poulsen, 1996). Maybe not so surprisingly, IRAP was shown to protect β-cells from IL-1 effects *in vitro* (Sandler *et al*, 1991). To exert its suppressive actions on the β-cell, IL-1 signals through multiple and complex pathways that obviously need gene transcription, mRNA translation, and *de novo* protein synthesis to result in an impeded β-cell function since the actions of the cytokine can be prevented by inhibitors of these processes (Welsh *et al*, 1991a). By means of 2-dimensional gel electrophoresis, the expression of at least 33 islet proteins has been shown to be altered by IL-1 (Mandrup-Poulsen, 1996). The upregulation of some of these proteins probably reflects defense mechanisms mounted by the β-cell. For instance, different heat shock proteins, catalase, hemoxygenase, glutathione peroxidase, and superoxide dismutase (MnSOD), in other cell types are expressed under conditions of toxic stress or injury, and are induced by IL-1 in the β-cell (Sandler *et al*, 1991; Welsh *et al*, 1995a). However, under basal conditions, rodent β-cell expression of these protective proteins is strikingly low and may contribute to the susceptibility of β-cells to autoimmune assaults and their inability to endure free radicals (Welsh *et al*, 1995a).

In order to ultimately find a means of intervening in the outbreak of IDDM, it is of paramount importance to pin down the subcellular mechanisms by which IL-1 exerts its inhibitory actions in the β-cell. Thus, the immediate events taking place after IL-1 occupancy of its cell surface receptor has been the subject of intense scrutiny during recent years. As in immune cells, a mechanism involving connection of IL-1 receptor binding with the remainder of intracellular signaling, being mediated through low molecular weight GTP-binding proteins has been suggested in the β-cell (Mandrup-Poulsen, 1996). GTP-binding protein interference has been shown to partially account for the suppression of insulin secretion by epinephrine, somatostatin, galanin and E-type prostaglandins (Robertson *et al*, 1991). At least seven G_α subunits have been identified in rat islets by PCR strategies and *in situ* hybridization (Zigman *et al*, 1994). However, in contrast to the pertussis toxin-sensitive GTP-binding proteins regulating adenylyl cyclase and phospholipase C (Neer, 1995), the putative proteins involved in IL-1 signaling in the β-cell appears to be of a different kind (Mandrup-Poulsen, 1996). Thus, pretreatment of β-cells with pertussis toxin prevented the suppressive effects of adrenergic agonists, but not those of IL-1 (Sjöholm, 1991a,b; 1992a,b; 1993; 1996a). The reported reduction in the content of cyclic AMP in IL-1-treated islets (Sjöholm, 1991a,b; 1992a,b) might be suspected to contribute to the inhibitory actions of the cytokine on insulin secretion and DNA synthesis, since both these processes are stimulated by cyclic AMP (Sjöholm, 1992b; 1993; 1996b; Ämmälä *et al*, 1994; Newgard and McGarry, 1995). Addition of the stimulatory cyclic AMP analog Sp-cAMP[S] was found to increase β-cell DNA synthesis and insulin secretion (Sjöholm 1996c; 1997), but failed to affect the inhibitory action of IL-1 on these parameters (Sjöholm 1991a; 1992a). These findings indicate that the decrease in cyclic AMP elicited by IL-1 treatment is not responsible for the inhibition of DNA synthesis or insulin secretion evoked by the cytokine. Recent data also indicate that IL-1 may function through tyrosine kinase activation, because its actions in the β-cell were prevented by tyrosine kinase inhibitors (Corbett *et al*, 1993a).

The expression of a number of β-cell genes and transcription factors is altered by IL-1. Among these, the immediate-early response genes, c-*fos* and c-*jun*, are rapidly (30 – 60 min) and transiently induced by IL-1 (Welsh, 1996). Additionally, IL-1 appears to activate p38 mitogen-activated protein kinase and JNK-1 (Mandrup-Poulsen, 1996) and the transcription factors AP-1 and ATF-2 (Mandrup-Poulsen, 1996; Welsh, 1996). In clonal insulinoma cells, IL-1 induces the expression of the antiproliferative gene p53, which may contribute to the growth inhibitory effect of IL-1 in this system (Sjöholm, 1991a,c; 1992a). Also, the transcription factor NF-κB is rapidly (15 – 30 min) translocated from the cytosol to the nucleus, by a tyrosine kinase-dependent mechanism (Eizirik *et al*, 1996).

In other cell types, IL-1 has been shown to activate intracellular proteases. Recent evidence also in the β-cell suggests that an important event in IL-1 action might be

protease activation, since a complete protection against IL-1 inhibition of insulin production and secretion in adult rat islets can be afforded by certain serine protease inhibitors (Welsh *et al*, 1991b; Sjöholm, 1991a), such as *N*α-*p*-tosyl-L-lysine chloromethyl ketone (TLCK). The tosyl-lysine group of TLCK forms a complex with enzymes recognizing basic and hydrophobic amino acid residues, whereas the chloromethyl ketone group accounts for irreversible inactivation of the enzyme by alkylating a catalytically active histidine.

IL-1 stimulates islet nitric oxide formation and causes apoptotic β-cell death?

Since the original discovery of the involvement of the noxious free radical gas nitric oxide (NO) in IL-1-mediated suppression of β-cell function (Southern *et al*, 1990), a considerable amount of compelling evidence suggest that a primary, mandatory event in IL-1 inhibition of β-cell function involves generation of NO (reviewed by Mandrup-Poulsen, 1996; McDaniel *et al*, 1996). This may be of particular clinical relevance, since epidemiological studies have demonstrated an increased incidence of IDDM in populations with high intake of dietary nitrites, possibly because of formation of diabetogenic nitrosoamines (Virtanen *et al*, 1994; Kostraba *et al*, 1992; Helgason *et al*, 1982). NO is highly unstable and is readily converted into its stable end products nitrite and nitrate (Bredt and Snyder, 1994). According to the contemporary conceptual framework, IL-1 increases β-cell formation of NO by inducing the expression of NO synthase (iNOS [McDaniel *et al*, 1996]) after a lag period of 3–6 h. The enzyme catalyzes the release of NO from L-arginine (Bredt and Snyder, 1994). This process requires transcription and enzyme synthesis and is preceded by induction of c-*fos*, c-*jun*, and the transcription factor NF-κB (Eizirik *et al*, 1996). However, c-*fos* and c-*jun* apparently do not regulate iNOS gene expression. It was recently shown that TLCK prevented both NF-κB expression and NO formation induced by IL-1β in the β-cell.

Aside from stimulating β-cell iNOS, IL-1 may also promote NO release from resident macrophages, and act as a chemoattractant to recruit other immune cells to the insulitis lesion. In the islet, iNOS expression seems restricted to the β-cell (McDaniel *et al*, 1996; Eizirik *et al*, 1996). The β-cell 130 kDa Ca^{2+}-independent iNOS resembles that expressed in macrophages, and is located on chromosome 17 (Eizirik *et al*, 1996). The enzyme appears to be feedback inhibited by NO itself, probably by decreasing iNOS transcription (Eizirik *et al*, 1996). By contrast, iNOS mRNA degradation occurs rapidly, and is not affected by IL-1 (Eizirik *et al*, 1996). The regulation of β-cell iNOS is complex, and has recently been reviewed (Eizirik *et al*, 1996). The functional significance of NO generation is underscored by findings showing a complete protection against IL-1-induced β-cell dysfunction by specific inhibitors of NO synthase, e.g. arginine analogs (Southern *et al*, 1990; Corbett *et al*, 1993a). There is also a constitively expressed Ca^{2+}-dependent NOS (cNOS), producing low amounts of NO needed to maintain vascular tone and neuronal synaptic transmission (Bredt and Snyder, 1994). It is of interest that aminoguanidine selectively inhibits iNOS (McDaniel *et al*, 1996). Being a highly reactive, low molecular weight radical, NO is able to rapidly interact with a variety of target molecules (Bredt and Snyder, 1995). For instance, NO seems to cause ultrastructural damage and internucleosomal DNA fragmentation characteristic of apoptosis in cultured rodent β-cells.

Apart from NO, oxygen radicals such as hydroperoxide and superoxide are also formed within the insulitis lesion (Mandrup-Poulsen, 1996). It seems that enzymes scavenging oxygen radicals could not protect from IL-1 toxicity, when being introduced into β-cells by means of liposomal delivery (Welsh *et al*, 1994a). Recently, the regulation of the expression of MnSOD, which removes free radicals superoxide and peroxynitrite, has been studied (Eizirik *et al*, 1996). MnSOD mRNA is induced by IL-1 independently of protein synthesis and NO formation, indicating that IL-1 activates genes involved in β-cell defense and injury by different mechanisms. Furthermore, NF-KB activation appears to be required for iNOS, but not for MnSOD, mRNA expression in the β-cell.

One may ask whether effects of NO are conveyed by cGMP formation, as has been reported in many other cells (Bredt and Snyder, 1994). This is an attractive hypothesis since NO activates guanylyl cyclase by binding to iron in the heme at the enzyme's active site, thereby altering its conformation to augment catalysis (Bredt and Snyder, 1994). However, in the β-cell here are a number of indirect evidence against such a role for cGMP. Neither cell-permeable cGMP analogs, nor the guanylyl cyclase agonist atrial natriuretic peptide (Sjöholm, 1997) affected glucose-regulated insulin release in spite of the fact that β-cells express high-affinity receptors for this peptide, whose receptor has been shown to be identical to guanylyl cyclase.

Admittedly, most of the studies on IL-1 and NO effects have been carried out on isolated islets from rodents, and their pathogenic significance in human β-cells is still a matter of debate (Welsh *et al*, 1994b; Eizirik *et al*, 1996). It seems, from the few studies carried out on human islets so far, that β-cells from this species are much more resistant to both IL-1 and NO than their rodent counterparts (Welsh *et al*, 1994b). In particular, combinations of various other cytokines produced within the insulitis lesion (IL-1, interferon-γ and tumor necrosis factor α) appear to be needed to cause functional inhibition in human β-cells, whereas rat β-cells are sensitive to IL-1 alone. Moreover, the human β-cell is less prone to form NO when exposed to cytokines, and seems also less susceptible to NO, than its rodent counterpart (Welsh *et al*, 1994b; Eizirik *et al*, 1996). Possibly, this can be explained by the higher expression of protective enzymes, e.g. heat shock proteins, catalase, hemoxygenase, glutathione peroxidase, and MnSOD, in human islets than in rodents (Welsh *et al*, 1995a).

It is noteworthy that serum from patients with IDDM, whose β-cells are succumbed by autoimmune assault, not only contains elevated levels of IL-1 (Hussain *et al*, 1996) but also causes exaggerated Ca^{2+} influx in clonal rat β-cells cells leading to DNA fragmentation characteristic of apoptosis (Juntti-Berggren *et al*, 1993), and that IL-1

activates apoptosis in these cells through NO generation. Very recently, a number of papers have presented results arguing in favor of apoptosis as the mode of β-cell death *in vivo* in IDDM. For instance, by crossing a transgenic mouse carrying a β-cell-specific T cell receptor onto a NOD.scid background, it was possible to obtain an animal carrying $CD4^+$ T cells bearing transgenic T cell receptor (but not $CD8^+$ T cells or B cells). In this model, IDDM rapidly occurred and the mode of β-cell death was by apoptosis (Kurrer *et al*, 1997). In another study, it was found that IL-1 upregulated β-cell expression of Fas which led to β-cell apoptosis, apparently without the involvement of NO. This mechanism of β-cell death was recently confirmed in a transgenic mouse model (Chervonsky *et al*, 1997), in which Fas ligand was specifically overexpressed in the β-cell driven by the insulin promoter. These animals developed autoimmune diabetes through heightened sensitivity to diabetogenic T cells, due to self-destruction of β-cells upon T cell-mediated induction of Fas.

Previous findings in isolated insulin-secreting cells (Cooney *et al*, 1995) also showed that the vitamin E γ-tocopherol, a major antioxidant in Western diets, could reverse the effects of IL-1, probably by detoxifying the nitrogen dioxide formed thereby preventing lipid peroxidation (Cooney *et al*, 1993). Interestingly, *in vivo* administration of E-vitamin was found to decrease or delay the onset of IDDM in BB rats (Murthy *et al*, 1992), and to reduce the incidence of IDDM in the NOD mouse (Hayward *et al*, 1992). Alluding to this, our previous studies on the role of prooxidants in β-cells has shown that quinone-induced oxidative stress activates programmed cell death in clonal insulinoma cells (Dypbukt *et al*, 1994).

Figure 1 provides a highly simplified view of intracellular events affected by IL-1 and NO in the β-cell.

Role of lipid signaling in IL-1-exposed islets

Lipid signaling is a versatile system by which cells transduce external messages into biological responses (reviewed by Divecha and Irvine, 1995; Nishizuka, 1995). In β-cells, plasma membrane phosphoinositide hydrolysis occurs early in secretory stimulation (Newgard and McGarry, 1995). Besides NO formation, generation of ceramide through the

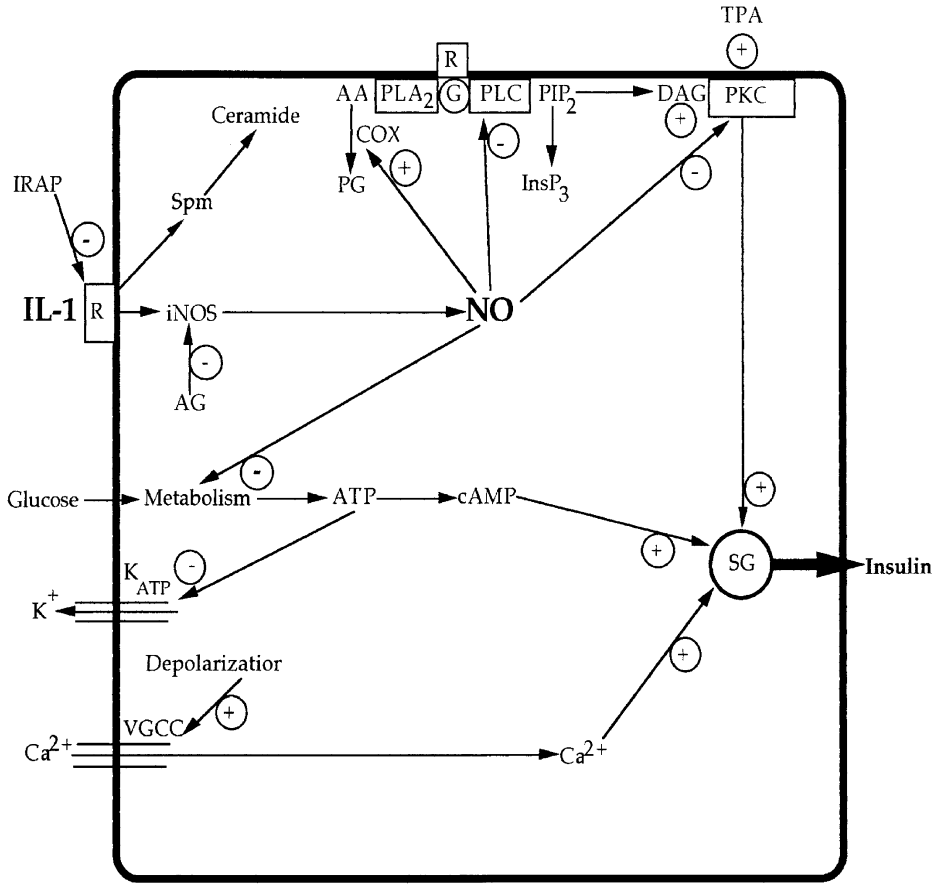

Figure 1 Working hypothesis for inhibitory effect of IL-1 and NO on β-cell function. +, stimulation; −, inhibition. AA, arachidonic acid: AG, aminoguanidine; ATP, adenosine trisphosphate; cAMP, adenosine 3′, 5′-cyclic monophosphate; COX, cyclooxygenase; DAG, 1,2-diacylglycerol; G, GTP-binding protein; IL-1, interleukin-1; iNOS, inducible nitric oxide synthase; $InsP_3$, inositol 1,4,5-trisphosphate; IRAP, IL-1 receptor antagonistic protein; K_{ATP}, ATP-regulated K^+ channel; NO, nitric oxide; PG, prostaglandin; PIP_2, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PLA_2, phospholipase A_2; PLC, phospholipase C; R, receptor; SG, secretion granule; Spm, sphingomyelin; TK, tyrosine kinase; TLCK, *N*α-*p*-tosyl-L-lysine chloromethyl ketone; TPA, 12-*O*tetradecanoylphorbol-13-acetate; VGCC, voltage-gated Ca^{2+} channel. See text for details

action of sphingomyelinase is a rapid and consistent event after exposure to IL-1 and other cytokines, e.g. interferon-γ and tumor necrosis factor α (Hannun and Obeid, 1995). This event was also recently confirmed the β-cell, and it was suggested that IL-1-induced ceramide and diacylglycerol generation may lead to activation of the c-*jun* NH_2-terminal kinase and transcription factor ATF2, but not NF-KB (Welsh, 1996). In contrast to the large wealth of information accumulating regarding products of phosphoinositide hydrolysis as regulatory molecules, the sphingolipids have been poorly appreciated and were until recently merely perceived as structural components of the plasma membrane. However, a rapidly growing body of evidence implicate ceramide, generated through sphingomyelin hydrolysis, as a novel second messenger, conveying antiproliferative signals by cytotoxic molecules such as IL-1 (reviewed by Hannun and Obeid, 1995). It seems that ceramide activates a p97 kinase thereby phosphorylating and activating p38 mitogen-activated protein kinase and JNK-1 (Mandrup-Poulsen, 1996). It is noteworthy that ceramide seems to counteract the effects of the phosphoinositide cleavage product diacylglycerol, the latter being an activator of PKC and linked to growth promotion and stimulation of insulin release (Sjöholm, 1991d; 1992b; 1993; 1996b; Ämmälä *et al*, 1994; Newgard and McGarry, 1995; Nishizuka, 1995; Arkhammar *et al*, 1994; Sjöholm *et al*, 1993a). Thus, although ceramide reportedly does not inhibit PKC, it has emerged as a second messenger mediating growth suppression and apoptosis (Hannun and Obeid, 1995) and may well prove to be as paramount as diacylglycerol in regulation of cell signaling. Additionally, specific targets for ceramide have been identified, including activation of a serine/threonine protein phosphatase of the 2A subfamily, the latter recently being identified by us in β-cells (Ämmälä *et al*, 1994; Sjöholm *et al*, 1993b; 1995; Larsson *et al*, 1997). The recent development of cell-permeant ceramide analogs has made elucidation of the role of ceramide in living cells become amenable and conclusive (Hannun and Obeid, 1995). It appears that ceramide regulates the expression of c-*myc* levels and the transcription factor NF-KB, and is also able to interact with phospholipase A_2 and cyclooxygenase (Hannun and Obeid, 1995), which are also affected by IL-1 (Hannun and Obeid, 1995; Mandrup-Poulsen, 1996). Whether any of these elements are targeted by ceramide also in the β-cell remains to be seen.

Previous findings show that exogenous ceramide suppresses β-cell growth and function, and also that artificially stimulating generation of ceramide in the β-cell by addition of the normally membrane-bound enzyme sphingomyelinase gave rise to qualitatively similar results (Sjöholm, 1995). This also mimicked the suppressive actions of IL-1, a pathophysiological effector of ceramide synthesis and β-cell death, thus adding further credence to a role of ceramide as a second messenger mediating cytotoxic signals by molecules such as IL-1. Entirely consistent with the above results are the previous findings that specific inhibition of serine/threonine protein phosphatase 2A with low concentrations of okadaic acid (Ämmälä *et al*, 1994; Larsson *et al*, 1997) or protein kinase C stimulation with phorbol ester (Sjöholm, 1991d; 1992b; 1993; 1996b; Ämmälä *et al*, 1994; Newgard and McGarry, 1995; Arkhammar *et al*, 1994; Sjöholm *et al*, 1993a) promotes insulin secretion and that insulin secretagogues transiently suppress β-cell PPase activities (Sjöholm *et al*, 1995). Conversely, nanomolar concentrations of okadaic acid or phorbol ester prevent apoptosis and c-*myc* downregulation evoked by ceramide (Hannun and Obeid, 1995). Thus, ceramide and diacylglycerol appear to be the Yin and Yang of cellular signaling through their opposing effects on protein phosphorylation.

NO impacts negatively on the β-cell insulin stimulus-secretion coupling

In a recent paper, the influence of the NO donor 3-morpholino-sydnonimine (SIN-1) on the regulation of discrete parts of the stimulus-secretion coupling in isolated pancreatic islets has been investigated (Sjöholm, 1996d). The insulin secretory response to the cardinal insulin secretagogue glucose was preferentially impeded following SIN-1 exposure, resembling the situation noted with IL-1 (Sandler *et al*, 1991). It is currently believed that glucose metabolism in the cytosolic Emden-Meyerhof pathway, and after funneling into mitochondria, further oxidative catabolism in the Krebs cycle, generates signals that promote insulin secretion (Newgard and McGarry, 1995). It is moreover believed that the ATP generated through the metabolism of the sugar blocks ATP-dependent K^+ channels in the plasma membrane, resulting in cell depolarization with the subsequent influx of Ca^{2+} through voltage-activated Ca^{2+} channels, an event that sets in motion the exocytotic release of insulin (Rorsman *et al*, 1990; Wollheim and Sharp, 1981). Thus, it is evident that the reported effects of SIN-1 may reflect NO interaction with any of these steps, or others, activated by hexose stimulation. One striking feature of NO is that it is able to bind to iron-sulfur enzymes, and thereby modulate their biological activity (Bredt and Snyder, 1994). One such example is the Krebs cycle enzyme *cis*-aconitase, which was previously shown to be a major inhibitory target for IL-1 and NO in the β-cell and possibly accounting for the reduced glucose oxidation by IL-1 (Welsh *et al*, 1991a). Obviously, inhibition of this enzyme would result in an impaired ATP production with secondary effects on ATP-dependent K^+ channels and Ca^{2+} entry. Additionally, by ADP ribosylation, NO can enhance incorporation of NAD into glyceraldehyde-3-phosphate dehydrogenase by modifying a cysteine at the active site of the enzyme, thereby potentially depressing glycolysis. Another biological feature of NO includes its ability to stimulate *S*-nitrosylation of various target proteins containing 'critical' SH groups (Bredt and Snyder, 1994; Lipton *et al*, 1993; Stamler, 1994). The precise nature of which proteins in the stimulus-secretion coupling that are targeted by NO in the thiol-dependent redox mechanisms reported (Sjöholm, 1996d), remains to be elucidated in forthcoming studies. However, in this context it is of interest to note that reversible redox reactions are of great regulatory significance in β-cell function (reviewed by Ammon and Mark, 1985).

Previous findings indicate that the stimulation of insulin secretion with carbachol was partially countered by SIN-1

(Sjöholm, 1996d), suggesting that NO may have modified phospholipase C by means of *S*-nitrosylation, as was reported previously in other tissues. Indirect evidence in favor of this view comes from the fact that co-incubation with the disulfide reducing agent DTT partially offset the inhibitory action of SIN-1 (Sjöholm, 1996d). Stimulating insulin release by directly activating heterotrimeric GTP-binding proteins, connected to phospholipase C and adenylyl cyclase, by AlF was not affected by SIN-1 (Sjöholm, 1996d). This finding indicates that NO apparently does not affect the GTP-binding proteins regulating insulin release in this system. By contrast, in other cells, NO has been reported to influence the function of GTP-binding proteins.

SIN-1 impeded the insulin secretory response to protein kinase C (PKC) activation by phorbol ester (Sjöholm, 1996d), suggesting PKC inactivation by NO, possibly through *S*-nitrosylation. This is an attractive hypothesis since PKC has critical thiol residues that that influence its kinase activity and which can be oxidized by NO (Gopalakrishna *et al*, 1993). However, somewhat surprising, co-addition of DTT failed to reverse the inhibitory effect of SIN-1 on PKC-regulated insulin release (Sjöholm, 1996d). This indicates either that SIN-1 acts unrelated to *S*-nitrosylation in this case, or, alternatively, that NO permanently inactivated PKC, as has been reported previously (Gopalakrishna *et al*, 1993).

Cyclic AMP-stimulated insulin release was not influenced by SIN-1 (Sjöholm, 1996d). This finding differs from previous data in other tissues, that showed NO inhibition of adenylyl cyclase activity by *S*-nitrosylation of vicinal thiols in the calmodulin binding domain of the cyclase, an effect reversed by DTT or glutathione. However, it appears that in the β-cell, the cyclic AMP system is less sensitive to thiol oxidation than other parts of the stimulus-secretion coupling (Ammon and Mark, 1985).

There are several reports indicating that NO can regulate Ca^{2+} channels in cardiac myocytes (Kirstein *et al*, 1995), although it is not clear whether this is achieved through *S*-nitrosylation. However, contrasting to these reports, the β-cell Ca^{2+} channel seems more resistant to NO, as SIN-1 exerted no discernable effects on K^+-stimulated insulin release (Sjöholm, 1996d).

In conclusion, these data suggest that NO inhibits insulin secretion from β-cells partly by *S*-nitrosylation of vicinal thiol residues, likely forming intramolecular disulfides, in key proteins in the stimulus-secretion coupling (Sjöholm, 1996d). The results seem to exclude heterotrimeric GTP-binding proteins, adenylyl cyclase or voltage-activated Ca^{2+} channels being inhibited by NO in the β-cell (Sjöholm, 1996d). This resembles the situation with IL-1, which does not appreciably inhibit the insulin secretory response to cyclic AMP-raising agents or depolarizing concentrations of L-arginine. By contrast, enzymes involved in glucose catabolism, phospholipase C, protein kinase C or proteins regulating intracellular Ca^{2+} handling may be targeted by NO (see Figure 1 for overview). A cell-permeant methyl ester of the Krebs cycle intermediate succinate was found to circumvent IL-1-induced inhibition of glucose-sensitive insulin release (Eizirik *et al*, 1996). However, this does not exclude that inactivation of the enzymes targeted by SIN-1 described above impacts negatively on the secretory response to other insulin secretagogues than glucose, e.g. neurotransmittors. These adverse effects of NO on the β-cell stimulus-secretion coupling may be of importance for the development of the impaired insulin secretion characterizing diabetes mellitus.

Role of prostaglandins in β-cell dysfunction induced by IL-1

Generation of the inflammatory mediators prostaglandins (PG), thromboxanes and leukotrienes through the concerted action of phospholipase A_2, cyclooxygenase and 12-lipoxygenase is a consistent event after exposure to IL-1 in many tissues. Also in β-cells does IL-1 evoke a marked but delayed increase in PGE_2 formation, the time course fitting the inhibitory phase of IL-1 action on insulin release (McDaniel *et al*, 1996). More recently, it was shown that IL-1 induces a cooperative expression of NO synthase and the inducible cyclooxygenase type 2 (iCOX) in rat islets, the activation of the latter enzyme apparently being mediated by NO and requiring RNA transcription (Corbett *et al*, 1993b). Recently it was also demonstrated that IL-1 enhances islet 12-lipoxygenase product generation by increasing the availability of nonesterified arachidonic acid through a NO-dependent mechanism. Furthermore, chemotactic leukotrienes may be produced through 5-lipoxygenase expressed in islet resident macrophages (McDaniel *et al*, 1996). Interestingly, ceramide may assist in the phosphorylation and activation of phospholipase A_2 and may thereby, together with NO, mediate the stimulatory effect of IL-1 on islet prostaglandin formation (Mandrup-Poulsen, 1996). It is possible that locally produced prostaglandins may exert critical regulatory actions on β-cell growth and function and it is conceivable that they adversely influence insulin secretion and glucose homeostasis *in vivo*. Indeed, experiments in intact animals and in humans revealed that prostaglandin infusion decreased the amount of insulin secreted in response to an intravenous glucose challenge (Robertson, 1979). As a corollary to this, certain cyclooxygenase inhibitors have been reported not only to augment glucose-induced insulin responses in normal humans, but also to improve glucose disposal rates and glucose-sensitive insulin secretion in patients with NIDDM (Robertson, 1979). Previous studies have shown that exogenous prostaglandins of the E-series cause long-term suppression of pancreatic β-cell mitogenesis and insulin secretion (Sjöholm, 1996e). There are also numerous reports indicating that prostaglandins function as inhibitors of short-term insulin secretion *in vitro* (reviewed by Robertson, 1979).

The mechanisms by which prostaglandins exert their multifarious actions still remain largely elusive. However, in other cell types cDNAs encoding thromboxane A_2 and prostaglandin EP_3 receptor subtypes have been cloned and characterized. In addition, at least in β-cells, prostaglandins reportedly inhibit inositol phosphate generation and cyclic AMP formation, two events invariably connected to promotion of mitogenesis and insulin secretion (Sjöholm, 1992b; 1993). Germane to this, pertussis toxin pretreatment

was found to partially alleviate the inhibition of phasic insulin secretion from a hamster insulinoma cell line (Robertson *et al*, 1991), arguing in favor of heterotrimeric GTP-binding proteins partially conveying the short-term inhibitory effect of prostaglandins in this cell line. By contrast, in long-term experiments involving normal β-cells reported (Sjöholm, 1996e), no such protection by pertussis toxin pretreatment was afforded. Thus, the finding that exogenous prostaglandins mimicked the inhibitory actions of IL-1 on β-cell mitogenesis and insulin secretion engendered interest in the possibility that endogenous prostaglandins indeed convey the suppressive signal by the cytokine, rather than merely being coexpressed. However, the findings that cyclooxygenase inhibitors failed to influence the inhibitory effects of IL-1 argues against this possibility (Sjöholm, 1996e). Nonetheless, the prostaglandins may be involved in exacerbating the proinflammatory response within the insulitis lesion.

Intervention in the IL-1/NO pathway may preserve β-cell function in IDDM

While insulin injection is clearly life-saving in IDDM patients, uniformly effective preventive or β-cell protective therapy is not currently available. If IL-1 is causing β-cell dysfunction in human IDDM through NO production, several processes in the IL-1-NO connection are appropriate targets for agents protecting β-cells from destruction and functional inhibition in IDDM. The selective iNOS blocker aminoguanidine has been given to nonobese diabetic (NOD) mice *in vivo* (Corbett *et al*, 1993c), and was found to delay the outbreak of overt diabetes in these animals, but did not prevent the disease. IRAP, the naturally occurring IL-1 receptor antagonist (Mandrup-Poulsen, 1996), should also be employed *in vivo* in a similar setting. Overexpression of the normally low abundant radical scavenger enzymes in islets may prove beneficial to dispose of toxic radicals in islets. Notably, liposomal delivery of heat shock protein 70 into β-cells, was found to protect these cells against IL-1 suppression (Sandler *et al*, 1991). As discussed above, antioxidant E-vitamins have showed promising results both *in vitro* and *in vivo* (Cooney *et al*, 1993; Murthy *et al*, 1992; Hayward *et al*, 1992). Corticosteroids such as dexamethasone have been reported by some groups to afford protection against IL-1, probably by posttranscriptionally inhibiting iNOS, but also through iCOX inhibition (McDaniel *et al*, 1996). Nicotinamide, tested *in vitro* with variable results is currently being clinically tried (Sperling, 1997), but the promising results by some groups, reporting a delayed IDDM onset, need to be confirmed. One may also envisage that inhibitors of ceramide synthesis, prostanoid formation, and agents interfering in apoptosis (e.g. blockers of plasma membrane Ca^{2+} channels and intervention in the Fas system), may eventually become of clinical utility. Finally, one promising approach would be gene therapy. Indeed, recently a hybrid gene consisting of IRAP and the insulin gene was constructed and transfected into clonal insulinoma cells (Welsh *et al*, 1995b). The construct was stably expressed by these cells and conferred resistance against the inhibitory actions of IL-1 in these cells (Welsh *et al*, 1995b). It remains to be seen, however, whether any of these approaches also could afford protection *in vivo* against human IDDM. If so, the immense morbidity and expense caused by IDDM may be significantly reduced.

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