Molecular Mechanisms of Insulin Secretion and Pancreatic B-Cell Dysfunction

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Regulation of pancreatic B-cell glucokinase and GLUT2 glucose transporter gene expression
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Introduction
Randle's original idea of a signal function of fuel metabolism for the initiation of insulin secretion by glucose is now generally accepted (for review see [1]): in the pancreatic B-cell glucose is both a fuel and, at millimolar concentrations, a physiological stimulus for insulin secretion and insulin biosynthesis.

The device in the pancreatic B-cell, which translates changes in millimolar blood glucose concentration into corresponding signal-generating metabolic flux rates for the initiation of insulin secretion, is composed of the plasma membrane low-affinity GLUT2 glucose transporter and glucokinase, a low-affinity glucose phosphorylating enzyme. These structures are present in the pancreatic B-cell in addition to the high-affinity GLUT1 glucose transporter and high-affinity hexokinase isoenzymes. Glucokinase, the flux-regulating enzyme, is strategically positioned at the entrance to the glycolytic chain and is therefore able to control the metabolic flux through this pathway (for details of this concept see [2]). Thus this enzyme functions, in the pancreatic B-cell, as the so-called signal recognition enzyme for glucose-induced insulin secretion [2, 3]. Recently, defective glucokinase function has also been associated with certain forms of human diabetes (for review see [4]).

Glucokinase, in contrast with other hexokinases, has a $K_m$ value of approximately 10 mM and is present in significant amounts only in pancreatic B-cells and hepatocytes [2, 3, 5–9]. Glucokinase also differs from other hexokinases in its enzymic properties: it has a low affinity for glucose, is not inhibited by glucose-6-phosphate and exhibits kinetic co-operativity with glucose (for review see [2, 3, 5–9]).

During recent years, some principles of the short- and long-term regulation of glucokinase enzyme activity have been elucidated in liver and pancreatic B-cells [2, 3, 5–9]. Only recently has the regulation of gene expression of the pancreatic B-cell glucose recognition apparatus attracted attention. We will consider here some aspects of this regulation. For further details the reader is referred to recent review articles [3, 7–13].

Regulation of pancreatic B-cell glucokinase enzyme activity
Two major factors affect the pancreatic B-cell glucokinase enzyme activity: the concentration of glucose and the activity level of the enzyme, which is dependent on the nutritional status. Changes in blood glucose concentration allow rapid adaptation of the metabolic flux rate, whereas the nutrition-dependent changes in enzyme activity represent a long-term modulation. Fasting reduces glucokinase activity in pancreatic B-cells, while refeeding restores enzyme activity (for review see [2]). A recently described regulatory protein involved in the short-term regulation of liver glucokinase, which is activated by fructose-6-phosphate [14], may also
be operative in pancreatic B-cells, but with lower efficiency than in liver [15]; its role has not yet been clearly defined. Although it is generally accepted that liver glucokinase is regulated by insulin at both the translational and transcriptional levels, there are indications that, in the regulation of pancreatic B-cell glucokinase activity, glucose also plays an important role [10]. However, it appears that this regulation of enzyme activity takes place at a post-translational rather than at a translational level [15].

**Regulation of pancreatic B-cell glucokinase gene expression**

The pancreatic B-cell and liver glucokinase genes have tissue-specific transcription initiation sites under the control of tissue-specific transcription factors. This implicates a tissue-specific regulation of the glucokinase gene [7-9, 13].

The glucokinase gene is expressed in pancreatic B-cells as a 2.8 kb mRNA species, in contrast with the 2.4 kb mRNA transcript that is expressed in liver cells [16, 17]. A 4.4 kb mRNA transcript has also been detected in pancreatic B-cells [16, 17]. Although the 2.8 kb transcript is expressed in pancreatic B-cells of neonatal rats, distinct expression of the 4.4 kb mRNA transcript does not occur until two weeks after birth [18]. Different transcription units give rise to each tissue-specific glucokinase mRNA species and also to the tissue-specific glucokinase isoenzymes. However, the enzymic properties of pancreatic B-cell and liver glucokinase are virtually identical, indicating that any differences are functionally insignificant. In insulin-producing tumour cells, variant alternatively-spliced glucokinase mRNA species appear to be associated with glucokinase proteins that lack normal enzymic properties [19-21] but it has not yet been decided what functional consequences these changes may have.

Glucokinase mRNA levels are dependent on nutritional status, with a reduction of almost 50% after 48 h of starvation and normalization after refeeding [17]. These changes are significantly less dramatic than in liver [17], indicating that the pancreatic B-cell promoter of the glucokinase gene is relatively insensitive to short-term regulatory influences. No significant change in glucokinase gene regulation (under the influence of changing diet [16], glucose concentration [22] or insulin concentration [23]) was detected in other studies. However, exercise training led to a remarkable decrease in the level of glucokinase mRNA in total pancreas [24].

**Regulation of pancreatic B-cell GLUT2 glucose transporter gene expression**

The low-affinity GLUT2 glucose transporter in the pancreatic B-cell has a high \( K_m \) (17 mM) for glucose (for review see [11, 12]) and a glucose transport capacity that is probably one hundred times higher than the capacity for glucose phosphorylation by glucokinase [25]. As in the liver, the GLUT2 glucose transporter gene is expressed in the early postnatal period in pancreatic B-cells [18] as a 2.8 kb mRNA species [26, 27].

Northern blot analysis of adult rat pancreas mRNA revealed a 50% reduction in GLUT2 glucose transporter mRNA only after a 48 h starvation period, while reconstitution of GLUT2 glucose transporter mRNA was evident within four hours after refeeding [17]. Similar results were obtained using in situ hybridization of rat pancreas sections after a hyperinsulinaemic, hypoglycaemic clamp [28]. In rat pancreatic islets, cultured at high glucose concentrations, GLUT2 glucose transporter mRNA levels were increased [25, 29]. In parallel, GLUT2 glucose transporter protein levels increased in pancreatic islets during long-term (seven days) tissue culture. However, at the same time, glucose transport capacity decreased; this is indicative of a modified glucose transporter protein function [25].

Koranyi et al. [23, 30] detected no significant regulation of GLUT2 glucose transporter gene expression that was dependent on the starvation period [30] or insulin treatment [23]. At first glance, the results favour glucose as the principal regulator of GLUT2 glucose transporter gene expression in pancreatic B-cells at the transcriptional and post-transcriptional levels, though a permissive action of insulin in this context cannot be excluded at present. Thus, although the GLUT2 glucose transporter in pancreatic B-cells is apparently regulated at both the transcriptional and post-transcriptional levels during starvation, the physiological relevance remains unclear, particularly in view of the high glucose transport capacity of GLUT2.

In some animal models of non-insulin dependent diabetes, though not in human diabetes, the GLUT2 glucose transporter is not expressed in pancreatic B-cells. It has been hypothesized that this may be the reason for insulin deficiency in these animals [31-35]; it appears possible that a complete lack of GLUT2 glucose transporter gene expression is incompatible with normal glucose-induced insulin secretion. This view is also supported by the observation that, in certain insulin-producing tumour cell lines, defective insulin secretion at
Physiological glucose concentrations is accompanied by a lack of GLUT2 glucose transporter gene expression. Thus it can be concluded that an almost complete loss of GLUT2 glucose transporter gene expression may be responsible for defective glucose-induced insulin secretion at physiological glucose concentrations. However, mere fluctuations of the GLUT2 glucose transporter gene and protein expression that occur normally under physiological conditions, i.e., during starvation and refeeding cycles, are unlikely to have a physiologically relevant regulatory function for glucose-induced insulin secretion.

**Genetically modified insulin-producing cell lines**

During the last decade, some permanent insulin-producing tissue culture cell lines became popular in pancreatic islet research. However, the disadvantage of these tumour cell lines is that, in contrast to normal pancreatic B-cells, they show an abnormal insulin secretory pattern in response to glucose stimulation. In particular, there is a significant increase in insulin release from these cells only at submillimolar and subphysiological millimolar glucose concentrations. These cells are unresponsive to glucose stimulation in the normal physiological glucose concentration range. Only cells of the recently established MIN insulinoma cell line (derived from transgenic mice), which have been transfected with the SV40 virus large T antigen under control of an insulin promoter, have been proposed to respond to stimulation with millimolar glucose concentrations by releasing insulin [36].

GLUT2 glucose transporter and glucokinase gene expression in RINm5F insulinoma cells and insulin-producing AtT-20ins anterior pituitary tumour cells differ in some respects from that in normal pancreatic B-cells [9]; there are remarkable differences between the relatively high abundance of glucokinase mRNA and the low glucokinase enzyme activity in these insulin-producing tumour cells [19–21, 37, 38]. In addition, these permanent insulin-producing cells predominantly express the high-affinity GLUT1 glucose transporter, while the low-affinity GLUT2 glucose transporter is underexpressed or even absent [37, 39]. In view of the importance of the low-affinity glucose-sensing apparatus of the pancreatic B-cell for stimulus-secretion coupling, the modulation of glucose transport and glucose phosphorylation by stable transfection may lead to the creation of an artificial pancreatic B-cell. Permanent insulin-producing cell lines are an excellent model for the manipulation of gene expression of such structures using molecular biology techniques (Table 1). Such cell lines have been transfected with both the low-affinity GLUT2 glucose transporter [37, 39] and the high-affinity GLUT1 glucose transporter [40, 41].

Hughes et al. [37] reported the stable transfection of an anterior pituitary tumour-derived insulin-producing AtT-20ins cell line with a rat GLUT2 glucose transporter cDNA. These GLUT2 glucose

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**Table 1**

Functional characteristics of some genetically modified insulin-producing cell lines

<table>
<thead>
<tr>
<th>Insulin-producing cell line</th>
<th>Transfected gene</th>
<th>Reference</th>
<th>Effects of transfection</th>
</tr>
</thead>
<tbody>
<tr>
<td>RINm5F</td>
<td>GLUT2</td>
<td>[39]</td>
<td>+ ++ ++ ++</td>
</tr>
<tr>
<td>AtT-20ins</td>
<td>GLUT2</td>
<td>[37]</td>
<td>++ + + +</td>
</tr>
<tr>
<td>RINr</td>
<td>GLUT1</td>
<td>[40]</td>
<td>+ ++ + +</td>
</tr>
<tr>
<td>AtT-20ins</td>
<td>GLUT1</td>
<td>[41]</td>
<td>+ ++ + +</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Functional characteristic</th>
<th>RINm5F</th>
<th>AtT-20ins</th>
<th>RINr</th>
<th>AtT-20ins</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-O-Methylglucose uptake</td>
<td>+ ++</td>
<td>++</td>
<td>-</td>
<td>+ ++</td>
</tr>
<tr>
<td>Insulin mRNA</td>
<td>+ ++</td>
<td>+</td>
<td>+</td>
<td>o</td>
</tr>
<tr>
<td>Insulin content</td>
<td>+ ++</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Insulin secretion</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>o</td>
</tr>
<tr>
<td>Ratio glucokinase/hexokinase</td>
<td>+ +</td>
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transporter-transfected cells revealed, at physiological glucose concentrations, not only a pronounced stimulation of 3-O-methylglucose transport, but also increased levels of insulin mRNA, increased insulin content and an improvement in the insulin secretory response to glucose stimulation. AtT-20ins anterior pituitary tumour cells have a disadvantage over RINm5F insulinoma cells in that the cotransfected insulin gene is under the control of a viral promoter [37], thus preventing the regulation of the natural insulin biosynthesis by the level of gene transcription in AtT-20ins cells from being reported. We therefore used the RINm5F rat insulinoma tissue culture cell line for such transfection experiments and transfected these cells [39] with human GLUT2 glucose transporter cDNA [42]. In addition to a significant stimulation of 3-O-methylglucose uptake at physiological millimolar glucose concentrations (the $K_m$ value for 3-O-methylglucose transport increased from 0.5 mM to 12 mM), the result was a pronounced increase in insulin mRNA levels and insulin content as well as an insulin secretory response to glucose stimulation at millimolar concentrations [39].

Both studies also revealed an increase in the glucokinase/hexokinase enzyme activity ratio in these GLUT2 glucose transporter-transfected cell lines [38, 39]. This increase was due to the induction of the glucokinase enzyme activity and was particularly pronounced in the GLUT2-transfected RINm5F cells [39]. Glucose appeared to induce glucokinase enzyme activity [39, 43, 44] at the level of translation or by post-translational modification, rather than at the transcriptional level, since glucokinase mRNA levels remained unchanged after incubation with high glucose concentrations [39]. Hughes et al. [37], in anterior pituitary tumour-derived AtT-20ins cells transfected with a rat GLUT2 glucose transporter cDNA, observed a maximal insulin secretory response at glucose concentrations of 10–50 μM, while Tiedge et al. [39], in RINm5F insulinoma cells transfected with human GLUT2 glucose transporter cDNA, observed a maximal insulin secretory response only at physiological millimolar glucose concentrations.

Both insulin-producing cell lines are tumour cell lines with a high hexokinase enzyme activity. In comparison with AtT-20ins cells, non-transfected RINm5F insulinoma cells contain higher glucokinase enzyme activities [6]. This probably enables GLUT2 glucose transporter-transfected RINm5F cells to generate a higher metabolic flux rate (at physiological millimolar glucose concentrations) in the initial rate-limiting step of glucose phosphorylation to produce the signal for initiation of glucose-induced insulin secretion. The low glucokinase enzyme activity, in relation to the high hexokinase enzyme activity, might explain the glucose sensitivity in the GLUT2-transfected AtT-20ins cells at such extremely low glucose concentrations. In addition, the glucokinase/hexokinase ratio was increased less through GLUT2 glucose transporter transfection in the AtT-20ins cells [37] than in the RINm5F cells [39]. Thus high hexokinase enzyme activity apparently limits the glycolytic flux rate in such GLUT2 glucose transporter-transfected insulin-producing tumour cells at high glucose concentrations (relative to normal pancreatic B-cells). A reduction in the high hexokinase activity of these tumour cells will be necessary to achieve glucose phosphorylation kinetics that can mirror the typical postprandial glucose concentration changes, and generate insulin secretory responses which are identical to those of normal pancreatic B-cells.

The crucial role of glucose metabolism, in the maintenance of a physiological insulin secretory response to glucose stimulation, has been supported by an observation in transgenic mice which expressed the yeast hexokinase (type B) under transcriptional control of the rat insulin II promoter [45]. Total hexokinase activity ($K_m < 0.25 \text{ mM}$) in pancreatic islets from these transgenic mice doubled, and was accompanied by an improved insulin secretory response to glucose in the concentration range 3–9 mM. The $K_m$ of yeast hexokinase is nearly five times higher [45] than the $K_m$ of the hexokinase isoenzyme which is expressed naturally in mouse pancreatic islets [46].

Shibasaki et al. [40] transfected RINr insulinoma cells and Hughes et al. [41] transfected AtT-20ins cells with the high-affinity GLUT1 glucose transporter. The results of these transfections indicated that additional expression of the GLUT1 glucose transporter in these insulin-producing tumour cells also increases 3-O-methylglucose uptake, insulin mRNA levels and insulin content [40, 41], albeit to a lesser extent than in the GLUT2-transfected cell lines. These changes were achieved at subphysiological glucose concentrations. Most important, however, was the observation that glucose was unable to increase insulin secretion in GLUT1 glucose transporter-transfected AtT-20ins cells [41]. This was surprising even though the increase in the $K_m$ and $V_{max}$ values for glucose was somewhat less pronounced in these GLUT1 cell lines than in the GLUT2 glucose transporter-transfected insulin-producing cell lines [41]. Thus even a high degree of GLUT1 glucose transporter
expression cannot wholly compensate for a lack of GLUT2 glucose transporter expression.

Conclusions
Low-affinity glucose transport and glucose phosphorylation play a central role in the signal generation for glucose-induced insulin secretion. Though the mutual roles of glucose and insulin in the gene expression of the GLUT2 glucose transporter and glucokinase in pancreatic B-cells have not yet been elucidated conclusively, it is clear that gene expression is regulated at both the transcriptional and translational levels, thus providing a basis for therapeutic interventions in diabetes treatment. In particular, the regulation of glucokinase gene expression is of great importance, since mutations of the glucokinase gene locus (detected in some patients with type II diabetes) are likely to be accompanied by reduced glucokinase enzyme activity, owing to defective gene expression [47].

Insulin-producing tissue culture cell lines are excellent models for transfection experiments. Such experiments provide evidence for the importance of the GLUT2 glucose transporter and glucokinase, the two major components of the glucose recognition apparatus of the pancreatic B-cell in stimulus-secretion coupling. The expression of a sufficient number of low-affinity GLUT2 glucose transporters in the plasma membrane is necessary for the physiological insulin secretory response to glucose stimulation of the pancreatic B-cell. However, sufficient glucokinase enzyme activity must also be expressed and the hexokinase enzyme activity should not be too high in relation to the glucokinase enzyme activity. Thus expression of the high-affinity GLUT2 glucose transporter and a high glucokinase/hexokinase enzyme activity ratio are the prerequisites for normal glucose responsiveness of the pancreatic B-cell. The GLUT1 glucose transporter cannot adequately replace the GLUT2 glucose transporter in this function.

The functional consequences of the transfection of insulin-producing cells, with a high- or low-affinity glucose transporter, provide additional support for the assumption that glucose phosphorylation by glucokinase is critical for stimulus-secretion coupling in the pancreatic B-cell. Future transfection experiments must aim to create insulin-producing cells with glucose responsiveness in the physiological concentration range. At present, insulin-producing tumour cells transfected with the low-affinity GLUT2 glucose transporter represent the closest adaptation of a tumour cell to physiological insulin secretory responsiveness. As tumour cells they can be produced in large quantities through tissue culture, and are suitable for transplantation into diabetic animals, so the function of such genetically modified insulin-producing cells, for normalization of a diabetic state, can be elucidated.

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Introduction

Ion channels have many important roles in governing the mechanisms of insulin secretion from the B-cells of the pancreatic islets of Langerhans. These cells synthesize, store and subsequently release insulin in response to a number of metabolic, hormonal and neural influences. The major physiological stimulus is, however, an increase in the plasma glucose concentration. Glucose initiates secretion by mechanisms that are dependent upon a rise in the B-cell free intracellular calcium ion concentration ([Ca2+]i). This is associated with calcium influx across the plasma membrane and not mobilization of calcium from intracellular calcium stores [1]. In order to adjust the rate of insulin release, in response to fluctuations in the availability of glucose, the B-cell has adopted several discrete con-