Insulin secretion in health and disease: genomics, proteomics and single vesicle dynamics

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Abstract

Defective insulin secretion from pancreatic islet β-cells is a *sine qua non* of Type II (non-insulin-dependent) diabetes. Digital imaging analysis of the nanomechanics of individual exocytotic events, achieved using total internal reflection fluorescence microscopy, has allowed us to demonstrate that insulin is released via transient or 'cavicapture' events whereby the vesicle and plasma membranes fuse transiently and reversibly. Such studies reveal that an increase in the number of abortive fusion events contributes to defective insulin secretion in *in vitro* models of Type II diabetes. Complementary analyses of genome-wide changes in β-cell gene expression, at both the mRNA and protein levels, are now facilitating the identification of key molecular players whose altered expression may contribute to the secretory defects in the diabetic β-cell.

Introduction

Type II (non-insulin-dependent) diabetes results when the capacity of the pancreas to release insulin is outstripped by the requirements for the hormone to regulate glucose and lipid metabolism in insulin-resistant tissues. Insulin secretory deficiencies play a critical role in the onset of disease: pancreatic β-cell failure is apparent prior to the onset of the full-blown disease, and progressive loss of β-cell function, in the face of unaltered insulin sensitivity, underlies the worsening of the disease after onset [1]. While recent studies have argued that a decrease in total β-cell mass may be a major or even the principal cause of secretory failure [2], against this view at least 70% decreases in β-cell mass appear to be required before the onset of human Type I (insulin-dependent) diabetes [3]. Thus the observed decreases in β-cell mass observed in advanced stages of Type II diabetes (30–40%) [1] are unlikely to be sufficient in themselves to provoke the disease. Moreover, a rodent model of TNDM (transient neonatal diabetes mellitus), which shows many of the characteristics of Type II diabetes, demonstrates a failure of the β-cell to respond appropriately to glucose, in the face of a normal or increased β-cell mass [4]. Correspondingly, very recent work on pancreatic islets isolated from Type II diabetic humans reveals severely impaired glucose metabolism and an almost total loss of glucose-stimulated insulin secretion [5,6]. Understanding of the mechanisms through which the healthy β-cell responds to glucose, and their failure in the Type II diabetic β-cells, is therefore likely to be of paramount importance in the discovery of new treatments of this increasingly prevalent disease [7].

Glucose-stimulated insulin secretion is generally accepted to involve the uptake of the sugar into the cell by a low-affinity glucose transporter, GLUT2 [8], and phosphorylation of the sugar to glucose 6-phosphate by a high-K_m hexokinase, glucokinase [9]. Oxidative metabolism of the sugar as far as CO_2 and water then proceeds with unusual (85–90%) efficiency in the β-cell, thanks in large part to very low levels of lactate dehydrogenase and plasma membrane lactate/monocarboxylate transporter activity [10–13] and high levels of mitochondrial glycerol phosphate dehydrogenase activity, an arrangement which ensures mitochondrial oxidation of glycolytically derived reducing equivalents. Increases in mitochondrial and, consequently, cytosolic free ATP concentration [14,15] then lead to (i) inhibition of AMP-activated protein kinase [16–18] and the release of insulin-containing LDCVs (large dense core secretory vesicles) from intracellular tethers [19] (see below) and (ii) closure of ATP-sensitive potassium channels at the plasma membrane [20]. The latter event prompts cell depolarization, and Ca^{2+} influx via voltage-gated (L-type) Ca^{2+} channels [21]. Increases in the concentrations of other second messengers including nicotinic acid–adenine dinucleotide phosphate [22] and mobilization of intravesicular Ca^{2+} [23] may also contribute to the generation of localized Ca^{2+} concentrations close to the dense core vesicle [24]. Overall, increased cytosolic Ca^{2+} concentrations [25] then provoke dispersal of the cortical actin network [19] and the fusion of insulin-containing LDCVs with the cell surface, the latter via an interaction with a vesicle-resident Ca^{2+} sensor, likely to be a member of the synaptotagmin family [26].

Key words: β-cell, genomics, glucose, insulin secretion, Type II (non-insulin-dependent) diabetes, vesicle dynamics.

Abbreviations used: LDCV, large dense core secretory vesicle; GFP, green fluorescent protein; EGFP, enhanced GFP; TIRF, total internal reflection fluorescence.

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Interestingly, the role of the β-cell to transduce a metabolic signal into a secretory event appears to require multiple metabolic specializations. These include the presence of unusually low levels of the detoxifying enzymes glutathione peroxidase and catalase, a change which renders the β-cell particularly vulnerable to cellular damage, e.g. from cytokines (in Type I diabetes) or reactive oxygen species (in Type II diabetes) [27].

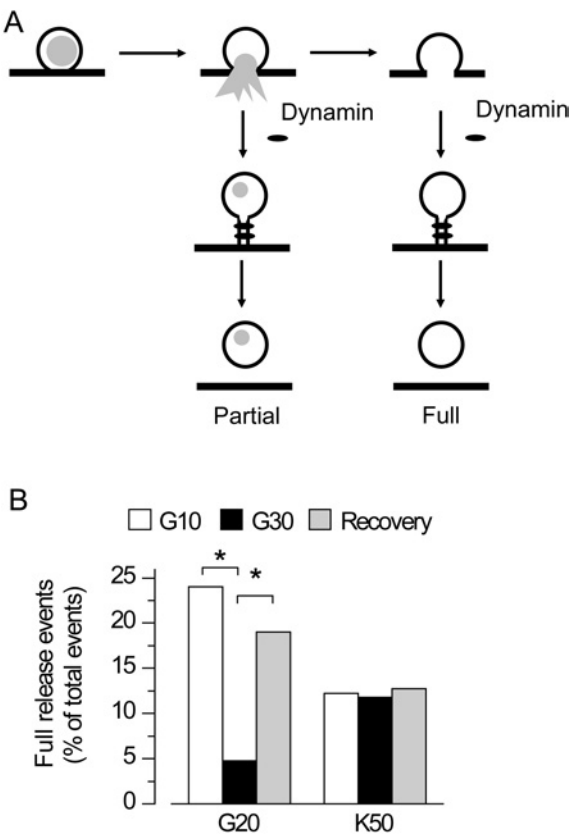
Vesicle dynamics: how does glucose make the secretory vesicle move?

A cardinal feature of normal glucose-induced insulin secretion is the existence of two phases of insulin release [28]; loss of 'first phase' insulin release is seen early in the development of Type II diabetes [29]. While several models have sought to explain this pattern in terms of intracellular signalling by glucose [30], an alternative model proposes that, within individual β-cells, multiple vesicle pools exist, notably 'readily releasable' and remotely located 'reserve' pools [31,32]. In an effort to visualize vesicle movement in living cells, we have developed a number of recombinant fluorescent tags which are efficiently targeted to the vesicle membrane or matrix. Studies using phogrin–EGFP [enhanced GFP (green fluorescent protein)] [33] revealed a crucial role of the motor proteins kinesin [34,35] and myosin Va [36] in glucose-stimulated movement of vesicles within the cell and in the final approach to the plasma membrane respectively, through mechanisms that are yet to be fully elucidated. One intriguing possibility is that changes in the phosphorylation state of one or more of the above motor proteins, perhaps mediated by AMP-activated protein kinase [17], are involved.

How does glucose stimulate the release of vesicles already in close proximity to the plasma membrane? To address this, we have targeted fluorescent probes to the vesicle interior including NPY (neuropeptide Y)–Venus (an enhanced form of yellow fluorescent protein [37]) (note that targeting of insulin as a GFP construct to the vesicle interior is poorly efficient [38]). Combined imaging of the latter construct with the vesicle membrane-targeted phogrin–EGFP, using evanescent wave [TIRF (total internal reflection fluorescence)] microscopy demonstrated that individual exocytotic events usually occur without complete dispersal of vesicle membrane components into the plasma membrane, i.e. 'full fusion' [37]. Instead, the vesicle's contents are released through a stable fusion pore whose closure is catalysed 1–2 s after the release event by the GTPase, dynamin-1 [39] (Figure 1A). This mechanism may allow more minute regulation of insulin release than can be achieved by 'all or none' events (involving the complete and irreversible fusion of single LDCVs), for example by allowing the release of only part of the vesicle's insulin content, or by permitting, if the fusion pore expands slowly, the selective release of low molecular mass vesicle cargoes such as ATP [40]. Such a mechanism would also allow the delivery of selected membrane proteins destined for eventual insertion into the plasma membrane [37]. Of note is that incubation of islets under hyperglycaemic conditions (as seen in Type II diabetes) causes a substantial increase in the number of incomplete fusion events (Figure 1B).

Figure 1 | Nanomechanics of partial insulin release events

(**A**) Model for the mechanisms underlying transient or 'cavicapture' release of vesicles [37]. (**B**) Culture of dispersed islet cells for 48 h under hyperglycaemic conditions [30 mM (G30) versus 10 mM (G10) glucose] leads to a reversible decrease in the number of 'full' fusion events, observed after stimulation with 20 mM glucose (G20) or 50 mM KCl (K50) during TIRF microscopy for 6 min (see the text). Recovery condition: islet cells previously cultured at 30 mM glucose for 48 h were incubated for a further 24 h at 10 mM glucose.



Genomics

What may underlie deficiencies in insulin secretion in Type II diabetes? Changes in the expression of key genes involved in β-cell glucose sensing seem likely to be involved. Marked alterations in the expression of these genes are apparent in rodent models of the disease such as the Zucker diabetic fatty rat [41]. Thus our recent genome-wide microarray analysis of these changes (Figure 2) demonstrates both similarities and differences with respect to changes identified in human Type II diabetic islets [42]. These include decreases in the expression of GLUT2, and genes involved in the exocytotic process (Figure 2) and in lipid biosynthesis [43].

Proteomics

The combination of subcellular fractionation with the large-scale identification of proteins with mass spectroscopy and bioinformatics analysis has opened up the possibility of performing 'subcellular proteomics' analysis in the healthy and diseased β-cell. While genetic and biochemical analyses have

Figure 2 | Changes in gene expression in poorly glucose-responsive islets from Zucker diabetic fatty rats (*fa/fa*; grey bars) compared with control rats (*fa/+*; black bars)

Islets were isolated from 7–8-week-old animals prior to culture for 48 h and RNA extraction and quantification of mRNA by microarray analysis (Affymetrix). Results shown are the means for four separate experiments. Cdc42, cell division cycle 42; G-6-P-D, glucose-6-phosphate dehydrogenase; NS, not significant; RIM, Rab3-interacting module; SUR1, sulphonylurea receptor 1.

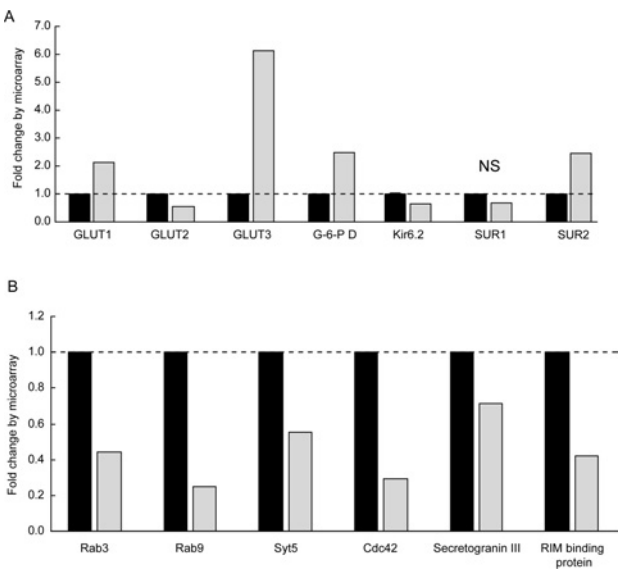
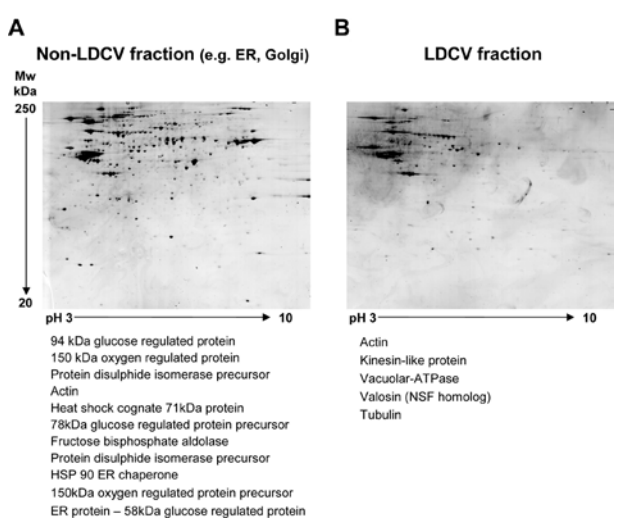


Figure 3 | Analysis of proteins from MIN6 cell LDCVs

(**A**) Endoplasmic reticulum (ER)/Golgi or (**B**) LDCV-enriched fractions were isolated by Optiprep™ fractionation [36] and subjected to two-dimensional gel electrophoresis. A list of proteins found in each fraction is indicated (M. Sanders and A. Varadi, unpublished work).



identified a number of proteins that regulate insulin vesicle movement, vesicle proteins and their complexes remain poorly characterized and several aspects of vesicle cycle regulation are poorly understood. We have pre-isolated secretory vesicles (an example is shown in Figure 3) that revealed the presence in a dense core secretory granule-enriched fraction of expected molecules, e.g. pumps involved in the accumulation of ions into vesicles and of motor proteins which may be involved in translocation of vesicles across the cell. This approach may ultimately provide novel insights into changes at the level of the proteome which contribute to defective insulin secretion in Type II diabetes.

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