For the ZDF rat, “Breaking Up Is Hard To Do”: dissociation of the GK:GKRP complex

Alex J. Lange
Department of Biochemistry, Molecular Biology, Biophysics, University of Minnesota Medical School, Minneapolis, Minnesota

GLUCOKINASE (GK) is the enzyme that converts glucose, a major fuel of the body, into glucose-6-phosphate (G-6-P) in liver, pancreatic beta cells, and some specialized neurons, as well as enteroendocrine and pituitary cells (12). G-6-P is the gateway to the major pathways of glucose utilization: glycolysis, the oxidation of glucose, the pentose phosphate pathway, and glycogenesis. GK performs different functions in the tissues where it is expressed. In liver, in the postprandial state when insulin is elevated, GK increases glucose utilization as part of decreasing net hepatic glucose output (HGO) to prevent the liver from contributing to hyperglycemia. In pancreatic beta cells, GK is thought to act as a glucose sensor leading to the release of insulin in the well-established cascade of events, glucose uptake via the transporter GLUT2, phosphorylation by GK, increased the ATP-to-ADP ratio, closing of K+-gated channels, membrane depolarization, and Ca2+ influx leading to secretion of insulin (13). Recently, GK has been shown to play a role in glucose sensing in the ventral medial hypothalamus, which is involved in regulation of the glucose homeostasis. Experimentally decreasing (siRNA) or increasing (activator) GK had the expected effects on Ca2+ oscillations in glucose-excitatory and glucose-inhibitory neurons (10).

Classically, the control of glycolysis was thought to be at the phosphofructokinase-1 (PFK1) reaction, which was considered the rate-limiting step in the pathway, by crossover experiments (5). The major role that GK plays in the control of pathway flux was elucidated by metabolic control analysis (8), where GK was shown to have a much higher flux control coefficient than PFK1 on the process of glycolysis in liver. This analysis established the shared roles of enzymes in a given pathway and revealed that GK contributed a lion’s share to the control of glycolysis and glycogenesis (7). This is still a simplification, although it is quantitative. The physiologic key is that GK is a high \( K_m \) (actually, the half-saturation constant \( S_0.5 \)) hexokinase, equal to the normal blood glucose concentration (5–8 mM), and therefore can respond to changes in blood glucose in the physiological range. GK, although monomeric, displays sigmoidal kinetics, with the inflection point at normal physiological concentrations of glucose. In an elegant structural study, Kamata, et al., (9) show that GK exists in three conformational states and has two catalytic cycles. Both the closed and open forms are active and the super-open form is inactive. GK enters into a fast (open-to-closed) or slow (super-open to open-to-closed) catalytic cycle depending on the concentration of glucose; at high glucose the enzyme goes into the fast cycle and at low glucose into the slow cycle, explaining the low affinity and sigmoidal saturation curve of the enzyme.

GK has major roles in pancreatic beta cells and liver in the control of blood glucose homeostasis. Mutations in GK lead to maturity-onset diabetes in the young (type 2 diabetes; T2D). Over 100 mutations that compromise some aspect of GK have been characterized (12). Several mutations have also been identified that lead to higher-than-normal GK activity, which leads to hypoglycemia (12). For these reasons, GK is a strong target for the pharmacological treatment of T2D. Small molecular weight GK activators (GKAs) that bind to GK and activate it are currently being developed as antidiabetic drugs (2, 4, 6). These GKAs, some currently in clinical trials, have great potential to reduce hyperglycemia in diabetic patients.

In the liver, and most likely in other tissues as well, regulation of GK activity goes beyond its kinetic properties. In the liver, GK regulatory protein (GKRP) sequesters GK in the nucleus in an inactive conformation. In response to high glucose, GK dissociates from GKRP and translocates to the cytoplasm where it is active. Low concentrations of sorbitol or fructose, as precursors of fructose-1-phosphate, also dissociate GK from GKRP, leading to translocation out of the nucleus and activation (1, 17).

Levels of GK in the nucleus are never depleted, regardless of metabolic state, providing food for thought that GK may have other noncatalytic functions in the nucleus. A proteomic approach to analyzing protein complexes has revealed that GK is also present in a mitochondrial complex containing BAD, a proapoptotic Bcl-2 family member that induces apoptosis by inhibiting the antiapoptotic molecule Bcl-XL. Danial et al (3) suggest that mitochondrial-bound GK performs a key role in apoptosis in response to various factors, such as extrinsic stimuli and metabolic states. Additionally, there are other binding partners that effect the compartmentation and activity of GK. For example, GK forms a complex with the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, where complexed GK activity is increased and GK may be sequestered in the cytosol (11, 14).

It has been established that glucose-induced suppression of net HGO is associated with increased glucose phosphorylation and active GK, and that the impaired suppression of net hepatic glucose production and the defective hepatic glucose uptake in response to increased plasma glucose seen in T2D is due to the failure of increased plasma glucose to enhance flux through GK.

In this issue of the American Journal of Physiology—Regulatory, Integrative and Comparative Physiology, Shin, et al. (16) report studies on GK in the Zucker diabetic fatty (ZDF) rat, a model that presents strong parallels to humans with T2D. The authors examine the role of hepatic GK on a physiological whole body level. By using clamping and tracer analysis they
were able to quantitate glucose turnover, total HGO, hepatic glucose cycling, and incorporation into glycogen and thereby get a comprehensive view of liver carbohydrate metabolism. In this model, they observed that glucose utilization is not increased when blood glucose is increased as it is in normal-weight rats. They demonstrated that this is due to a defect in the glucose-dependent translocation of GK to the cytosol, leaving it sequestered in the nucleus in an inactive state. Therefore, GK is incapable of increasing glucose phosphorylation, and there is no decrease in net HGO. They pinpoint the inability of GK to translocate in response to glucose to the inability of glucose to dissociate GK from the inhibitory grip of GKRP in the nucleus. This is based on the rescue of GK translocation by the addition of sorbitol at low concentration, which dissociates GK from GKRP. Sorbitol restores glucose phosphorylation and decreases net HGO, rescuing the normal hepatic response to glucose. Clearly, these are two distinct mechanisms bringing about the dissociation of GK from GKRP. How they are related and what differentiate them are questions currently under investigation.

Several implications can be drawn from the authors’ observations. First, these observations demonstrate the importance of liver GK in the maintenance of normal glucose homeostasis. However, because of clamping and tracer methodologies, liver metabolism is experimentally more accessible, and we may not fully appreciate the role of GK in the control of whole body glucose metabolism as guided from glucose-sensitive neurons in the hypothalamic regions of the brain. Second, since this defect was discovered in an early stage of diabetes, it could be a cause of the progression to diabetes seen in the adult ZDF rat. We must remember that the etiology of T2D is complex and likely involves multiple defects that conspire to generate the phenotype. Third, the ZDF rat model may be limited as far as parallels with T2D in humans is concerned. One wonders whether the same dissociation defect is seen in humans with T2D and, furthermore, how this step is affected by GKAs that are in the pharmaceutical pipeline. Will the GKAs be able to overcome the defect?

Clearly, basic research on the regulation of GK remains an important area of study. It is important to understand the subtleties that come to bear on the efficacy of antidiabetes therapies utilizing GK as a target.

NOTE ADDED IN PROOF

While this editorial focus was in review, an in-depth review of GK regulation in liver and pancreatic beta cells was published (Baltruch S, Tiedge M. Glucokinase regulatory network in pancreatic beta-cells and liver. Diabetes 55, Suppl 2: S55–S65, 2006).

REFERENCES