Glucokinase and glucose homeostasis: proven concepts and new ideas

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Abstract
The enzyme GK (glucokinase), which phosphorylates glucose to form glucose 6-phosphate, serves as the glucose sensor of insulin-producing β-cells. GK has thermodynamic, kinetic, regulatory and molecular genetic characteristics that are ideal for its glucose sensor function and allow it to control glycolytic flux of the β-cells as indicated by control-, elasticity- and response-coefficients close to or larger than 1.0. GK operates in tandem with the K+ and Ca2+ channels of the β-cell membrane, resulting in a threshold for glucose-stimulated insulin release of approx. 5 mM, which is the set point of glucose homeostasis for most laboratory animals and humans. Point mutations of GK cause ‘glucokinase disease’ in humans, which includes hypo- and hyper-glycaemia syndromes resulting from activating or inactivating mutations respectively. GK is allosterically activated by pharmacological agents (called GK activators), which lower blood glucose in normal animals and animal models of T2DM. On the basis of crystallographic studies that identified a ligand-free ‘super-open’ and a liganded closed structure of GK (Grimsby, Sarabu, Corbett and others (2003) Science 301, 370–373; Kamata, Mitsuya, Nishimura, Eiki and Nagata (2004) Structure 12, 429–438), on thermostability studies using glucose or mannoheptulose as ligands and studies showing that mannoheptulose alone or combined with GK activators induces expression of GK in pancreatic islets and partially preserves insulin secretory competency, a new hypothesis was developed that GK may function as a metabolic switch per se without involvement of enhanced glucose metabolism. Current research has the goal to find molecular targets of this putative ‘GK-switch’. The case of GK research illustrates how basic science may culminate in therapeutic advances of human medicine.

Determinants of the glucose homeostatic set point
Glucose homeostasis in man and many animals is maintained by feedback designed to keep the blood glucose (G) close to a set point characteristic for each species. This set point is close to 5 mM. Critical elements of the loop include: (1) G-sensitive insulin and glucagon-producing β- and α-cells, respectively; (2) the hormones insulin and glucagon; (3) hormone-sensitive tissues that consume, store or produce glucose, i.e. liver, skeletal and heart muscle and adipose tissue; and (4) the regulated parameter G. The G set point is determined by the G thresholds for stimulating insulin or glucagon secretion. When G exceeds 5 mM insulin secretion increases above basal and when glucose falls below this level glucagon release is augmented above basal. Note that stimulation of glucagon release by lowering glucose is actually due to reducing the glucose inhibition of secretion stimulated by other agonists (e.g. amino acids or adrenaline). Importantly, the G set point is close to the cross-over point of the glucose dependency curves for hormone secretion rates of these two opposing endocrine cell types.

Glucokinase as β-cell glucose sensor and the role of metabolic coupling factors
The G phosphorylating enzyme glucokinase (GK) serves as β-cell glucose sensor [1,2] and is the main topic of this paper. (Note that the mechanism of G sensing by the α-cells is not understood!). GK, also called hexokinase IV or D, converts glucose to glucose-6-phosphate with MgATP2− as the cofactor. The thermodynamic, kinetic and molecular genetic characteristics of GK are ideally suited for its G sensor function: the reaction is virtually irreversible; the glucose S0.5 is about 7.5 mM at pH 7.4 and the enzyme is physiologically saturated with MgATP2− (Km about 0.4 mM); there is no direct feedback inhibition by any of the reaction products; GK shows cooperative kinetics with glucose (the Hill coefficient is about 1.7 and the inflection point about 4.0 mM G); GK has an allosteric activator site as shown by the activating effect of at least 10-point mutation of different amino acids located in an area of the enzyme that is situated about 20 Å away from the substrate binding site and also serves as the contact site of a new class of blood G lowering drugs called GK activators (GKAs) [3–7]; GK is inhibited by GK regulatory protein (GKRP), a process competitive with G and enhanced by fructose-6-phosphate but counteracted by fructose-1-phosphate [8]; GK controls glycolytic flux and G oxidation of the β-cells because of GK’s high control strength for these pathways (the control coefficient is close to 1.0).

Key words: drug discovery, genetics, glucose, glucokinase, glucose homeostasis, therapeutics.

Abbreviations used: G, glucose; GK, glucokinase; GKA, GK activators; GKRP, GK regulatory protein; GK-AI, GK activity index; GSIR, glucose-stimulated insulin release; MM, mannoheptulose; MODY-2, diabetes mellitus in young people; PNDM, permanent neonatal diabetes mellitus; PHHI, persistent hyperinsulinaemic hypoglycaemia in infancy.

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Table 1 | GK induction in cultured rat islets by G and MH, alone and combined

Isolated rat islets were cultured for 3–5 days in RPMI containing additional leucine (7 mM), glutamine (7 mM) and 10% G-free fetal calf serum. G and MH were added as indicated. The n = 3 for every condition and 700–1000 islets were used for each experiment. Means ± S.E.M. are shown. The relatively high variation of media insulin is explained by the fact that islet counts per dish during the induction phase of the experiment differed considerably between experiments.

<table>
<thead>
<tr>
<th>Additions (mM)</th>
<th>GK (pmol/µg protein/h)</th>
<th>G 50.5 (mM)</th>
<th>nH</th>
<th>Islet protein (µg/islet)</th>
<th>Media insulin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>63 ± 15</td>
<td>7.38 ± 0.66</td>
<td>1.75 ± 0.14</td>
<td>0.31 ± 0.10</td>
<td>239 ± 120</td>
</tr>
<tr>
<td>G 10</td>
<td>226 ± 12</td>
<td>6.33 ± 0.57</td>
<td>1.74 ± 0.07</td>
<td>0.40 ± 0.07</td>
<td>3395 ± 523</td>
</tr>
<tr>
<td>MH 7</td>
<td>118 ± 13</td>
<td>8.12 ± 0.38</td>
<td>1.60 ± 0.02</td>
<td>0.33 ± 0.03</td>
<td>602 ± 113</td>
</tr>
<tr>
<td>G 10 + MH 7</td>
<td>443 ± 99</td>
<td>5.71 ± 0.63</td>
<td>1.80 ± 0.18</td>
<td>0.33 ± 0.03</td>
<td>1127 ± 584</td>
</tr>
</tbody>
</table>

Induction of β-cell GK by G and mannheptulose (G and MH alone or combined) and first attempts to outline a new ‘GK switch’ hypothesis

GK expression is regulated differentially in a tissue-specific manner, by G in pancreatic β-cells and by insulin in hepatocytes [11–14]. This biochemical characteristic of the system is explained by the presence of two distinct tissue-specific promoters, one upstream operating in the β-cells and some other glucose responsive cells (e.g. certain neurons) and the other downstream controlling hepatic GK expression [15–18]. The physiological significance of this molecular design feature is far reaching. We have reinvestigated this problem and have focused on G induction of pancreatic islet GK using a new spectrometric activity assay that eliminates interferences by low K m hexokinases and by N-acetylglucosamine kinase that have handicapped previous studies. Using isolated rat islets cultured for 3–5 days and G-free foetal calf serum we established that the enzyme is constitutively expressed at a basal rate of 63 pmol/microgram protein/h (Table 1). G concentration dependency studies showed that GK is increased maximally about 6-fold over basal. The G dependency curve for induction is sigmoidal, has an EC50 for G of 8.8 mM and an apparent Hill coefficient of 2.9, not significantly different from the kinetic constants of the enzyme itself (Figure 1). The findings could be explained in many different ways: GK mRNA and/or protein biosynthesis might be increased as a result of G stimulated metabolism, GK induction could be indirect and caused by stabilization of the enzyme or it could be caused by a combination of these and other factors and processes. To distinguish between such possibilities we used MH, a non-metabolized competitive inhibitor of GK as a potential inducer, alone or combined with G (Table 1). MH is indeed able to induce GK (about 2-fold at 7.0 mM). Since MH binding to GK is facilitated by G [19,20] we also tested the effect on GK induction of combined exposure to G and MH. If metabolism and/or insulin played an important role in this process then MH should block GK induction by G. If, however, MH were to stabilize the enzyme and if this effect were critical...
Rat islets were cultured for 3–5 days at different G levels and GK activity was then measured. There were at least three experiments for each data point and the means are given. (A) shows the observed islet GK activities (solid circles) and also the predicted values (solid triangles) computed by using the known kinetic constants of GK and the actual islet V_max corrected for basal activity. The two curves are virtually indistinguishable. (B) shows the observed and the expected relative effects of G on GK induction using a similar approach as in (A). Both curves were fitted to the Hill equation. The n_H for the observed curve (solid circles) was 2.92 ± 0.86, not different from that of the expected curve (1.73, solid triangles). The G_{S_0.5} values were also close (8.8 versus 7.7 mM).

MH might actually enhance G induction of GK. And that is what we found. The data suggest that substrate or inhibitor binding to the active site is more important for GK induction than metabolism or insulin release. Note that insulin or GKA was unable to induce GK in the absence of G. The GK induction was paralleled by well-preserved glucose responsiveness of β-cells when assessed in a perifusion experiment following the induction phase. Here again pre-treatment with insulin or GKAs alone was without effect. Thermostability experiments with recombinant GK provided additional support for a critical role of substrate stabilization in GK induction. MH alone protected GK against heat inactivation although less effective than G alone. GKAs alone were not able to protect the enzyme in this test system. If MH interacts only with GK and not with other cellular constituents it follows that GK per se and not G metabolism or insulin released from β-cells, acting as an autocrine stimulus, functions as signal to maintain, in part at least, the conditions required for GSIR (e.g. sufficient insulin stores or priming of insulin granules for release). These results and the demonstration of two distinct GK structures (the wide-open and the closed forms) [5–7] complementing the kinetic models of GK catalysis [10] inspired us to propose a new hypothesis that GK may function as a direct molecular switch independent of metabolism, i.e. the ‘GK switch’ (Figure 2). Recent reports that GK may interact with and neutralize the proapoptotic protein BAD leading to activation of the antiapoptotic BCL-2 [21] and that an inactive HK mutant may serve as a growth switch in plants [22] required comparable molecular explanations. The G stimulated translocation from the hepatic nucleus to the cytosol, also activated by G, MH and 5-thio-D-glucose (another GK inhibitor) [20], can be conceptualized as another example of such a switch. Targets of the hypothetical ‘GK switch’ of islet cells and hepatocytes are unknown. The implications of such a switch could be very significant.

‘Glucokinase disease’, a grand experiment of nature that proves the GK glucose sensor hypothesis

The tenets of the GK glucose sensor concept received their proof from the biochemical genetic analysis of a complex of genetic syndromes described as ‘glucokinase disease’ [1,2,9]. About 200 mutations of the GK gene have been found to be linked to autosomal dominant hypo- and hyperglycaemia in humans. Inactivating mutations in one or both alleles are associated with a mild form of diabetes mellitus in young people (MODY-2) or severe permanent neonatal diabetes mellitus (PNDM), respectively. Activating mutations cause persistent hyperinsulinaemic hypoglycaemia in infancy (PHHI) and only heterozygous cases are known to date. Close to 40 point mutations have been analysed in great detail using recombinant glutathione S-transferase (GST)-GK [23]. The kinetic constants and other characteristics of wild-type and mutant enzymes were used to predict the β-cell threshold for GSIR by mathematical modelling, based on several assumptions. The 5 mM threshold for GSIR is reached when β-cell glycolysis is 25–30% of its maximal rate as determined by the kinetic constants of normal GK. In cases of inactivating mutations much higher G levels are needed to achieve equivalent glycolytic rates and, conversely, much lower glucose levels are sufficient in cases with an activating mutation. The β-cell GK content is determined by the ambient G concentration and G binding stabilizes the enzyme decreasing its turnover.
Figure 2 | Stabilization of the closed form of GK by G or MH as basis of a ‘GK switch’

The diagram was designed on the basis of the mnemonic/slow transition models of GK kinetics and the open and closed crystal structures of GK in the absence and presence of ligands (G and GKA combined), respectively. GKA facilitate binding of G and MH. It is postulated that the closed conformations b, c and d are stable (switched on) and serve as messengers and that the open form a is unstable and lacks messenger function (switched off).

and thus raising its cellular level (see the section on ‘GK Switch’ hypothesis). This could occur without increased metabolism and stimulation of insulin release or enhancement of de novo synthesis of GK. The threshold of GSIR is a function of the relative GK activity index (GK-AI), a measure of the enzyme’s catalytic capacity: GK-AI = (k\textsubscript{cat}/S\textsubscript{0.5}) (2.5/2.5 + ATP K\textsubscript{m}). With two normal alleles the threshold is by definition 5 mM and the relative GK-AI is 1.0. When the index falls the threshold for GSIR and the basal blood sugar rise concomitantly but when the index rises the threshold for GSIR and basal blood sugar fall predictably. It is clear that adaptation has a profound impact on the outcome of the modelling. If it were lacking the predicted threshold for GSIR in classical GK-linked MODY with severe GK defects indicated by a GK-AI of less than 0.2 would be much higher than observed. The impact of adaptation is less pronounced in milder cases of MODY-2 and in PHHI. Five natural activating GK mutations have been found in patients with PHHI (T65I, W99R, Y214C, V455M and A456V). Their blood sugars range from 1.5 to 3.5 mM. As noted before [3,5–7], these activating mutations are all clustered in a circumscribed area about 20 Å away from the substrate binding site. This site represents a hitherto unknown allosteric activator site that may be responsive to an endogenous activator still to be discovered. There are a number of GK mutations in linkage to hyperglycaemia with no significant kinetic defect of the enzyme (A53S, G72R, H137R, G264S, R275C, V367M and K414E) or showing paradoxical activation (V62M) [23]. Some are instability mutants but for others an explanation for the hyperglycaemia is still lacking. These puzzling mutations and the close to 100 missense mutations still to be analysed biochemically are a rich treasure chest for new discoveries about GK and its role in G homeostasis.

**GKAs as potential antidiabetic drugs**

The basic and biochemical genetic studies had identified GK as a drug target. Beginning in the early 1990s a search was undertaken using a screening system with recombinant GK inhibited by GKRP in the presence of fructose 6-phosphate. The search was for a mimetic of fructose 1-phosphate to remove GKRP inhibition of GK, a typical strategy in drug searches. These efforts resulted in the surprising discovery of direct, non-essential activators of the enzyme involving an allosteric activator site [5–7,24,25]. Drug contact points have been identified by crystallography: V62, R63, M210, I211, Y214, Y215, M235, V452, V455. Three of the natural activating mutants (V62M, Y214C and V455M) are GKA contact sites and the other two (T65I and W99R) are located within or close to the activator site. GKAs increase the k\textsubscript{cat} slightly and lower the glucose S\textsubscript{0.5} markedly.
They may lower the Hill coefficient at near maximal concentrations but have no effect on the ATP $K_m$ when G is saturating. Since the first disclosure of GKAs in 2001 [26,27] three additional, chemically different GKAs have been reported [7,24]. The GKAs have profound effects on $\beta$-cell and hepatocyte function and lower blood sugar in normal and diabetic animals. The threshold for GSIR is lowered predictably, G-dependent respiration of islets is stimulated and hepatic glucose production is curbed. The therapeutic potential of these compounds as antidiabetic drugs is obvious. One danger lies in hypo-glycaemia. There is however little to fear as long as low dosages are used because GK activation would be moderate and the Hill coefficient would not be affected preserving the in-built anti-hypoglycaemic safety of the GK glucose sensor.

**Perspective**

It is 40 years since GK was discovered in liver [28–30] and 36 years since it was demonstrated in pancreatic islets [31]. During these four decades we have witnessed a stunning evolution of this molecule to be finally understood as a critical player in glucose homeostasis. Important milestones in this ongoing appreciation of GK’s role were the formulation of the GK glucose sensor concept [31,32], the discovery of the crystal structure of GK [5–7], the discovery of an allosteric activator site of GK [3,4,9] serving as the target of a new class of drugs (GKAs) that stimulate the enzyme [5–7,24] and the biochemical genetic characterization of ‘glucokinase disease’ [1,2,9,23]. To further advance the field we offer here a new working hypothesis that the enzyme may function as a direct metabolic messenger in pancreatic islet cells and hepatocytes (as ‘GK switch’). The stage is set for future intensive and rewarding research on the biochemistry and structure of the GK glucose sensor, on the genetics of GK disease and on the therapeutic efficacy and safety of GKAs for diabetic subjects.

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**References**


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