Mechanisms of pancreatic B-cell dysfunction and glucose toxicity in non-insulin-dependent diabetes

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Introduction
The insulin response to food ingestion is determined by the direct actions of glucose and certain amino acids on the pancreatic B-cell together with indirect actions generated through activation of both hormonal and neural arms of the enteroinsular axis [1-3]. These signals are normally integrated at the level of the pancreatic B-cell such that insulin is secreted to appropriately regulate nutrient metabolism and glucose homeostasis. In non-insulin-dependent diabetes mellitus (NIDDM), defects in the mechanisms that regulate insulin secretion make a major contribution to the glucose intolerance and metabolic disarray associated with the disease [4-6]. Possible molecular mechanisms underlying pancreatic B-cell dysfunction in NIDDM include site-specific defects in the stimulus-secretion coupling pathway and changes in B-cell function consequent to alterations in external influences on the B-cell. The participation and interaction of the various pathways to disturbances of insulin secretion in NIDDM are considered below in the framework of our present understanding of the regulation of B-cell function and stimulus-secretion coupling.

Susceptible points in B-cell stimulus-secretion coupling
Glucose insensitivity of the pancreatic B-cell lies at the heart of defective insulin secretion in NIDDM [4-6]. Glucose is the principal regulator of B-cell function and also amplifies the insulinotropic actions of all other secretagogues, including entero-

Abbreviations used: NIDDM, non-insulin-dependent diabetes mellitus; [Ca2+], cytoplasmic Ca2+ concentration; K+-ATP, ATP-sensitive K+ channel.

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certain is the extent to which such defects (summarized in Table 1) are primary genetic manifestations or secondary features acquired from abnormalities of the B-cell environment and external influences. A future search for mutations of key functional proteins in the B-cell secretory machinery will undoubtedly help to address this issue. These discussions have an important bearing on the debate, conducted elsewhere, on whether the primary defect in NIDDM is at the level of the B-cell rather than at the site of insulin action [4, 5, 12].

**Insulin synthesis and trafficking**

Point mutations of the insulin gene and other mutations which may affect the intracellular processing and trafficking of proinsulin have been observed in small numbers of human subjects [13]. Although the frequency of such mutations is low, mechanisms which potentially lead to the abnormal release of proinsulin from the B-cell are relevant to the hyperproinsulinaemia encountered in NIDDM.

**Stimulus-secretion coupling**

Abnormalities have been detected in NIDDM at almost all known stages of the pancreatic B-cell stimulus-secretion coupling pathway [6]. Brief consideration of the principal site-specific lesions contributing to defective insulin secretion in specific examples of NIDDM is given below.

**GLUT2 glucose transporter**

A variable reduction in expression of the B-cell GLUT2 glucose transporter has been recorded in human NIDDM and several animal models with diabetes [8, 14]. A reversible loss of GLUT2 expression has been reported in db/db mice on an undefined background genome [15]. However, since glucose transport is not normally rate limiting in B-cell glucose metabolism [8], abnormalities of GLUT2 seem to be generally less important than defects that affect more distal steps in the insulin secretory pathway.

**Glucokinase**

An alteration of glucokinase can be expected to disturb insulin secretion by virtue of the role of the enzyme in determining the rate of signal generating metabolic flux in the B-cell [8]. Most notable in this context is the detection of functionally significant mutations of the glucokinase gene in a high proportion of MODY (maturity-onset diabetes of the young) patients with defective insulin secretion [16]. Mutations at this locus are not common in classical NIDDM [17]. However, the apparent link between glucokinase activity and defective insulin secretion highlights the need for more detailed studies on possible changes in the activity of the enzyme in the diabetic B-cell.

**Glucose-6-phosphatase (glucose cycle)**

A high rate of glucose cycling (namely metabolism of glucose to glucose-6-phosphate and back to glucose) catalysed by glucokinase and glucose-6-phosphatase has been demonstrated in the islets of ob/ob mice, GK rats and rats treated with streptozotocin during the neonatal period [18]. Consumption of one molecule of ATP for each molecule of glucose cycled might, by decreasing the ATP pool, interfere with the regulation of K⁺-ATP channels and contribute to defective insulin secretion.

**Mitochondrial FAD-glycerophosphate dehydrogenase**

Activation of mitochondrial FAD-linked glycerophosphate dehydrogenase by [Ca²⁺], is proposed to optimize ATP generation from glucose in B-cells.

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through preferential stimulation of oxidative, as opposed to total, glycolysis [19]. The enzyme appears therefore to serve an important role in the glucose-sensing function in the B-cell. Alterations of mitochondrial FAD-linked glycerophosphate dehydrogenase activity have been described in various animal models of experimentally-induced and spontaneous NIDDM, including adult rats treated neonatally with streptozotocin, GK rats, fa/fa rats and C57BL/KsJ db/db mice [20].

**Generation of ATP**

Despite the key role of ATP generation and increase of ATP/ADP ratio in the closure of K⁺-ATP channels, few studies have actually addressed directly the link between defective insulin secretion and perturbations of ATP generation. One problem is undoubtedly the difficulty in monitoring functionally important changes of ATP specifically adjacent to K⁺-ATP channels. Nevertheless, a compromise in the generation of cellular ATP has been clearly shown to be associated with the glucose unresponsiveness of fetal rat pancreatic B-cells [21].

**K⁺-ATP channels**

Early studies of pancreatic B-cell membrane potential and ⁶⁸Rb efflux, from preloaded islets of C57BL/KsJ db/db mice and diabetic Chinese hamsters, demonstrated an association between abnormalities in the regulation of K⁺ permeability and defective insulin secretion [22-24]. Cell-attached and inside-out configurations of the patch-clamp technique have been used recently to directly assess the regulation of K⁺-ATP channels in B-cells isolated from animal models. Studies using the neonatal-streptozotocin-treated rat model and GK rats indicate that the inhibitory effect of glucose on B-cell K⁺-ATP channels is impaired in NIDDM [25, 26]. However, sensitivity of the channels to direct application of ATP using inside-out patches was intact. This suggests that glucose insensitivity of the K⁺-ATP channels in these models reflects impaired cellular ATP generation rather than a defect in the K⁺-ATP channel per se.

**Regulation of [Ca²⁺], and contractile proteins**

Disturbances in the regulation of transmembrane Ca²⁺ fluxes and [Ca²⁺], represent a common feature associated with defective insulin secretion in animal models of NIDDM, including C57BL/KsJ db/db mice, Spiny mice and neonatal-streptozotocin-treated rats [6]. Abnormal regulation of [Ca²⁺], undoubtedly plays a major role in the defective stimulus-secretion of the B-cell in NIDDM. However, the extent to which such abnormalities reflect disturbances at earlier stages of the secretory process is uncertain. Site-specific lesions in voltage-dependent Ca²⁺ channels, intracellular Ca²⁺ transport and the effects of Ca²⁺ on the exocytotic machinery remain to be established. Apparent disturbances in microtubules and microfilaments involved in exocytosis have been reported in diabetic Spiny mice and C57BL/KsJ db/db mice [27, 28].

**Alterations of external input and B-cell dysfunction**

NIDDM is associated with significant disturbances in the local environment of the B-cell, including profound changes in the concentrations of nutrients and hormones with established effects on insulin secretion [4-6]. As illustrated by specific examples given below, such alterations can undoubtedly contribute to B-cell dysfunction. In certain cases, most notably hyperglycaemia-induced glucose toxicity, alterations in external environment may actually lead to the production of site-specific defects in the B-cell.

**Local modulators of secretion**

Disturbances in location and relative proportions of the various islet cell types are commonly observed in diabetes [29]. Such alterations undoubtedly disrupt the normal functional interactions between B-cells and the surrounding A- and D-cells [30]. Local paracrine effects and actions of glucagon and somatostatin dependent on normal vascular flow through the islets may be affected. However, possibly more important are changes in the secretion and action of a large number of other proposed local modulators of insulin secretion [3, 7, 31]. These include biogenic amines, pancreastatin, islet amyloid polypeptide (IAPP), opiate peptides, thyrotropin-releasing hormone, corticotropin-releasing factor, peptide YY, atrial natriuretic peptide, diazepam-binding inhibitor and other less well-known peptides. Pancreastatin and IAPP, which are released from the B-cell, have been proposed to contribute to B-cell dysfunction through inhibition of insulin secretion [7, 32, 33].

**Alterations of autonomic tone**

The islet autonomic innervation plays a subtle and possibly important role in the fine-tuning of insulin secretion [3, 34]. Alterations of autonomic tone undoubtedly occur in NIDDM, as illustrated by the well-known deleterious effect of hyperglycaemia on
nerve conduction velocity [35]. The B-cell innervation may be also disrupted in diabetes as illustrated by ultrastructural observations in diabetic Spiny mice and Chinese hamsters [36, 37] and by alterations in the levels of neuropeptides in the islets of various diabetic models [38]. Classical and peptidergic neurotransmitters include acetylcholine, noradrenaline, cholecystokinin, somatostatin, galanin, gastrin-releasing peptide, vasoactive intestinal polypeptide and neuropeptide Y [3, 7, 34]. Alterations in the actions of these neurotransmitters in diabetes may contribute to the amplification or suppression of insulin secretion through parasympathetic and sympathetic nerves, respectively.

**B-cell hyperactivity**

There is good evidence for a link between B-cell hyperactivity and defective insulin responses in some forms of experimental and spontaneous diabetes [6]. Thus in various animal models of NIDDM, loss of an insulin response to glucose shows a close association with the extent of hyperinsulinaemia [6]. Interventions such as fasting in ob/ob mice, which decreases the extent of hyperinsulinaemia and hyperglycaemia, have also been reported to improve insulin secretory responsiveness [39]. Defective insulin secretion in glucose-infused rats may also partly result from induction of B-cell hyperactivity in addition to possible direct deleterious effects of the accompanying hyperglycaemia [40, 41]. Such observations suggest that hyperinsulinaemia, arising from excessive B-cell stimulation by overactive entero-insular axis, contributes to defective insulin secretion in many susceptible animal syndromes of NIDDM [38].

**Glucose toxicity**

Studies in man and experimental animals, using cultured islets, both in vivo and in vitro, indicate that long-term exposure to a hyperglycaemic environment results in a gradual impairment of insulin secretion [5, 42]. Such observations have reaffirmed the concept of glucose toxicity and the view of hyperglycaemia as an inducer, as well as a consequence of B-cell dysfunction [43]. Pancreatic B-cell glucose toxicity is particularly well illustrated by the induction of defective insulin secretion in rats subjected to partial pancreatectomy or to glucose infusions [5, 6, 42]. Interestingly, there seems to be significant species and strain variability in susceptibility to the deleterious actions of hyperglycaemic culture conditions on the function of isolated islets. The B-cells of certain strains of mice seem particularly resilient to the potentially adverse effects of hyperglycaemia [44]. In contrast, human islets and islets isolated from genetically susceptible rodent species clearly display glucose toxicity manifested in defective insulin secretion [41, 45-47]. Two potentially important considerations arise from these observations. First, species differences in susceptibility might be due to differences in endogenous protection against the mechanisms involved in mediation of glucose toxicity. Secondly, the apparent requirement for a genetically susceptible B-cell might imply that the deleterious effects of hyperglycaemia may become manifest by imposing additional functional strain on existing weakspots in the insulin secretory pathway of predisposed B-cells.

**Role of glycation in B-cell glucose toxicity**

Despite compelling evidence that B-cell glucose toxicity makes an important contribution to defective insulin secretion in established NIDDM, few studies have addressed the molecular mechanisms involved. A component of the glucose toxicity induced experimentally in vivo or in tissue culture may be secondary to chronic glucose-induced stimulation of B-cell hyperactivity [40, 41]. This aspect is discussed above. However, it is hard to transpose this situation to the B-cell in NIDDM, where glucose responsiveness is already compromised. With this in mind, recent studies have been conducted to assess the role of glycation of B-cell proteins in glucose toxicity and defective insulin secretion. Using the newly established glucose-responsive BRIN-BD11 cell line [48], it has been shown that detrimental effects of chronic exposure to hyperglycaemia on insulin secretory responsiveness are associated with a substantial glycation of intracellular proteins [49]. Since glycation can be expected to influence the function of key proteins in the B-cell secretory machinery, it is not unreasonable to propose that glycation of important regulatory proteins serves as a cardinal mediator of B-cell glucose toxicity. Furthermore, ongoing studies indicate that insulin is also a target for glycation in B-cells exposed to hyperglycaemia and that glycation impairs insulin-mediated glucose disposal at peripheral tissues [50, 51]. In this respect, it appears that glycation of insulin in NIDDM provides a novel link in the vicious spiral that, by promoting insulin resistance, exacerbates hyperglycaemia and consequently B-cell dysfunction.

**Conclusions**

Pancreatic B-cell dysfunction in NIDDM represents the combined effects of site-specific defects in the
stimulus–secretion coupling pathway compounded by an array of diabetes-induced deleterious external influences on the B-cell (Figure 1). Studies in animals with NIDDM indicate heterogeneity in the molecular mechanisms underlying defective insulin secretion. However, growing evidence suggests that abnormalities in the early steps of B-cell glucose recognition and toxic effects of glucose mediated through glycation of B-cell proteins may be of particular importance.

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Nitric oxide toxicity in pancreatic islet cells: role of protein biosynthesis, calcium influx and arachidonic acid metabolism

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Introduction
Nitric oxide (NO) appears to be an important mediator of the inflammatory attack against islet cells in autoimmune diabetes. The lysis of islet cells by activated macrophages in vitro is NO dependent [1] and can be mimicked by exposure of islet cells to chemical NO donors [2]. The lytic effect of interleukin-1 (IL-1) on islets is also mediated by NO [3]. Finally, inhibition of the β-islet cell response to glucose, by incubation with IL-1 under non-lytic conditions, requires NO synthase activity [4].

The mechanism of islet cell lysis by NO has not been elucidated. The discovery that nicotinamide protects against NO toxicity [2] and func-