Proceedings of the Nutrition Society (1997), **56**, 243–262

Pancreatic B-cell dysfunction and glucose toxicity in non-insulin-dependent diabetes

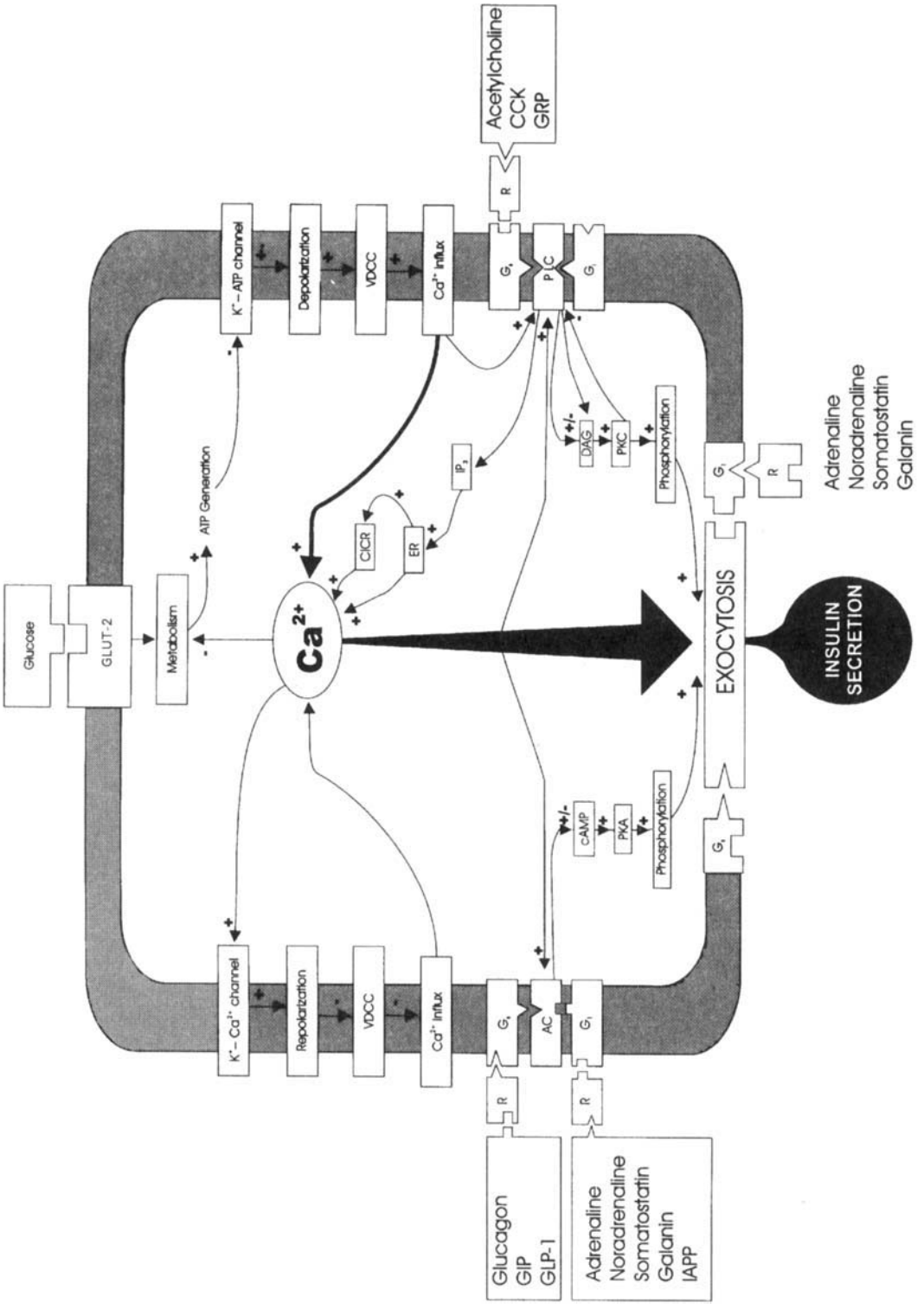
BY PETER R. FLATT, YASSER H. A. ABDEL-WAHAB, ALISON C. BOYD, CHRISTOPHER R. BARNETT AND FINBARR P. M. O'HARTE

School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA

Regulation of insulin secretion in response to feeding is determined by the direct actions of glucose and amino acids on the pancreatic B-cell together with indirect effects mediated by activation of hormonal and neural arms of the entero-insular axis (Berggren *et al.* 1992; Morgan, 1992; Havel *et al.* 1994; Flatt, 1996). These signals are integrated at the level of the pancreatic B-cell such that insulin is secreted to regulate nutrient metabolism and glucose homeostasis appropriately. In non-insulin-dependent diabetes mellitus (NIDDM), defects in insulin action and the mechanisms that regulate insulin secretion are key to the glucose intolerance and metabolic disarray associated with the disease (Kahn & Porte, 1990; Leahy, 1990; Flatt *et al.* 1992). Possible molecular mechanisms underlying pancreatic B-cell dysfunction in NIDDM include site-specific defects in the stimulus–secretion coupling pathway and alterations in B-cell responsiveness consequent to environmental changes in diabetes. Deleterious effects of hyperglycaemia, so-called glucose toxicity (Unger & Grundy, 1985), represent an important means through which changes in internal environment impair B-cell function and both the secretion and action of insulin. The participation and interaction of site-specific defects and environmental factors in pancreatic B-cell dysfunction of NIDDM are considered in the framework of our current understanding of the regulation of B-cell function and stimulus–secretion coupling (Flatt, 1992; Flatt & Lenzen, 1994). Particular attention is drawn to the results of our recent and ongoing studies indicating an important role for glycation of functionally-important B-cell proteins in the glucose toxicity and functional demise of insulin-secreting cells.

B-CELL STIMULUS–SECRETION COUPLING

Glucose insensitivity of the pancreatic B-cells is the hallmark of defective insulin secretion in NIDDM (Kahn & Porte, 1990; Leahy, 1990; Flatt *et al.* 1992). Glucose is not only the principal regulator of B-cell function, but this sugar also modulates the insulinotropic potency of virtually all other secretagogues, including entero-insular hormones and neurotransmitters (Berggren *et al.* 1992). Signals generated by these stimuli are normally integrated at the level of the B-cell through a network of second-messenger pathways leading to insulin secretion. It follows, therefore, that a lesion at any one point in the sequence of events linking B-cell glucose recognition to hormone discharge might contribute to defective insulin secretion and glucose intolerance in NIDDM. Potential sites contributing to defective B-cell stimulus–secretion coupling in NIDDM include all known steps in the secretory process (for recent comprehensive review, see Flatt, 1992; Flatt & Lenzen, 1994). As illustrated in Fig. 1, the normal secretory pathway by which glucose induces insulin secretion begins with the transport of the sugar into the B-cell by means of the high-capacity GLUT-2 glucose transporter. Phosphorylation of glucose to glucose-6-phosphate by glucokinase (*EC* 2.7.1.2) and subsequent metabolism, involving mitochon-



https://doi.org/10.1079/PNS19970029 Published online by Cambridge University Press

drial FAD-glycerophosphate dehydrogenase (GDH), results in the optimal generation of ATP. The increased cellular ATP : ADP results in closure of ATP-sensitive K^+ channels (K–ATP channels) in the B-cell membrane, membrane depolarization and opening of voltage-dependent Ca^{2+} channels. Ca^{2+} influx and the increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) triggers the exocytotic machinery culminating in insulin secretion. A secondary effect of elevation of $[Ca^{2+}]_i$ is activation of adenylate cyclase (*EC* 4.6.1.1) and phospholipase C (*EC* 3.1.4.3). Generation of cyclic AMP, diacylglycerol and inositol 1,4,5-trisphosphate serve to amplify $[Ca^{2+}]_i$ signal by effects on cellular stores and by promoting phosphorylation of proteins that sensitize the secretory process to Ca^{2+}. These same enzymes are also directly activated by specific membrane receptors for hormonal and neural elements of the entero-insular axis (Berggren *et al.* 1992; Flatt, 1996).

SITE-SPECIFIC DEFECTS IN B-CELL DYSFUNCTION

Site-specific defects in the B-cell stimulus–secretion pathway (Flatt *et al.* 1992) have been revealed in studies investigating defective insulin secretion using islets derived from various animal models of NIDDM (Fig. 2). The extent to which these defects are due to genetic predisposition (primary defects) or represent secondary (acquired) features consequential to alterations in B-cell environment or external influences in diabetes remains to be established. The main site-specific defects uncovered in specific examples of NIDDM are discussed briefly.

GLUT-2 transporter

Decreased expression of the GLUT-2 glucose transporter has been demonstrated in both human NIDDM and in several animal models of diabetes (Thorens *et al.* 1992; Unger, 1992; Ohneda *et al.* 1993). In diabetic mice, this decrease has been shown to be reversible and caused by environmental factors, although the role played by hyperglycaemia is uncertain (Thorens *et al.* 1992; Ogawa *et al.* 1995). Since glucose transport is not normally a rate-limiting step for B-cell glucose metabolism, it is questionable whether abnormalities in the expression of the GLUT-2 glucose transporter play a particularly notable role relative to defects at more influential distal sites in the secretory process (Ohneda *et al.* 1993). Furthermore, rare mutations of GLUT-2 detected in individuals with NIDDM are unlikely to be a major determinant of the disease (Tanizawa *et al.* 1994*a*; Shimada *et al.* 1995).

Glucokinase

Alterations in glucokinase activity can be predicted to disturb insulin secretion in altering the rate of signal-generating metabolic flux in the B-cell (Matchinsky, 1990; Lenzen, 1992). Alterations of glucokinase and hexokinase (*EC* 2.7.1.1) activities have been linked with disturbances of insulin secretion (Matchinsky, 1996). Most notable are the functionally-important mutations of glucokinase detected in a subset of individuals with

Fig. 1. Overview of mechanisms involved in glucose-induced insulin secretion. AC, adenylate cyclase (*EC* 4.6.1.1); CICR, Ca^{2+}-induced Ca^{2+} release; ER, endoplasmic reticulum; Gi, Gs, inhibitory and stimulatory G-protein respectively; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C (*EC* 3.1.4.3); R, receptor agonist; VDCC, voltage-dependent Ca^{2+} channels; IP_3, inositol 1,4,5-triphosphate; DAG, diacylglycerol; IAPP, islet amyloid polypeptide; CCK, cholecystokinin; GRP, gastrin-releasing peptide; GLP-1, glucagon-like peptide 1 (7–36) amide; GIP, gastric inhibitory polypeptide.

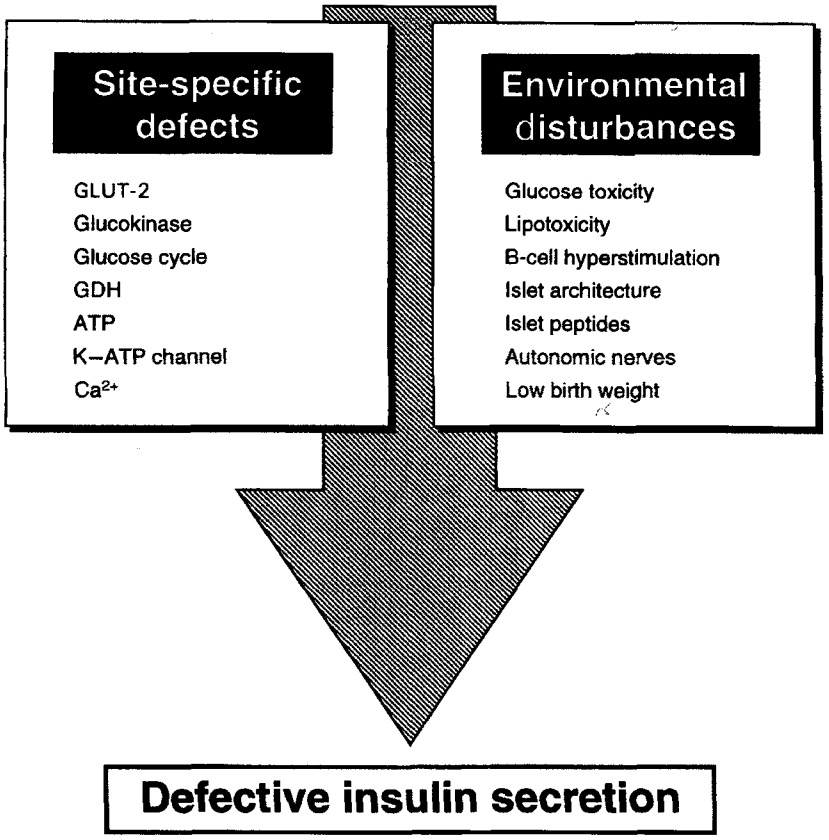


Fig. 2. Site-specific defects and environmental disturbances possibly contributing to defective insulin secretion in non-insulin-dependent diabetes mellitus. GDH, FAD-glycerophosphate dehydrogenase; glucokinase, *EC* 2.7.1.2.

maturity-onset diabetes of the young (MODY) who display abnormal insulin secretion (Velho *et al.* 1992). However, it appears that mutations of the glucokinase gene are not commonly associated with classical NIDDM (Tanizawa *et al.* 1994*b*; Stone *et al.* 1996).

Glucose-6-phosphatase (glucose cycling)

An abnormally high rate of glucose cycling, i.e. the conversion of glucose to glucose-6-phosphate by glucokinase followed by reconversion back to glucose by the action of glucose-6-phosphatase (*EC* 3.1.3.9), has been reported in islets of various animal models of NIDDM. These include *ob/ob* mice, GK rats and rats treated with streptozotocin during the neonatal period (Kahn *et al.* 1994). Consumption of one molecule of ATP for every molecule of glucose cycled might, by decreasing the ATP pool, interfere with the regulation of K–ATP channels and contribute to defective insulin secretion in NIDDM. Further studies with human islets will allow evaluation of the importance of this defect.

Mitochondrial FAD-glycerophosphate dehydrogenase

Activation of mitochondrial GDH by $[Ca^{2+}]_i$ results in the preferential metabolism of glucose by aerobic rather than anaerobic glycolysis (Sener & Malaisse, 1992). Thus, by

optimizing the generation of ATP from glucose, GDH participates, like glucokinase, in the crucial glucose-sensing process of the B-cell. Alterations in the activity of islet GDH have been demonstrated in human NIDDM and various animal models of experimentally-induced and spontaneous diabetes, including adult rats treated neonatally with streptozotocin, GK rats, *fa/fa* rats and C57BL/KsJ *db/db* mice (Malaisse, 1994; Fernandez-Alvarez *et al.* 1994). Other animal models of NIDDM, such as *ob/ob* mice, have normal islet GDH activity (Malaisse, 1994). However, other studies challenge the view that the irregularities of GDH observed may not represent an acquired defect consequent to diabetes (MacDonald *et al.* 1996). The development of a polymorphic genetic marker for GDH will enable assessment of its role in the pathogenesis of human NIDDM (Ferrer *et al.* 1996). However, it has recently been suggested that mutations in this gene are unlikely to contribute to MODY or NIDDM in white Europeans (Warren-Perry *et al.* 1996).

Generation of ATP

Generation of ATP and alteration in intracellular ATP : ADP is a key step linking metabolic and cationic events in the insulin secretory pathway. However, very few studies have actually addressed the possible link between defective insulin secretion and perturbations of the intracellular ATP generation. One problem is the difficulty of monitoring functionally important alterations in cellular ATP adjacent to the plasma membrane K–ATP channels. Nevertheless, compromised ATP generation has been shown to be associated with the glucose unresponsiveness of fetal rat pancreatic B-cells (Rorsman *et al.* 1989).

ATP-sensitive potassium channels

Disturbances in the regulation of K^+ permeability and defective insulin secretion have been observed in spontaneously diabetic *db/db* mice and diabetic Chinese hamsters (Meissner & Schmidt, 1976; Berglund *et al.* 1980; Frankel & Sehlin, 1987). Patch-clamp studies using both the cell-attached and inside-out configurations have been used to directly investigate the regulation of the K–ATP channels. Studies using the neonatal-streptozotocin-treated rat model and GK rats have indicated that the inhibitory action of glucose on B-cell K–ATP channels is impaired in NIDDM (Tsuura *et al.* 1992*a*,*b*). However, the inhibitory effect of ATP applied directly to inside-out patches was preserved. This would suggest that the glucose insensitivity of the K–ATP channel reflects impaired ATP generation rather than a defect in structure or function of the channel itself. Recent studies have ruled out a possible role of genetic variations in K–ATP channel genes in MODY and human NIDDM (Zhang *et al.* 1995; Iwasaki *et al.* 1996; Tanizawa *et al.* 1996).

Cytoplasmic free calcium ions

Disturbances in the regulation of B-cell Ca^{2+} fluxes and $[Ca^{2+}]_i$ represent a common feature in animal models with defective insulin secretion. Abnormalities have been demonstrated in spontaneously-diabetic *db/db* mice, Spiny mice and neonatal-streptozotocin-treated rats (for review, see Flatt *et al.* 1992). Since Ca^{2+} plays a key role in B-cell signal transduction, any perturbation in Ca^{2+} regulation will result in disturbed insulin secretion. However, the extent to which abnormalities in the regulation of $[Ca^{2+}]_i$ reflect disturbances at earlier stages in the stimulus–secretion coupling pathway is unclear. Site-

specific lesions in voltage-dependent Ca^{2+} channels, intracellular Ca^{2+} transport and the effects of Ca^{2+} on the exocytotic machinery are yet to be uncovered. Studies in glucose-infused rats indicate that hyperglycaemia may decrease B-cell Ca^{2+}-channel mRNA (Iwashima *et al.* 1993).

DISTURBANCES OF B-CELL ENVIRONMENT

Mutations and functional alterations in key proteins involved in the B-cell secretory machinery undoubtedly contribute to defective insulin secretion. However, such mutations will be exposed in early life during the functional maturation of the B-cell. Since the normal onset of NIDDM is in later life, important interactions are likely to exist between site-specific lesions and environmental factors in determining the functional demise of the B-cell.

NIDDM is associated with a significant number of disturbances of the B-cell environment including alterations in the concentrations of nutrients and hormones which affect insulin secretion (Kahn & Porte, 1990; Leahy, 1990; Flatt *et al.* 1992). As outlined in Fig. 2, such alterations can make an important contribution to B-cell dysfunction. This is particularly well illustrated with respect to hyperglycaemia-induced glucose toxicity. Such environmentally-induced changes may themselves manifest site-specific lesions within the B-cell secretory machinery.

Glucose toxicity

Investigations in human subjects and experimental animals *in vitro* using cultured isolated islets indicate that long-term exposure to a hyperglycaemic environment results in a gradual impairment of insulin secretion (Leahy, 1990; Simonson *et al.* 1992). This has given support to the concept of glucose toxicity and the view of hyperglycaemia as an inducer, as well as a consequence of B-cell dysfunction (Unger & Grundy, 1985).

Glucose toxicity and B-cell dysfunction. Glucose toxicity is well illustrated by the induction of defective insulin secretion in rats subjected to partial pancreatectomy or to continuous glucose infusions (Leahy, 1990; Flatt *et al.* 1992; Simonson *et al.* 1992). Interestingly, studies using isolated islets have revealed both species and strain differences in susceptibility to glucose toxicity. The B-cells of some strains of mice are particularly resistant to the adverse effects of hyperglycaemia (Svensson *et al.* 1993). In contrast, human islets and islets isolated from certain strains of rats are extremely susceptible to the detrimental effects of hyperglycaemia on insulin secretion (Bedoya & Jeanrenaud, 1991; Kaiser *et al.* 1991; Davalli *et al.* 1992; Eizirik *et al.* 1992). Other studies have shown rapid reversal of glucose toxicity on restoration of normoglycaemia (Anello *et al.* 1996). However, not all B-cell defects in spontaneous diabetes are normalized by this manoeuvre (Abdel-Halim *et al.* 1995), indicating the interplay between genetic and environmental factors in the functional disarray. Recent experiments in our laboratory have examined the effects of culture at high glucose concentration on the acute insulin secretory responsiveness of glucose-responsive BRIN-BD11 cells (McClenaghan *et al.* 1996). As shown in Fig. 3, exposure to a hyperglycaemic environment for 72 h markedly impaired the acute insulin-releasing action of glucose. These and other studies have shown that such differences cannot be attributed merely to changes in cellular insulin stores (Barnett *et al.* 1993).

Glycation and B-cell glucose toxicity. Despite accumulating evidence that glucose toxicity makes an important contribution to defective insulin secretion in NIDDM, relatively few studies have investigated the underlying molecular mechanism. Recent studies have been performed to assess the role of glycation of B-cell proteins in glucose

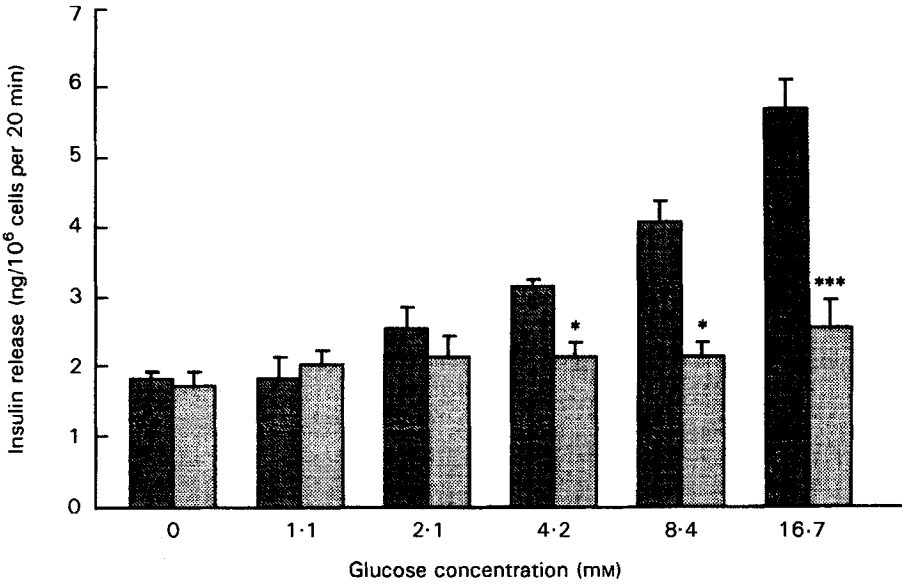


Fig. 3. Glucose toxicity and the impaired responsiveness of insulin-secreting cells to acute glucose challenge. BRIN-BD11 cells were cultured for 72 h at 11·1 (■) or 66·6 (□) mM-glucose before evaluation of acute (20 min) secretory responsiveness to glucose. Values are means with their standard errors represented by vertical bars for six to eight observations. Culture at 66·6 mM glucose significantly decreased the acute secretory response to glucose concentrations of 4·2–16·7 mM: compared with culture at 11·1 mM-glucose: $*P < 0.05$, $***P < 0.0001$. (Data from Barnett *et al.* 1993.)

toxicity and defective insulin-secretion. Using BRIN-BD11 cells (McClenaghan *et al.* 1996), it has been demonstrated that the detrimental effects of hyperglycaemia on B-cell function were associated with a substantial increase in the glycation of intracellular proteins (Fig. 4). The extent of glycation was increased 3–5-fold by increasing the glucose

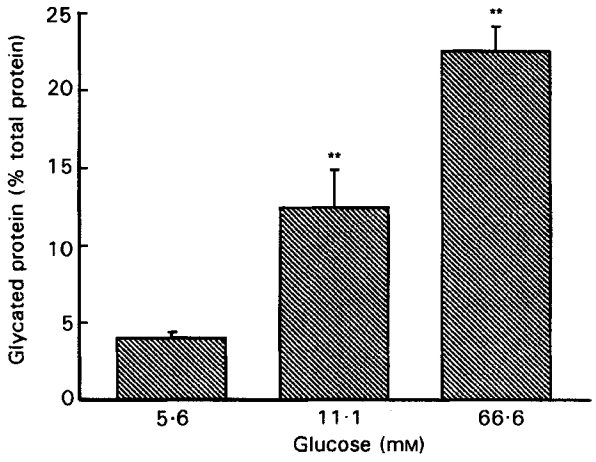


Fig. 4. Glycation of cellular proteins in insulin-secreting cells exposed to increasing concentrations of glucose in culture. BRIN-BD11 cells were cultured at 5·6, 11·1 or 66·6 mM-glucose for 72 h. Cells were extracted and dialysed against saline (9 g NaCl/l) before separation of glycated and non-glycated proteins by affinity chromatography. Results are expressed as a percentage of total protein. Values are means with their standard errors represented by vertical bars for six observations. Exposure to 11·1–66·6 mM-glucose significantly increased the extent of glycation: $**P < 0.01$.

https://doi.org/10.1079/PNS19970029 Published online by Cambridge University Press

concentration in culture from 5·6 mM to either 11·1 or 66·6 mM respectively. Since glycation can be expected to influence the activity of enzymes and other functional proteins in the B-cell, it is probable that glycation is an important mediator of B-cell glucose toxicity and defective insulin secretion.

Evidence for glycation of insulin and proinsulin. Our ongoing studies concerning identification of glycated B-cell proteins have initially focused on the most important protein in the B-cell, i.e. the insulin molecule itself (Abdel-Wahab *et al.* 1997). Incubation of insulin with glucose *in vitro* followed by analysis using reversed-phase HPLC revealed two peaks, corresponding to glycated and non-glycated insulins (O'Harte *et al.* 1996; Fig. 5). Studies have also shown that incubation of insulin *in vitro* with glucose induced a concentration- and time-dependent increase in the glycation of insulin (Abdel-Wahab *et al.* 1993) as measured by radioimmunoassay after separation of glycated and non-glycated

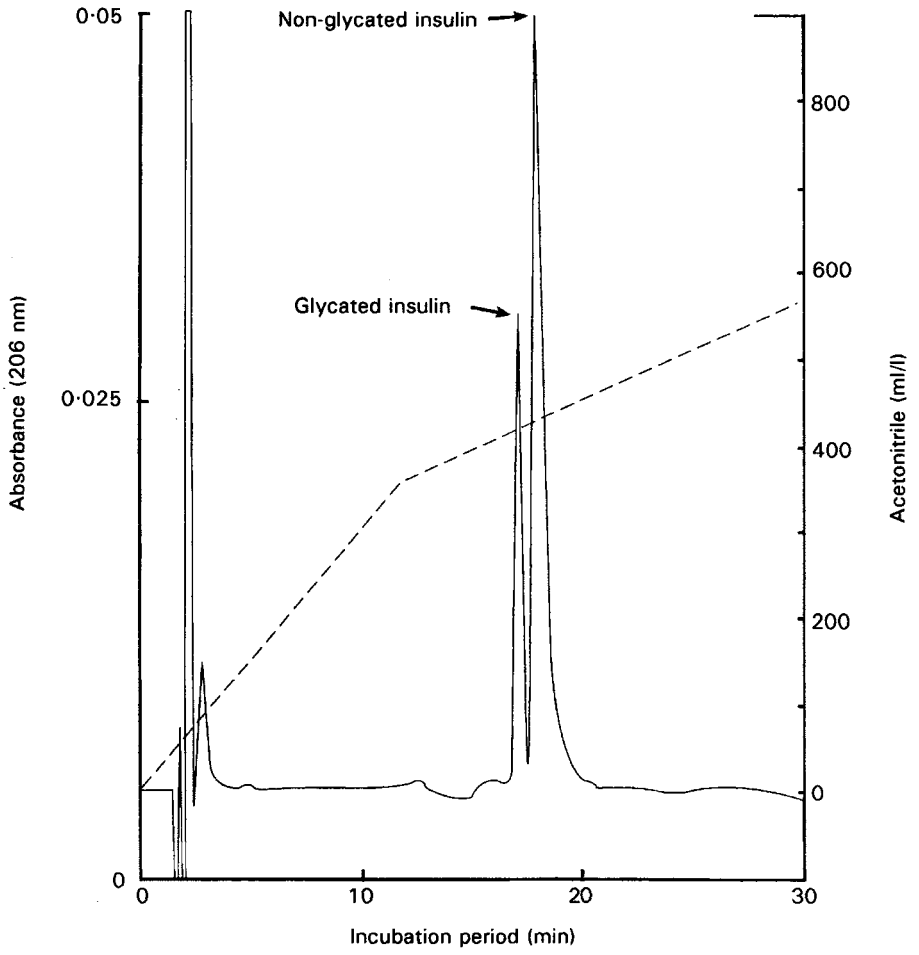


Fig. 5. Reversed-phase HPLC separation of glycated and non-glycated insulin following incubation with 220 mM-D-glucose for 24 h at 37°. Bovine insulin was incubated in phosphate-buffered saline (9 g NaCl/l; pH 7·4) containing 220 mM-glucose for 24 h at 37°. Samples were subjected to reversed-phase HPLC using a Supelcosil LC-8 (Supelco, Poole, Dorset) column equilibrated with 10 ml trifluoroacetic acid/l water at a flow-rate of 1·0 ml/min. For further details, see O'Harte *et al.* (1996).

https://doi.org/10.1079/PNS19970029 Published online by Cambridge University Press

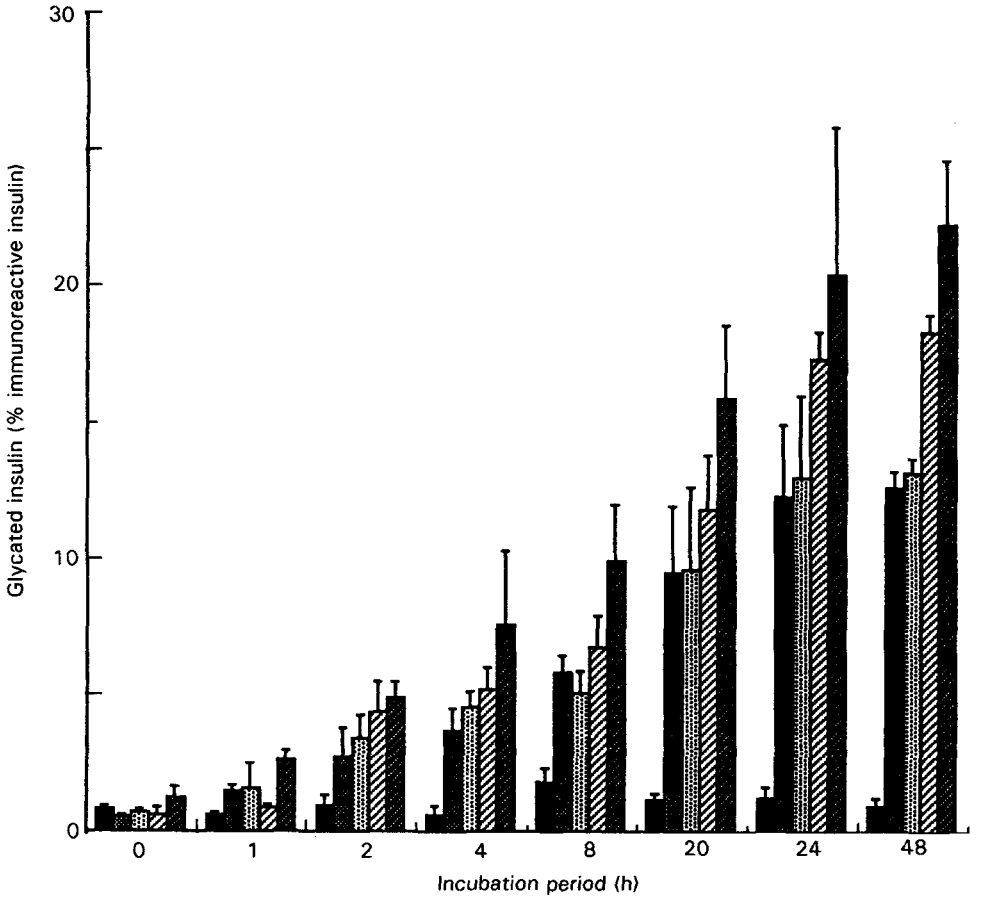


Fig. 6. Glucose concentration- and time-dependent glycation of insulin. Bovine insulin was incubated in phosphate-buffered saline (9 g NaCl/l, pH 7·4) containing 0–220 mM-glucose for 1–48 h at 37°. Samples were dialysed and glycated- and non-glycated-insulin fractions were separated by affinity chromatography. Percentage glycation of immunoreactive insulin is shown as means with their standard errors represented by vertical bars for three observations. (■), 0 mM; (▩), 27·7 mM; (▦), 55·5 mM; (▨)

insulins by affinity chromatography using Glycogel B (Pierce & Warriner, Chester, Cheshire; Fig. 6). Similar studies (Abdel-Wahab *et al.* 1997) have shown that metabolites of glucose, such as glyceraldehyde-3-phosphate and glucose-6-phosphate, are significantly more reactive than glucose itself (Fig. 7(A)). Furthermore, the precursor proinsulin molecule is also susceptible to glycation (Fig. 7(B)).

Glycation of insulin and proinsulin within the B-cells. The characteristics of the GLUT-2 glucose transporter in the B-cell membrane ensure that rapid fluctuations in extracellular glucose concentrations are matched by similar changes in glucose concentrations within the B-cells. It follows, therefore, that the hyperglycaemia of NIDDM will be mirrored by elevated glucose concentrations within the pancreatic B-cells. It is not surprising, therefore, that culture of BRIN-BD11 cells under hyperglycaemic conditions was associated with the concentration-dependent glycation of cellular immunoreactive insulin (Abdel-Wahab *et al.* 1997; Fig. 8(A)). Comparison with the characteristics of extracellular glycation (Figs. 6–7) suggests that the milieu within the B-

https://doi.org/10.1079/PNS19970029 Published online by Cambridge University Press

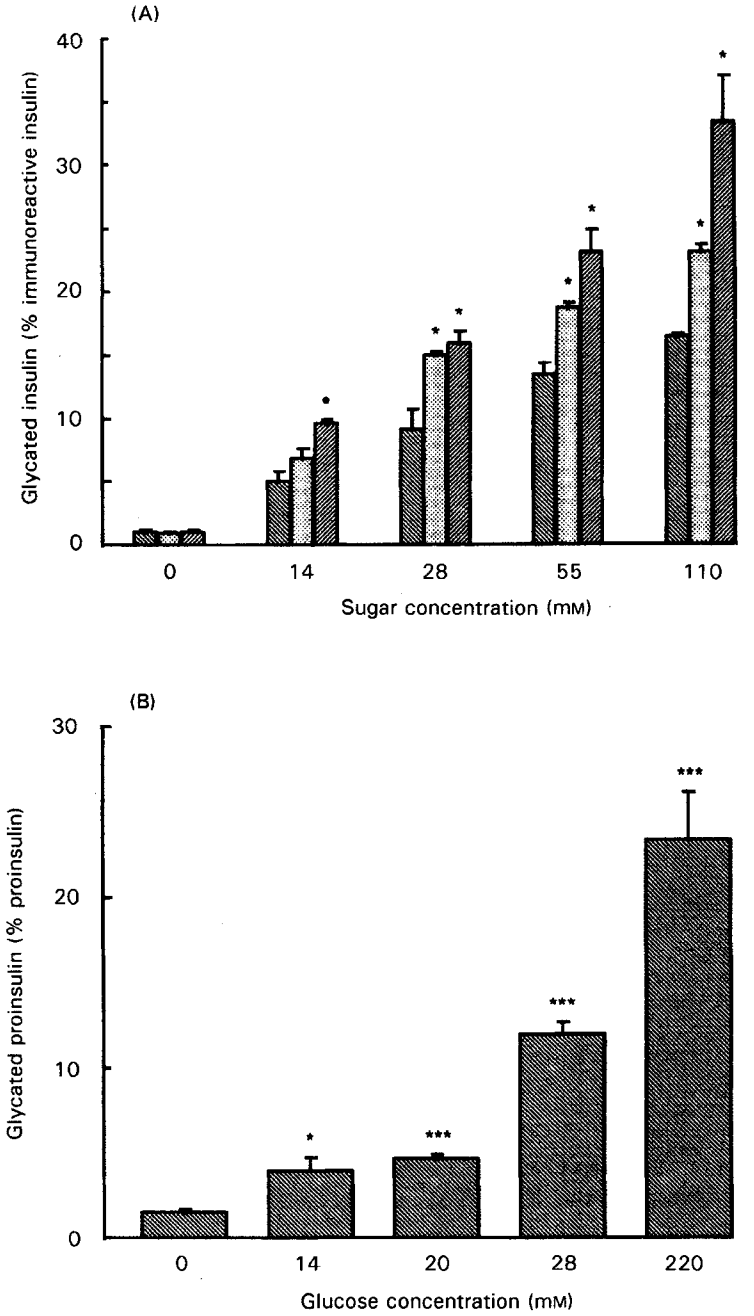


Fig. 7. Glycation of insulin and proinsulin *in vitro* in presence of glucose and glucose metabolites. Bovine insulin (A) or recombinant human proinsulin (B) were incubated in phosphate-buffered saline (9 g NaCl/l, pH 7·4) containing glucose (▨) or metabolites, glyceraldehyde-3-phosphate (▩) and glucose-6-phosphate (▨), for 24 h at 37°. Samples were dialysed, subjected to affinity chromatography, and glycated and non-glycated peptides were quantified by radioimmunnoassay using an antibody with full cross-reactivity to insulin and proinsulin. Values are means with their standard errors represented by vertical bars for three to four observations. (A) Mean values were significantly different from those for same concentration of glucose: $*P < 0{\cdot}05$. (B) Mean values were significantly different from those for 0 mM-glucose: $*P < 0{\cdot}05$, $***P < 0{\cdot}001$. (Data from Abdel-Wahab *et al.* 1997.)

https://doi.org/10.1079/PNS19970029 Published online by Cambridge University Press

cell is particularly favourable for glycation of insulin. It is also apparent from Fig. 8(B) that the glycation of cellular insulin (or proinsulin) is rapid, occurring within 2 h exposure to hyperglycaemia. In accordance with these observations, glycated insulin has been shown to be elevated in the pancreas of various animal models of diabetes, including hydrocortisone-treated rats, streptozotocin-treated mice and genetically-obese–diabetic *ob/ob* mice (Abdel-Wahab *et al.* 1996). As shown in Fig. 9, glycated insulin was elevated in both pancreatic stores and plasma of *ob/ob* mice. Further analysis of the glycated fractions in the pancreas (Abdel-Wahab *et al.* 1996) revealed substantial glycation of both proinsulin and insulin stores (Fig. 10).

Decreased biological activity of glycated insulin. In order for glycation of insulin to be of pathophysiological significance in NIDDM, it is essential to show that glycation to a level achievable in the B-cell *in vivo* is associated with a measurable decrease in insulin biological activity. As shown in Fig. 11, a preparation of 20% glycated insulin clearly exhibited diminished ability to lower plasma glucose concentrations in normal mice compared with non-glycated insulin (Abdel-Wahab *et al.* 1994). Extension of these observations to human subjects infused with HPLC-purified peptides (Hunter *et al.* 1996) revealed a decreased potency of glycated insulin, as evidenced by lower glucose infusion rates to maintain steady-state euglycaemia (Fig. 12).

Involvement of glycation of B-cell proteins in non-insulin-dependent diabetes mellitus. On the basis of the recent observations referred to previously, glycation of B-cell proteins may be considered as a potentially-important novel compounding factor in the B-cell dysfunction, glucose intolerance and metabolic disarray of NIDDM. As illustrated in Fig. 13, it is possible to envisage two interacting vicious spirals through which hyperglycaemia-induced glycation of insulin and other functionally-important B-cell proteins contribute directly to both the defective insulin secretion and insulin resistance of NIDDM. Furthermore, since glycation of proinsulin may interfere with enzymic processing, glycation of this prohormone might contribute to the incomplete processing and elevated levels of circulating proinsulin encountered in NIDDM (Flatt *et al.* 1992; Abdel-Wahab *et al.* 1996). Future studies are required to develop this hypothesis and to identify the proteins and the glycation sites involved.

Lipotoxicity

Recently, much attention has focused on possible detrimental effects of elevated lipid concentrations on B-cell function in NIDDM (Grill *et al.* 1994; Unger, 1995; Prentki & Corkey, 1996). Studies *in vivo* in human subjects and using isolated islets in tissue culture have clearly demonstrated B-cell lipotoxicity, resulting in impaired insulin secretory responses to glucose and other stimuli (Sako & Grill, 1990*a*; Zhou & Grill, 1994; Paolisso *et al.* 1995). The mechanisms likely to be involved include alterations in B-cell glucose metabolism and ATP generation due to activity of the glucose–fatty acid cycle (Randle *et al.* 1994; Zhou *et al.* 1996). Recent studies by Zhou *et al.* (1996) have shown that stimulation of fatty acid oxidation in islets inhibits glucose oxidation by decreasing pyruvate dehydrogenase (*EC* 1.2.4.1; PDH) enzyme activity with parallel enhancement of PDH kinase. Future studies will assess the effects of lipid-lowering agents and inhibitors of fatty acid oxidation on B-cell dysfunction in NIDDM.

Pancreatic B-cell hyperactivity

Studies of experimental and spontaneous diabetes have provided evidence for a link between B-cell hyperactivity and defective insulin secretion (Flatt *et al.* 1992). In various

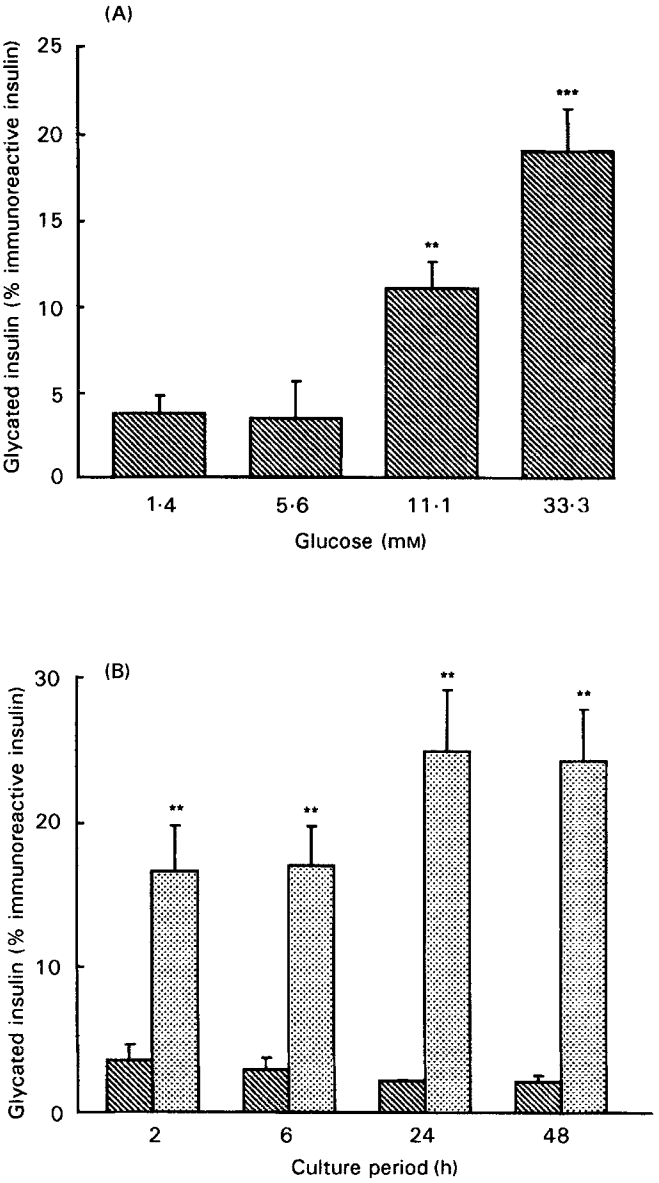


Fig. 8. Glucose concentration- and time-dependent glycation of insulin in clonal B-cells maintained in tissue culture. BRIN-BD11 cells were cultured for: (A) 72 h with 1·4–33·3 mM glucose, or (B) 2–48 h with 5·6 (▨) or 33·3 (▩) mM-glucose. Cells were extracted, dialysed and glycated and non-glycated insulin fractions were separated by affinity chromatography. Insulin was determined by radioimmunoassay in both separated fractions, and the percentage glycated insulin was calculated. Results are expressed as means with their standard errors represented by vertical bars for four to six observations. (A) Exposure to 11·1–33·3 mM-glucose significantly increased glycation compared with 1·4 mM and 5·6 mM: $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$ respectively. (B) Exposure to 33·3 mM-glucose increased glycation at each time point compared with 5·6 mM-glucose at the same time: $^{**}P < 0{\cdot}01$. (Data from Abdel-Wahab *et al.* 1997.)

https://doi.org/10.1079/PNS19970029 Published online by Cambridge University Press

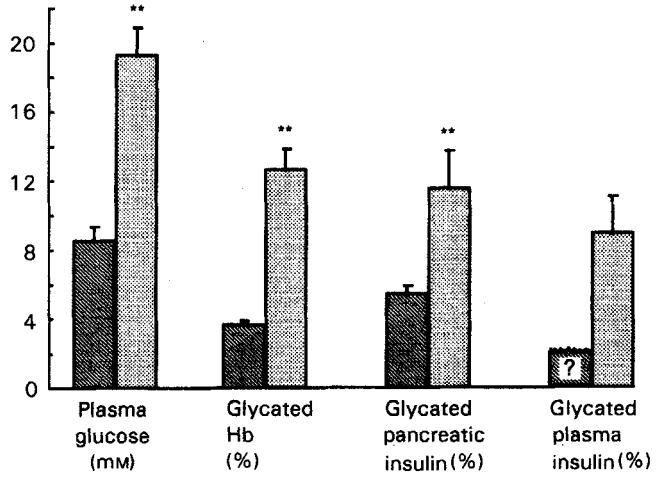


Fig. 9. Plasma glucose, glycated haemoglobin (Hb) and glycated pancreatic and plasma insulin in adult lean (▩) and genetically-obese–diabetic *ob/ob* (▩) mice. Glycated and non-glycated insulins were measured in pancreatic extracts and pooled plasma samples by radioimmunoassay following separation by affinity chromatography. Quantification of glycated plasma insulin in normal mice was not possible due to the low circulating insulin concentrations in these animals. Values are means with their standard errors represented by vertical bars for five observations. Mean values were significantly different from those of control animals: $**P < 0{\cdot}01$.

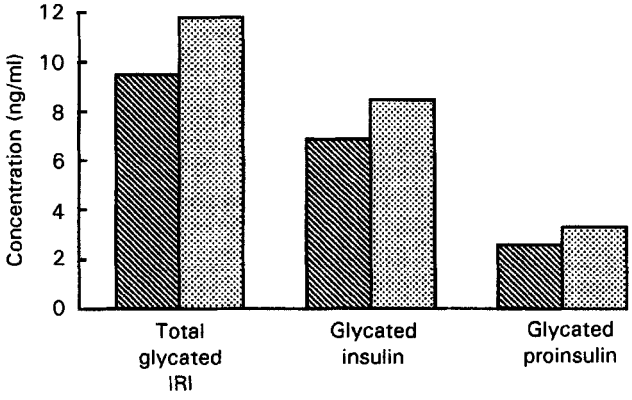


Fig. 10. Glycated insulin and proinsulin in pancreatic extracts of adult lean (▩) and genetically obese-diabetic *ob/ob* (▩) mice. Total glycated immunoreactive insulin (IRI; insulin + proinsulin), glycated insulin and glycated proinsulin are shown as single determinations from pooled pancreatic extracts (n 5–8). Following isolation of glycated peptides by affinity chromatography, insulin and proinsulin were separated by HPLC before measurement by radioimmunoassay with a fully cross-reacting insulin antibody. (Data from Abdel-Wahab *et al.* 1997.)

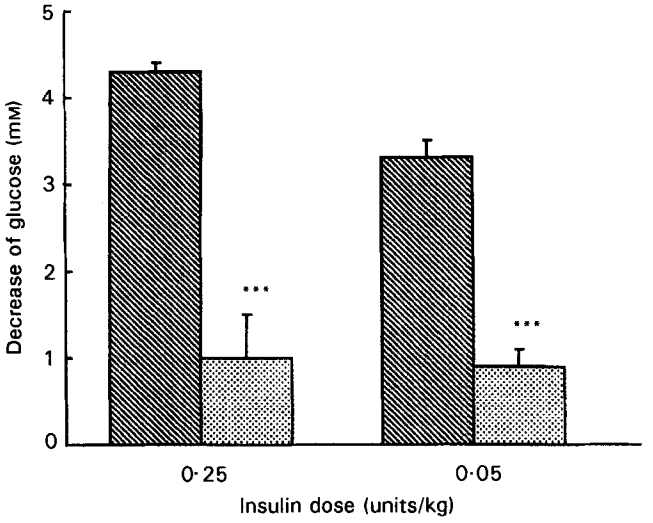


Fig. 11. Insulin-induced decrease in plasma glucose in normal mice injected with glycated (▨) or non-glycated (▩) insulin with 2 g glucose/kg body weight. Effects of glycated and non-glycated insulin at 0·05 and 0·25 units/kg on glucose-lowering activity were examined 30 min after intraperitoneal injection with 2 g D-glucose (390 g/l)/kg body weight. The extent of glycation of the former insulin preparation was 20 %. Values are means with their standard errors represented by vertical bars for five observations. Glycated insulin exhibited decreased biological potency compared with non-glycated insulin at both concentrations tested: ***$P < 0{\cdot}001$. (Data from Abdel-Wahab *et al.* 1994.)

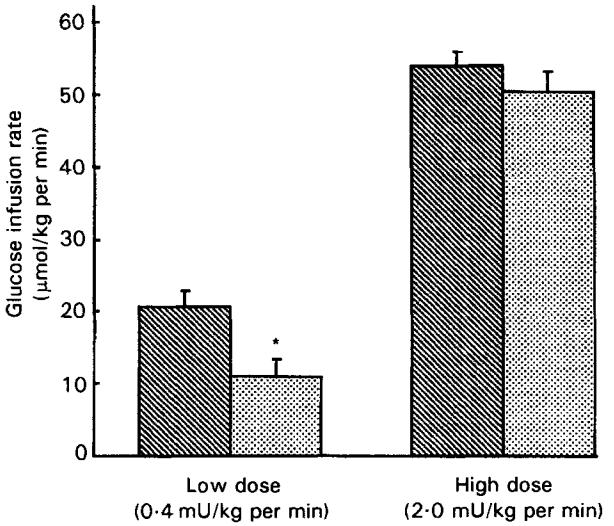


Fig. 12. Glucose infusion rates to maintain euglycaemia in human subjects in response to glycated (▨) and non-glycated (▩) insulin. The effect of HPLC-purified glycated and non-glycated human insulin (2 h at 0·4 mU/kg per min) on peripheral glucose uptake was compared on two separate occasions in seven healthy overnight-fasted 20–22-year old male volunteers. Values are means with their standard errors represented by vertical bars. Mean value was significantly different from the glucose infusion rate to maintain steady-state euglycaemia using non-glycated insulin: *$P < 0{\cdot}02$. (Data from Hunter *et al.* 1996.)

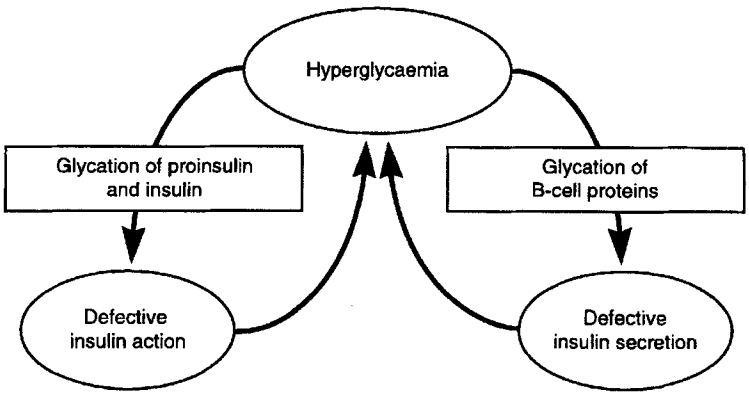


Fig. 13. Glucose toxicity and involvement for glycation of insulin and other B-cell proteins in the defective secretion and action of insulin in non-insulin-dependent diabetes mellitus.

models of NIDDM, loss of an insulin response to glucose shows a close correlation with the extent of hyperinsulinaemia (Flatt *et al.* 1992). Interventions, such as fasting in *ob/ob* mice, which decrease the extent of hyperinsulinaemia and hyperglycaemia have also been reported to improve insulin secretory responsiveness (Flatt & Bailey, 1981). Defective insulin secretion in glucose-infused rats may partly reflect induction of B-cell hyperactivity as well as the direct deleterious effects of hyperglycaemia (Sako & Grill, 1990*b*; Kaiser *et al.* 1991). Such observations suggest that hyperinsulinaemia due to excessive B-cell stimulation caused by an overactive entero-insular axis may contribute to defective insulin secretion in syndromes of NIDDM (Bailey & Flatt, 1988).

A component of the B-cell glucose toxicity induced *in vivo* or in tissue culture has been suggested to be secondary to chronic glucose-induced hyperstimulation of insulin secretion (Sako & Grill, 1990*b*; Kaiser *et al.* 1991; Bjorklund & Grill, 1993; Leahy *et al.* 1993). Such studies have used diazoxide to pharmacologically restrain insulin secretion during artificial exposure of normal B-cells to hyperglycaemia. Extrapolation of these observations to the natural environment of glucose-insensitive B-cells in spontaneous diabetes is not straightforward. However, it appears likely that the mechanism by which hyperglycaemia induces B-cell glucose toxicity involves both B-cell hyperactivity and glycation of functionally-important cellular proteins.

Islet architecture and peptides

Alterations in the relative proportions of the various islet cell types in diabetes (Kloppel *et al.* 1992) can be expected to modify interactions between the B-cells and the surrounding A-cells and D-cells leading to disturbances in insulin secretion (Marks *et al.* 1992). Such effects may be compounded further by alterations in islet vasculature and blood flow which normally progresses from the B-cell core to peripherally-located A-cells and D-cells (Marks *et al.* 1992). Changes in the secretion and actions of various other local modulators of insulin secretion are also likely to be important (Berggren *et al.* 1992; Holst, 1992; Flatt, 1996). These include atrial natriuretic peptide, biogenic amines, corticotrophin-releasing factor, diazepam-binding inhibitor, islet amyloid polypeptide (IAPP), neuropeptide Y, opiate peptides, pancreastatin, peptide YY, thyrotropin-releasing hormone and a growing number of other less-well-known peptides. Most notable in this list are pancreastatin and

IAPP. Both are released from the B-cell and have been proposed to contribute to B-cell dysfunction by exerting an inhibitory tone on insulin secretion (Tatemoto *et al.* 1986; Flatt *et al.* 1992; Clark, 1992).

B-cell innervation

The islet autonomic innervation plays an important role in the fine-tuning of insulin secretion (Holst, 1992). Alterations in autonomic tone occur in NIDDM, as illustrated by the well-known deleterious effect of hyperglycaemia on nerve conduction velocity. The possibility that the B-cell innervation may also be disrupted in diabetes is illustrated well by ultrastructural observations in diabetic Spiny mice and Chinese hamsters (Orci *et al.* 1970; Diani *et al.* 1983), and by alterations in the levels of neuropeptides in the islets of various diabetic models (Bailey & Flatt, 1988). Alterations in the actions of the classical and peptidergic neurotransmitters in diabetes, including acetylcholine, noradrenaline, cholecystokinin, somatostatin, galanin, gastrin-releasing peptide, vasoactive intestinal peptide and neuropeptide Y (Holst, 1992), may contribute to the amplification or suppression of insulin secretion through parasympathetic and sympathetic nerves respectively (Flatt, 1996).

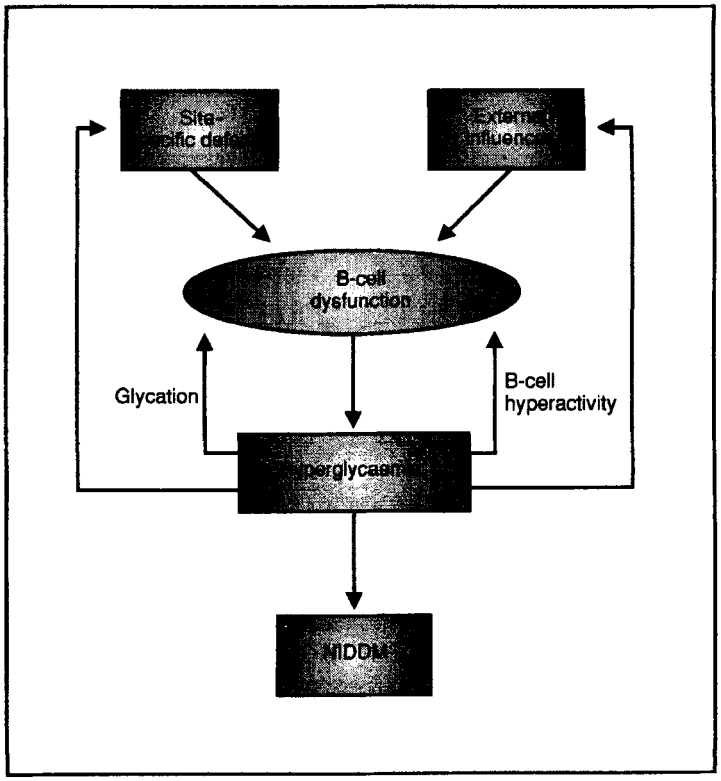


Fig. 14. Scheme for interactions between site-specific defects, external disturbances, glycation of B-cell proteins and secretory hyperactivity in pancreatic B-cell dysfunction and hyperglycaemia of non-insulin-dependent diabetes mellitus (NIDDM).

CONCLUDING REMARKS

Pancreatic B-cell dysfunction in NIDDM is the consequence of site-specific defects in the stimulus–secretion coupling pathway and numerous diabetes-related changes in external influences on the B-cell (Fig. 14). There is undoubtedly a great heterogeneity in the molecular mechanisms underlying defective insulin secretion in NIDDM as evidenced in animals and human subjects (Kahn & Porte, 1990; Leahy, 1990; Flatt *et al.* 1992). However, accumulating 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.

The authors' research on mechanisms of pancreatic B-cell dysfunction and glucose toxicity is generously supported by the British Diabetic Association, Wellcome Trust, Department of Health and Social Services (N. Ireland), NI DevR Funding and University of Ulster Research Committee.

REFERENCES

Abdel-Halim, S. M., Ostenson, C.-G., Andersson, A., Jansson, L. & Efendic, S. (1995). A defective stimulus-secretion coupling rather than glucotoxicity mediates the impaired insulin secretion in mildly diabetic F1 hybrids of GK-Wistar rats. *Diabetes* **44**, 1280–1284.

Abdel-Wahab, Y. H. A., Barnett, C. R. & Flatt, P. R. (1993). Characteristics of extracellular and intracellular glycation of insulin. *Diabetic Medicine* **9**, Suppl. 2, S12–S13.

Abdel-Wahab, Y. H. A., O'Harte, F. P. M., Barnett, C. R. & Flatt, P. R. (1994). Studies on the effect of glycation of insulin on biological action in isolated mouse diaphragm muscle. *Biochemical Society Transactions* **22**, 238S.

Abdel-Wahab, Y. H. A., O'Harte, F. P. M., Barnett, C. R. & Flatt, P. R. (1997). Characterisation of insulin glycation in insulin-secreting cells maintained in tissue culture. *Journal of Endocrinology* **152**, 59–67.

Abdel-Wahab, Y. H. A, O'Harte, F. P. M., Ratcliff, H., McClenaghan, N. H., Barnett, C. R. & Flatt, P. R. (1996). Glycation of insulin in the islets of Langerhans of normal and diabetic animals. *Diabetes* **45**, 1489–1496.

Anello, M., Rabazzo, A., M., Degano, C., Caltabiano, V., Patane, G., Vigneri, R. & Purrello, F. (1996). Fast reversibility of glucose-induced desensitization in rat pancreatic islets: Evidence for an involvement of ion fluxes. *Diabetes* **45**, 502–506.

Bailey, C. J. & Flatt, P. R. (1988). The enteroinsular axis in models of hyperinsulinaemic and hypoinsulinaemic diabetes. In *Frontiers in Diabetes Research: Lessons from Animal Diabetes*, vol. 2, pp. 217–224 [E. Shafrir and A. E. Renold, editors]. Chichester: John Libbey.

Barnett, C. R., Wilson, A. M., McClenaghan, N. H., Yoon, T. W., Swanston-Flatt, S. K., Ah-Sing, E. & Flatt, P. R. (1993). Effects of chronic hyperglycaemia on the function of insulin-secreting cells maintained in tissue culture. *Diabetic Medicine* **10**, Suppl. 3, S24.

Bedoya, F. J. & Jeanrenaud, B. (1991). Insulin secretory response to glucose by perifused islets from lean (fa/fa) rats chronically infused with glucose. *Diabetes* **40**, 15–19.

Berggren, P.-O., Rorsman, P., Efendic, S., Ostenson, C.-G., Flatt, P. R., Nilsson, T., Arkhammar, P. & Juntti-Berggren, L. (1992). Mechanisms of action of entero-insular hormones, islet peptides and neural input on the insulin secretory process. In *Nutrient Regulation of Insulin Secretion*, pp. 289–318 [P. R Flatt, editor]. London: Portland Press Ltd.

Berglund, O., Sehlin, J. & Taljedal, I.-B. (1980). Influence of the murine gene diabetes on rhubidium ion efflux from perifused islets. *Diabetologia* **19**, 45–49.

Bjorklund, A. & Grill, V. (1993). B-cell insensitivity in vitro: Reversal by diazoxide entails more than one event in stimulus-secretion coupling. *Endocrinology* **132**, 1319–1328.

Clark, A. (1992). Islet amyloid: An enigma of type 2 diabetes. *Diabetes Metabolism Reviews* **8**, 117–132.

Davalli, A. M., Pontiroli, A. E., Socci, C., Bertuzzi, F., Fattor, B., Braghi, S., Di Carlo, V. & Pozza, G. (1992). Human islets chronically exposed *in vitro* to different stimuli become unresponsive to the same stimuli given acutely: Evidence supporting specific desensitization rather than β-cell exhaustion. *Journal of Clinical Endocrinology and Metabolism* **74**, 790–794.

Diani, A. R., Peterson, T. & Gilchrist, R. J. (1983). Islet innervation of nondiabetic and diabetic Chinese hamsters. I Acetylcholinesterase histochemistry and norepinephrine fluorescence. *Journal of Neural Transmission* **56**, 223–238.

Eizirik, D. L., Korbutt, G. S. & Hellerstrom, C. (1992). Prolonged exposure of human pancreatic islets to high glucose concentrations *in vitro* impairs the β-cell function. *Journal of Clinical Investigation* **90**, 1263–1268.

Fernandez-Alvarez, J., Conget, I., Rasschaert, J., Sener, A., Gomis, R. & Malaisse, W. J. (1994). Enzymatic, metabolic and secretory patterns in human islets of type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* **37**, 177–181.

Ferrer, J., Aoki, M., Behn, P., Nestorowicz, A., Riggs, A. & Permutt, M. A. (1996). Mitochondrial glycerol-3-phosphate dehydrogenase: Cloning of an alternatively spliced human islet-cell cDNA, tissue distribution, physical mapping, and identification of a polymorphic genetic marker. *Diabetes* **45**, 262–266.

Flatt, P. R. (editor) (1992). In *Nutrient Regulation of Insulin Secretion*. London: Portland Press Ltd.

Flatt, P.R. (1996). Hormonal and neural control of endocrine pancreatic function. In *Textbook of Diabetes*, 2nd ed, pp. 9.1–9.17 [J. C. Pickup and G. Williams, editors]. Oxford: Blackwell.

Flatt, P. R. & Bailey, C. J. (1981). Development of glucose intolerance and impaired plasma insulin response to glucose in obese hyperglycaemic (*ob/ob*) mice. *Hormone and Metabolic Research* **13**, 556–560.

Flatt, P. R., Bailey, C. J., Berggren, P.-O., Herberg, L. & Swanston-Flatt, S. K. (1992). Defective insulin secretion in diabetes and insulinoma. In *Nutrient Regulation of Insulin Secretion*, pp. 341–386 [P. R. Flatt, editor]. London: Portland Press Ltd.

Flatt, P. R. & Lenzen, S. (editors) (1994). In *Frontiers of Insulin Secretion and Pancreatic B-Cell Research*. London: Smith-Gordon & Co. Ltd.

Frankel, B. J. & Sehlin, J. (1987). Abnormalities in glucose-stimulated insulin release, ^{45}Ca uptake and ^{86}Rb efflux in diabetic Chinese hamster islets. *Diabetes* **36**, 648–653.

Grill, V., Sako, Y., Bjorklund, A. & Inoue, K. (1994). Mechanisms underlying the influence of a diabetic state on pancreatic B-cell function. In *Frontiers of Insulin Secretion and Pancreatic B-cell Research*, pp. 473–479 [P. R. Flatt and S. Lenzen, editors]. London: Smith-Gordon & Co. Ltd.

Havel, P. J., Dunning, B. E. & Taborsky, G. J. Jr (1994). Autonomic control of insulin secretion. In *Frontiers of Insulin Secretion and Pancreatic B-Cell Research*, pp. 343–352 [P. R. Flatt and S. Lenzen, editors]. London: Smith-Gordon & Co. Ltd.

Holst, J. J. (1992). Role of classical and peptidergic neurotransmitters in insulin secretion. In *Nutrient Regulation of Insulin Secretion*, pp. 23–39 [P. R. Flatt, editor]. London: Portland Press Ltd.

Hunter, S. J., Wiggam, M. I., Boyd, A. C., O'Harte, F. P. M., Ennis, C. N., Gamble, R., Sheridan, B., Barnett, C. R., McNulty, H., Bell, P. M. & Flatt, P. R. (1996). Glycation of human insulin reduces its biological potency as assessed by euglycaemic clamps in normal man. *Diabetic Medicine* **13** (Suppl. 1), S17.

Iwasaki, N., Kawamura, M., Yamagata, K., Cox, N. J., Karibe, S., Ohgawara, H., Inagaki, N., Seino, S., Bell, G. I. & Omori, Y. (1996). Identification of microsatellite markers near the human genes encoding the B-cell ATP-sensitive K^+ channel and linkage studies with NIDDM in Japanese. *Diabetes* **45**, 267–269.

Iwashima, Y., Pugh, W., Depaoli, A. M., Takeda, J., Seino, S., Bell, G. I. & Polonsky, K. S. (1993). Expression of calcium channel mRNAs in pancreatic islets and down regulation after glucose infusion. *Diabetes* **42**, 948–955.

Kahn, A., Ostenson, C.-G. & Efendic, S. (1994). Glucose cycling in pancreatic islets. In *Frontiers of Insulin Secretion and Pancreatic B-cell Research*, pp. 103–111 [P. R. Flatt and S. Lenzen, editors]. London: Smith-Gordon & Co. Ltd.

Kahn, S. E. & Porte, D. Jr (1990). The pathophysiology of type II (non-insulin-dependent) diabetes mellitus: Implications for treatment. In *Ellenberg and Rifkin's Diabetes Mellitus: Theory and Practise*, 4th ed., pp. 436–456 [H. Rifkin and D. Porte Jr, editors]. New York: Elsevier.

Kaiser, N., Corcos, A. P., Sarel, I. & Cerasi, E. (1991). Monolayer culture of adult rat pancreatic islets on extracellular matrix: Modulation of β-cell function by chronic exposure to high glucose. *Endocrinology* **129**, 2067–2076.

Kloppel, G., Gepts, W. & In't Veld, P. A. (1992). Morphology of the pancreas in normal and diabetic states. In *International Textbook of Diabetes*, pp. 223–259 [K. G. M. M. Alberti, R. A. Defronzo, H. Keen and P. Zimmet, editors]. Chichester: John Wiley.

Leahy, J. L. (1990). Natural history of β-cell dysfunction in NIDDM. *Diabetes Care* **13**, 992–1010.

Leahy, J. L., Bumbalo, L. M. & Chen, C. (1993). Diazoxide causes recovery of β-cell glucose responsiveness in 90% pancreatectomized diabetic rats. *Diabetes* **43**, 173–179.

Lenzen, S. (1992). Glucokinase: Signal recognition enzyme for glucose-induced insulin secretion. In *Nutrient Regulation of Insulin Secretion*, pp. 101–124 [P. R. Flatt, editor]. London: Portland Press Ltd.

McClenaghan, N. H., Barnett, C. R., Ah-Sing, E., Abdel-Wahab, Y. H. A., O'Harte, F. P. M., Yoon, T.-W., Swanston-Flatt, S. K. & Flatt, P. R. (1996). Characterization of a novel glucose-responsive insulin-secreting cell line, BRIN-BD11, produced by electrofusion. *Diabetes* **45**, 1132–1140.

MacDonald, M. J., Efendic, S. & Ostenson, C.-G. (1996). Normalization by insulin treatment of low mitochondrial glycerol phosphate dehydrogenase and pyruvate carboxylase in pancreatic islets of the GK rat. *Diabetes* **45**, 886–890.

Malaisse, W. J. (1994). Pancreatic B-cell FAD-glycerophosphate dehydrogenase deficiency in non-insulin-dependent diabetes: An up-dated review. In *Frontiers of Insulin Secretion and Pancreatic B-cell Research*, pp. 445–452 [P. R. Flatt and S. Lenzen, editors]. London: Smith-Gordon & Co. Ltd.

Marks, V., Samols, E. & Stagner, J. (1992). Intra-islet interactions. In *Nutrient Regulation of Insulin Secretion*, pp. 41–57 [P. R. Flatt, editor]. London: Portland Press Ltd.

Matchinsky, F. M. (1990). Glucokinase as glucose sensor and metabolic signal generator in pancreatic β-cells and hepatocytes. *Diabetes* **39**, 647–652.

Matchinsky, F. M. (1996). A lesson in metabolic regulation inspired by the glucokinase glucose sensor paradigm. *Diabetes* **45**, 223–241.

Meissner, H. P. & Schmidt, H. (1976). The electrical activity of the pancreatic B-cells of diabetic mice. *FEBS Letters* **67**, 371–374.

Morgan, L. M. (1992). Insulin secretion and the enteroinsular axis. In *Nutrient Regulation of Insulin Secretion*, pp. 1–22 [P. R. Flatt, editor]. London: Portland Press Ltd.

Ogawa, Y., Norma, Y., Davalli, A. M., Wu, Y.-J., Thorens, B., Boner-Weir, S. & Weir, G. C. (1995). Loss of glucose-induced insulin secretion and GLUT2 expression in transplanted B-cells. *Diabetes* **44**, 75–79.

O'Harte, F. P. M., Hojrup, P., Barnett, C. R. & Flatt, P. R. (1986). Identification of the site of glycation of human insulin. *Peptides* **17**, 1323–1330.

Ohneda, M., Johnson, J. H., Inman, L. R., Chen, L., Suzuki, K.-I., Goto, Y., Alam, T., Ravazzola, M., Orci, L. & Unger, R. H. (1993). GLUT2 expression and function in β-cells of GK rats with NIDDM: Dissociation between reductions in glucose transport and glucose stimulated insulin secretion. *Diabetes* **42**, 1065–1072.

Orci, L., Lambert, A. E., Amherdt, M., Cameron, D., Kanazawa, Y. & Stauffacher, W. (1970). The autonomic nervous system and the B-cell: Metabolic and morphological observations made in Spiny mice (*Acomys cahirinus*). *Acta Diabetologica* **7**, Suppl. 1, 184–226.

Paolisso, G., Gambardella, A., Amato, L., Tortioriello, R., D'Amore, A., Varricchio, M. & D'Onofrio, F. (1995). Opposite effects of short- and long-term fatty acid infusion on insulin secretion in healthy subjects. *Diabetologia* **38**, 1295–1299.

Prentki, M. & Corkey, B. E. (1996). Are the B-cell signalling molecules malonyl-CoA and cytosolic long-chain acyl-CoA implicated in multiple tissue defects of obesity and NIDDM? *Diabetes* **45**, 273–283.

Randle, P. J., Priestman, D. A., Mistry, S. & Halsall, A. (1994). Mechanisms modifying glucose oxidation in humans. *Diabetologia* **37**, Suppl. 2, S155–S161.

Rorsman, P., Arkhammar, P., Bokvist, K., Hellerstrom, C., Nilsson, T., Welsh, M., Welsh, N. & Berggren, P.-O. (1989). Failure of glucose to elicit a normal secretory response in fetal pancreatic β-cells results from glucose-insensitivity to the ATP-regulated K^+ channels. *Proceedings of the National Academy of Sciences USA* **86**, 4505–4509.

Sako, Y. & Grill, V. (1990*a*). A 48-hour lipid infusion in the rat time-dependently inhibits glucose-induced insulin secretion and B-cell oxidation through a process likely coupled to fatty acid oxidation. *Endocrinology* **127**, 1580–1589.

Sako, Y. & Grill, V. (1990*b*). Coupling of β-cell desensitization by hyperglycemia to excessive stimulation and circulating insulin in glucose-infused rats. *Diabetes* **39**, 1580–1583.

Sener, A. & Malaisse, W. J. (1992). Hexose metabolism in pancreatic islets. Ca^{2+}-dependent activation of the glycerol phosphate shuttle by nutrient secretagogues. *Journal of Biological Chemistry* **267**, 13251–13256.

Shimada, F., Makino, H., Iwaoka, H., Miyamoto, S., Hashimoto, N., Kanatsuka, A., Bell, G. I. & Yoshida, S. (1995). Identification of two novel amino acid polymorphisms in beta cell/liver (GLUT2) glucose transporter in Japanese subjects. *Diabetologia* **38**, 211–215.

Simonson, D. C., Rossetti, L., Giaccari, A. & DeFronzo, R. A. (1992). Glucose toxicity. In *International Textbook of Diabetes*, pp. 635–667 [K. G. M. M. Alberti, R. A. DeFronzo, H. Keen and P. Zimmet, editors]. Chichester: John Wiley.

Stone, L. M., Kahn, S. E., Fujimoto, W. Y., Deeb, S. S. & Porte, D. Jr. (1996). A variation at position – 30 of the β-cell glucokinase gene promoter is associated with reduced B-cell function in middle-aged Japanese-American men. *Diabetes* **45**, 422–428.

Svensson, C., Sandler, S. & Hellerstrom, C. (1993). Lack of long-term β-cell glucotoxicity *in vitro* in pancreatic islets isolated from two mouse strains (C57BL/6J; C57BL/KsJ) with different sensitivities of the β-cells to hyperglycaemia *in vivo*. *Journal of Endocrinology* **136**, 289–296.

Tanizawa, Y., Riggs, A. C., Chiu, K. C., Janssen, R. C., Bell, D. S. H., Go, R. P.-C., Roseman, J. M., Acton, R. T. & Permutt, M. A. (1994*a*). Variability of the pancreatic islet beta cell/liver (GLUT2) glucose transporter gene in NIDDM patients. *Diabetologia* **37**, 420–427.

Tanizawa, Y., Chiu, K. C., Elbein, S. C., Turner, R. C. & Permutt, M. A. (1994*b*). Role of glucokinase in the defective insulin secretion of late-onset non-insulin dependent diabetes. In *Frontiers of Insulin Secretion and Pancreatic B-Cell Research*, pp. 437–444 [P. R. Flatt and S. Lenzen, editors]. London: Smith-Gordon & Co. Ltd.

Tanizawa, Y., Matsubara, A., Ueda, K., Katagiri, H., Kuwano, A., Ferrer, J., Permutt, M. A. & Oka, Y. (1996). A human pancreatic islet inwardly rectifying potassium channel: cDNA cloning, determination of genomic structure and genetic variations in Japanese NIDDM patients. *Diabetologia* **39**, 447–452.

Tatemoto, K., Efendic, S., Mutt, V., Makk, G., Feistner, G. J. & Barchas, J. D. (1986). Pancreastatin, a novel pancreatic peptide that inhibits insulin secretion. *Nature* **324**, 476–478.

Thorens, B., Wu, Y.-J., Leahy, J. L. & Weir, G. C. (1992). The loss of GLUT2 expression by glucose-unresponsive β-cells of db/db mice is reversible and is induced by the diabetic environment. *Journal of Clinical Investigation* **90**, 77–85.

Tsuura, Y., Ishida, H., Okamoto, Y., Tsuji, K., Kurose, T., Horie, M., Imura, H., Okada, Y. & Seino, Y. (1992*a*). Impaired glucose sensitivity of ATP-sensitive K^+ channels in pancreatic β-cells in streptozotocin-induced NIDDM rats. *Diabetes* **41**, 861–865.

Tsuura, Y., Ishida, H., Okamoto, Y., Kato, S., Sakamoto, K., Horie, M., Ikeda, H., Okada, Y. & Seino, Y. (1992*b*). Glucose sensitivity of ATP-sensitive K^+ channels is impaired in β-cells of the GK rat: A new genetic model of NIDDM. *Diabetes* **42**, 1446–1456.

Unger, R. H. (1992). GLUT-2 and the pathogenesis of type II diabetes. *Diabetes Nutrition and Metabolism* **5**, 65–69.

Unger, R. H. (1995). Lipotoxicity in the pathogenesis of obesity-dependent NIDDM, genetic and clinical implications. *Diabetes* **44**, 863–870.

Unger, R. H. & Grundy, S. (1985). Hyperglycaemia as an inducer as well as a consequence of impaired islet cell function and insulin resistance: Implications for the management of diabetes. *Diabetologia* **28**, 119–121.

Velho, G., Froguel, P., Clement, K., Pueyo, M. E., Rakotoambinina, B., Zouali, H., Passa, P., Cohen, D. & Robert, J. J. (1992). Primary pancreatic beta cell secretory defect caused by mutations in glucokinase gene in kindreds of maturity onset diabetes of the young. *Lancet* **340**, 444–448.

Warren-Perry, M. G., Stoffel, M., Saker, P. J., Zhang, Y., Brown, L. J., MacDonald, M. J. & Turner, R. C. (1996). Mitochondrial FAD-glycerophosphate dehydrogenase and G-protein-coupled inwardly rectifying K^+ channel: No evidence for linkage in maturity-onset diabetes of the young or NIDDM. *Diabetes* **45**, 639–641.

Zhang, Y., Warren-Perry, M., Sakura, H., Adedlman, J., Stoffel, M., Bell, G. I., Aschcroft, F. M. & Turner, R. C. (1995). No evidence for mutations in a putative B-cell ATP-sensitive K^+ channel subunit in MODY, NIDDM or GDM. *Diabetes* **44**, 597–600.

Zhou, Y. P., Berggren, P.-O. & Grill, V. (1996). A fatty acid-induced decrease in pyruvate dehydrogenase activity is an important determinant of B-cell dysfunction in the obese diabetic db/db mouse. *Diabetes* **45**, 580–586.

Zhou, Y. P. & Grill, V. (1994). Long-term exposure of rat pancreatic islets to fatty acids inhibits glucose-induced insulin secretion and biosynthesis through a glucose-fatty acid cycle. *Journal of Clinical Investigation* **93**, 870–876.