Glucokinase and glucose transporter expression in liver and islets: implications for control of glucose homeostasis

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The liver/islet high-K\textsubscript{m} facilitated glucose transporter (GLUT-2) and the glucose phosphorylating enzyme, glucokinase, are key regulators of the rate of glucose metabolism in liver and islets, the two 'glucose-sensing' tissues in mammals. Increases in circulating glucose stimulate insulin secretion from the \(\beta\)-cells of the islets of Langerhans and convert the liver from an organ of net glucose output to one of glucose storage, processes mediated by increased glucose metabolism in both tissues. Increases in islet glucose metabolism result in elevated levels of a number of intracellular compounds that are recognized as second messengers for insulin secretion, including intracellular Ca\textsuperscript{2+}, inositol phosphates, arachidonic acid, diacylglycerol and ATP/ADP ratios (reviewed in [1–5]). The \(\beta\)-cell glucose transporter, which has a \(K_m\) for glucose of 17 mM and which has the same primary sequence and similar kinetics to liver GLUT-2 [6], probably plays a permissive role, by allowing rapid equilibration of intracellular and extracellular glucose. The rate of glucose metabolism is then largely controlled by glucokinase [2, 7–9]. Perhaps the most compelling single piece of evidence supporting a regulatory role for the enzyme is that the curves for glucose dependence of glucose uptake (metabolism) and glucokinase activity in islets are virtually superimposable (see [2]). The curve for glucokinase activity as a function of glucose concentration in both liver and islets is sigmoidal, and is particularly steep over the physiological range of glucose concentrations (4–10 mM). This means that even modest increments in circulating glucose can quickly be translated into substantial increases in glucose metabolism, through rapid transporter-mediated equilibration across the plasma membrane and phosphorylation by glucokinase.

It has been known for some time that islets isolated from fasted animals exhibit both an attenuated insulin secretory response to glucose and a reduction in glucokinase activity that is thought to be sufficient to account for the dampened response of glucose usage (metabolism) and glucokinase activity in islets to increased glucose concentrations (4–10 mM). This means that even modest increments in circulating glucose can quickly be translated into substantial increases in glucose metabolism, through rapid transporter-mediated equilibration across the plasma membrane and phosphorylation by glucokinase.

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Abbreviation used: GLUT-2, high-\(K_m\) facilitated glucose transporter.

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molecular biology have led to an understanding of the structural basis for the differently sized messages (see below). (4) Regulation of liver glucokinase expression in response to dietary and/or hormonal changes is complex, and appears to involve changes in transcription, mRNA stability, and translation, and is further mediated by the long half-life of the glucokinase protein (30 h).

Islet glucokinase has been cloned from a normal rat cDNA library in our laboratory and from an insulinoma library and by amplification of total pancreas mRNA with the polymerase chain reaction by Magnuson & Shelton [26]. Comparison of the sequence with that for liver glucokinase [15] reveals that the two cDNAs are identical over a major portion, stretching from the codon for amino acid 15 (nucleotide 161, using the numbering system of Andreone et al. [15] for liver glucokinase) to the polyadenylation site at nucleotide 2329. The sequence encoding the first 15 amino acids, however, is clearly different, as shown in Fig. 1. A striking distinction is that within the first 15 amino acids, islet glucokinase contains nine residues with formal charge, whereas liver glucokinase has only two. The islet clone encodes an active glucokinase polypeptide in liver, demonstrating its expression in bacteria [26a]. It is possible that the charged N-terminus of islet glucokinase imparts a capacity for electrostatic interactions with other components of islet cells. In fact, subcellular fractionation of islet cells results in the equal distribution of a high-Km glucose phosphorylating activity to cytosolic and particulate fractions.

N-terminally, an ideal partner for electrostatic interaction with glucokinase would be the islet GLUT-2, since if such a complex forms, it would represent a highly efficient 'glucose-sensing apparatus'. We have recently prepared an antibody against the islet-specific N-terminal glucokinase peptide [26a] that specifically recognizes a protein of approx. 52,000 daltons in islet cell extracts, and that may allow immunocytochemical analysis of the intracellular distribution of glucokinase in the near future.

The above information, coupled with recent studies describing the structure of the rat glucokinase gene [25, 26], has led to a fairly complete understanding of the origin of distinct glucokinase mRNA and protein species in liver and islets. Each mature mRNA is comprised of 10 exons from the single glucokinase gene. The liver and islet transcripts both contain exons 2-10, but exon 1 is different in the tissue-specific mRNAs. The liver-specific exon 1 is located more proximal to the other exons, and is at least 12 kb downstream of the islet-specific exon 1 [26]. The islet-specific exon 1 is larger than its liver counterpart, accounting for the differently sized mRNAs [16, 26]. Although the exact factors and cis-acting sequences controlling differential regulation of the glucokinase gene in liver and islets remain to be identified, the structure of the gene does appear to provide a physical separation of the relevant regulatory regions.

Differential regulation of expression of GLUT-2 in liver and islets has also recently been observed by our group [28]. Continuous insulin infusion for 5 or 12 days, with consequent hypoglycaemia (55 mg/100 ml compared with 120 mg/100 ml in controls), results in a reduction of islet GLUT-2 mRNA levels, measured both by hybridization in situ and Northern blot analysis, to undetectable levels. The reduction in GLUT-2 mRNA is accompanied by loss of a 17-MDa component of glucose transport activity present in normal islets from insulin-infused rats and the abolition of glucose-stimulated insulin secretion [28]. In contrast, such infusions have no consistent effects on GLUT-2 levels in liver, as shown in Fig. 2. Unlike the glucokinase system these tissue-specific alterations in GLUT-2 expression appear to be accomplished without alteration in the structure of the mRNA in the two tissues, since they are the same size, and GLUT-2 clones from liver and islet cDNA libraries have the same sequence in both rats [6] and humans [29]. This suggests that there may be physiological circumstances in which glucokinase and GLUT-2 expression are differentially controlled, with consequent specific modulation of glucose metabolism in liver or islet cells. An example of differential alteration in GLUT-2 and glucokinase expression is given in...
Fig. 2. Hepatic glucokinase mRNA levels are increased by 3-fold in rats infused with insulin for 5 days relative to saline-infused controls; a more prolonged insulin infusion of 12 days had no effect. In contrast, insulin infusion for either 5 or 12 days resulted in no consistent change in GLUT-2 mRNA levels in liver.

Despite the interesting and sometimes dramatic alterations in glucokinase mRNA and protein levels described above, the changes in activity of the enzyme in liver or islet tissues in response to dietary manipulation are relatively modest. Decreases in islet glucokinase activity in response to 24-72 h of fasting are estimated at between 30 and 65% [10, 11]. The reduction in liver glucokinase activity in response to 24 h of fasting is similarly modest [12, 30]. Nevertheless, these changes may be sufficient to alter glucose metabolism, and as a consequence, the 'glucose-sensing' function of these tissues. Thus, the islets exhibit markedly attenuated insulin secretion in response to glucose when isolated from fasted rats. Reduced hepatic glucokinase activity in the fasted state may also prevent efficient deposition of glucose as glycogen in the liver by the 'direct' pathway, and thus act as a signal for the operation of the 'indirect' pathway, wherein glycogen is synthesized largely from gluconeogenic precursors [30]. Reduced insulin secretion in fasting may also delay the induction of glucokinase in liver and allow glucagon to stimulate liver gluconeogenesis in a relatively unopposed fashion for a significant period after termination of a fast. Preliminary studies suggest that GLUT-2 mRNA levels are not significantly altered in response to fasting and refeeding in either liver or islets (S. D. Hughes & C. B. Newgard, unpublished work). Normally, glucose transport capacity is far in excess of the rate of glucose metabolism in either liver or islets, so that modest changes in transport activity would likely have little effect on overall glucose disposal in these tissues. Loss of GLUT-2 function may become critical, however, in pathophysiological conditions, such as in non-insulin-dependent diabetes mellitus, where insulin resistance may lead to significant hyperinsulinaemia for prolonged periods and comprise GLUT-2 expression in islets. In short, continuing molecular and biochemical studies on the two components of the 'glucose-sensing apparatus' are warranted and will continue to be highly relevant to our understanding of the control of mammalian glucose homoeostasis.

The work from our laboratory described in this manuscript was supported by a Research and Development Award from the American Diabetes Association, and by a grant from the American Heart Association, Texas Affiliate (to C.B.N.).


Received 27 April 1990.

The lactating mammary gland of the rat and the starved-refed transition: a model system for the study of the temporal regulation of substrate utilization

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Abbreviation used: NEFA, non-esterified fatty acids.

Vol. 18