Regulated expression of GLUT2 in diabetes studied in transplanted pancreatic β cells

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Introduction

Non-insulin-dependent, type II diabetes is characterized by an inability of the pancreatic β cells to secrete insulin in response to elevation in extracellular glucose concentrations. This secretory defect is specific to the glucose signalling pathway since other non-glucose secretagogues such as arginine still induce normal insulin secretion [1]. Glucose-induced insulin secretion requires glucose to be taken up by β cells, to be phosphorylated by glucokinase, a high-$K_m$ hexokinase isozyme, and further catabolized through the glycolytic pathway [2]. As a result of glucose metabolism there is an increase in the cytosolic ATP/ADP ratio which induces the closure of an ATP-dependent $K^+$ channel. The consequent depolarization of the plasma membrane [3] induces the opening of voltage-gated $Ca^{2+}$ channels, leading to an increase in cytoplasmic $Ca^{2+}$ which triggers the eventual exocytosis of insulin secretory granules [4].

A characteristic of this signalling system is that insulin secretion is initiated when the extracel-
lular glucose concentration reaches ~5 mM and increases with glucose concentrations following a sigmoidal dose–response curve, with a maximum rate of secretion achieved at ~20 mM glucose. Increase in the ATP/ADP ratio is dependent on the glycolytic flux, which must change according to the variations in extracellular glucose concentrations. The rate-limiting step in glucose metabolism in β cells is its phosphorylation by glucokinase, which has a relatively high $K_m$ for glucose of ~6 mM [5]. In β cells, in the normal state, glucose rapidly equilibrates across the plasma membrane and uptake is not limiting for metabolism. This is due to the presence in the plasma membrane of a very high level of GLUT2 [6] which differs functionally from the other transporter isoforms by its relatively high $K_m$ for glucose (~17 mM) [7]. Therefore, the simultaneous expression in β cells of high-$K_m$ GLUT2 and high-$K_m$ glucokinase is probably important for the normal functioning of the glucose signalling pathway.

GLUT2 and glucokinase are so far the best characterized molecules of the glucose signalling pathway. In glucose-unresponsive β cells, little regulation of islet glucokinase has been reported, although an increase was measured in the islets of db/db mice [8,9]. In contrast, expression of GLUT2 is considerably reduced in rodent models of type II diabetes. In the Zucker diabetic rat [10], the neonatal streptozocin rat [11], the db/db mouse [12] and at the onset of diabetes in the BB/W rat when β cells no longer respond to glucose [13], a striking decrease in GLUT2 expression is observed which can range from ~50% in mildly diabetic Zucker or neonatal streptozocin rats to 90–95% in more severely diabetic rats or in the db/db mice. The level at which GLUT2 expression is regulated is at the mRNA level in most models studied (Zucker diabetic rats, db/db mouse) although it is not yet clear whether the regulation is transcriptional or post-transcriptional or occurs at both levels. In the Zucker fa/ta rats, in which diabetes is induced by dexamethasone, the decreased GLUT2 expression is not accompanied by a decrease in its mRNA level, suggesting that in this rat model control of GLUT2 expression may be at the translational or post-translational level [14].

Whether GLUT2 decreased expression is the cause or the result of the glucose unresponsiveness of β cells is a question not yet completely settled. It has been calculated that for GLUT2 to become rate-limiting in glucose metabolism, transporter expression should be decreased by at least 10-fold since the rate of glucose uptake is vastly superior to the rate of phosphorylation in normal β cells [15]. Since glucose-unresponsiveness appears when GLUT2 expression is still ~50% of the normal value, this decreased expression may not be sufficient to account for the loss of glucose sensing. Although this reasoning may be correct, it should still be considered that β cells are very heterogeneous in terms of glucose metabolism and insulin secretory response [16] and, at the molecular level, in their level of glucokinase expression [17]. It has been suggested that some β cells act as glucose sensors or pacemakers for the stimulation of insulin secretion by the whole islets. In that respect, while GLUT2 expression in control islets is homogeneous in all β cells, in islets from diabetic animals the reduction in transporter expression is not uniform: some cells still express high levels of GLUT2 whereas others have completely lost transporter expression [11]. How this differential expression of GLUT2 in diabetes correlates with the heterogenous expression of glucokinase is not yet known. If the loss of GLUT2 expression occurs first in the pacemaker cells, then the observed decreased expression, which is usually measured by Western blot analysis of total islet lysate, may not reflect the most important events taking place during the development of diabetes.

An important aspect of regulated GLUT2 expression in diabetes is that decreased expression is the only morphological marker of diabetic β cells known so far. In addition, GLUT2 expression in diabetes is reduced only in β cells while its expression in liver, intestine and kidney is either unchanged or slightly increased [12]. There is thus a very striking diabetes-specific and β-cell-specific regulation of GLUT2 expression, the molecular basis of which is unknown. Elucidating the causes of this particular regulation in diabetes may be important to understand the causes of the β-cell dysfunction associated with diabetes. As a first approach to determine the causes of the reduction in transporter expression, we performed cross-transplantation experiments in which islets from db/db mice were placed under the kidney capsule of control db/+ mice, or conversely, islets from control mice were transplanted into diabetic mice [12]. When the islets from db/db mice were left for 2 weeks in control mice, GLUT2 was re-expressed to normal levels. In contrast, when islets from control mice were transplanted to db/db mice, GLUT2 expression was completely abolished.

These experiments indicated that the loss of GLUT2 expression in diabetes is reversible and can be induced by exposing the islets to the diabetic
environment of a db/db mouse. This suggested that factor(s) present in the environment of diabetic mice could induce the disappearance of the transporter. Since db/db mice are hyperglycaemic and hyperinsulinaemic, we performed the same transplantation of normal islets in streptozocin diabetic mice. These mice are hyperglycaemic but have very low insulin levels. In these animals, GLUT2 also disappeared from the grafted islets, indicating that insulin was not the factor causing GLUT2 disappearance. Whether glucose could lead to the observed decreased GLUT2 expression is also not likely. Indeed exposing islets in vitro to high glucose concentrations rather leads to an increased GLUT2 expression [15]. The same is true in islets of glucose-infused rats which had high blood sugar levels for several days and GLUT2 was not decreased in fa/fa rats, which are also hyperglycemic. Also, preventing the development of hyperglycaemia in Zucker diabetic rats by acarbose treatment did not prevent decreased GLUT2 expression, suggesting that glycaemia was not the factor causing transporter disappearance [18].

**Experimental approach and results**

To better define the control of GLUT 2 expression in diabetes, we need to expose diabetic β cells to the diabetic environment of type 1 or type II diabetic animals before retrieving them for biochemical and functional analysis. We have therefore developed a new experimental approach which consists of encapsulating islets or insulinoma cells before transplantation in the peritoneum of recipient rats or mice. After different periods of time the capsules can be retrieved to study GLUT2 expression at the protein, mRNA or transcriptional level and these data can be correlated with the glucose-induced insulin secretory response.

Encapsulation of islets or insulinoma cells is performed using semi-permeable polyacrylonitrile-polyvinylchloride membranes which isolate the cells from the host’s immune system but which allow nutrients and insulin to cross the membrane. The encapsulated islets can be placed in a perfusion chamber and glucose-induced insulin secretion can be monitored, showing that the cells are still alive and respond normally to glucose. These membranes display an excellent biocompatibility and can be left in the peritoneum of receiver animals for several weeks although we retrieve them usually within 2 weeks of the date of transplantation. Using these capsules, we first transplanted normal islets in the peritoneum of control or streptozocin-diabetic rats. Ten days post-transplantation, the capsules were retrieved, immersed in fixative and sections were prepared for immunocytochemical detection of GLUT2 and insulin. Islets transplanted into normal rats display a normal staining pattern of GLUT2 while transplantation into diabetic rats leads to an almost complete disappearance of transporter expression. Insulin staining is, however, equivalent in both conditions, although it appears more heterogeneous in islets transplanted in streptozocin compared with control rats, probably because the hyperglycaemia induced degranulation of the β cells. These experiments are now being completed to determine whether correction of the hyperglycaemia by phlorizin treatment or by transplanting a sufficient number of islets would change the expression pattern of GLUT2. Also, the time course of disappearance of the transporter will be measured as well as the secretory response of the transplanted islets after retrieval from the animals.

An extension of this work is the transplantation of insulinoma cells (INS-1) [19] which express GLUT2 to high level [20]. After encapsulation these cells can be transplanted and retrieved for GLUT2 expression analysis. Also, these cells have been transfected with a plasmid containing a 1.3 kb region of the GLUT2 promoter followed by the reporter gene chloramphenicol acetyl transferase (CAT). These cells have been encapsulated and transplanted into rats for 10 days. After retrieval of the capsules, the cells have been extracted and the lysate processed for CAT activity measurement. Our preliminary data indicate that CAT activity can be measured from these retrieved cells and that exposure of the cells to the diabetic environment of streptozocin rats leads to a reduction in CAT activity. This last result therefore suggests a transcriptional control of GLUT2 expression in diabetes.

**Summary**

GLUT2 disappearance is a marker of the β cell glucose-unresponsiveness associated with diabetes. Understanding the factor(s) leading to this dysfunction may shed light on pathogenesis of diabetes. Since the regulation of GLUT2 expression in diabetes can so far only be studied in in vivo experiments, we developed a novel experimental approach to study the genetic regulation of GLUT2 in diabetes. By encapsulating islets or cell lines in semi-permeable membranes, these cells can be exposed to the diabetic environment of rats or mice and can be retrieved for analysis of GLUT2 expression and for the change in the secretory response to glucose. Immunocytochemical analysis of transpor-
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ter expression reveals changes in protein expression while transcriptional analysis of GLUT2 gene expression could be performed in cells transfected with promoter–reporter gene constructs. Using this last approach we hope to be able to characterize the promoter regions involved in the β cell- and diabetes-specific regulation of GLUT2 expression and possibly to determine which factors are responsible for this regulation.


Received 16 March 1994