Glucokinase, Glucose Homeostasis, and Diabetes Mellitus

Franz M. Matschinsky, MD

Address
University of Pennsylvania Medical School, Department of Biochemistry and Biophysics, 501 Stemmler Hall, 36th & Hamilton Walk, Philadelphia, PA 19104, USA.
E-mail: matsch@mail.med.upenn.edu

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The enzyme glucokinase (GK) regulates the rate of glucose metabolism in many tissues, including liver, the pancreatic β cells, certain neurons, enteroendocrine cells, and the pituitary, serving as a glucose sensor in many of these. Thus, GK plays a critical role in glucose homeostasis. Spontaneous mutants of GK in humans result in autosomal-dominant hypo- and hyperglycemia syndromes described as "GK disease." GK activator drugs have been discovered that lower blood glucose in normal and diabetic animals and promise to be useful in the treatment of type 2 diabetes mellitus. There is no question that the GK molecule and related issues will continue to be a fruitful topic for future research.

Introduction

This article deals with a highly complex system in seven paragraphs: 1) catalytic function and structure of the glucokinase (GK) molecule; 2) tissue distribution and expression control of GK; 3) distinct, tissue-specific functional roles of GK; 4) "GK disease"; 5) system analysis of the GK network; 6) "glucokinase activators" (GKAs) as promising antidiabetic drugs; and 7) conclusions.

Catalytic Function and Structure of GK

Glucokinase or hexokinase IV is one of four glucose-phosphorylating isoenzymes in mammalian tissues, which has very distinct structural and catalytic characteristics [1]. A word about nomenclature is needed: GK is not specific for D-glucose (it also phosphorylates mannose, fructose, and glucosamine) and is, thus, strictly and biochemically speaking not a "GK," but glucose is its exclusive physiologic substrate and calling the enzyme GK is biologically defendable and perhaps also preferable because of tradition. In order to appreciate GK and its role in glucose homeostasis, it is mandatory to know its kinetic and structural characteristics (Table 1).

Glucokinase has a turnover number of 60 to 70/sec and prefers the α anomer of glucose as substrate. The apparent $S_{0.5}$ for anomerized D-glucose is approximately 7.5 mM. The K_m (Michaelis constant) for the cosubstrate $MgATP^{2-}$ is approximately 0.4 mM. The reaction shows cooperativity with glucose, as indicated by a Hill coefficient of approximately 1.70. The cooperativity index of GK, Ra ($81^{1/h}$), is approximately 12, indicating that the glucose concentration needs to be increased 12-fold (instead of 81-fold for a hyperbolic enzyme) to increase the enzyme saturation or velocity from 10% to 90% [2]. The inflection point is the glucose concentration at which the enzyme is most sensitive to alterations of the glucose levels and is approximately 3.5 mM for GK. GK is not inhibited by its product glucose-6-phospate, nor is it modified by any other intermediate or cofactor of glucose metabolism in distinction to the other three hexokinases. GK is inhibited by GK regulatory protein, a 68-kD molecule, found primarily in the nucleus of hepatocytes [3]. This inhibition is competitive with glucose, and is enhanced by fructose-6-phosphate but reduced by fructose-1-phospate. GK is also inhibited by long-chain acyl-coenzyme A at micromolar concentrations.

It needs to be appreciated that GKs from different species differ kinetically (*eg,* the glucose $S_{0.5}$ ranges from 1.5 mM [in the Chilean frog] to 12.0 mM [in the Gilt Head Sea Bream]) [4]. However, the Hill coefficients are remarkably constant, ranging from 1.4 to 1.7. Furthermore, the enzyme is absent in certain vertebrate species: bats, cats, chinchillas, horses, alpacas, cattle, sheep, and goats [4]. The enzyme is a monomer of approximately 50 kD. The crystal structure of GK has been elucidated both in the liganded and in the free forms, which have very marked structural differences [5••,6,7••]. In the presence of α-D-glucose and a GKA drug, the enzyme exists in a closed form, whereas without ligands present an open form is seen. This detailed structural information has been of tremendous help in the explanation of the unique kinetic characteristics of GK and of the effects that disease-causing missense mutations have on structure and function of the enzyme. For example, the presence of two forms has been used to explain the cooperative kinetics of this monomeric enzyme [7••]. Enzyme kineticists had previously proposed the "slow transition" and "mnemonic" models to help understand cooperativity with glucose [8]. These two models had already envisioned that the enzyme does exist in two kinetically distinct, interconvertible forms and that

Table 1. Characteristics of human glucokinase*

Molecular size	50 kD, monomer
k_{cat}	60–70 sec^{-1}
Glucose $S_{0.5}$	~ 7.5 mM
ATP K_m	~ 0.4 mM
Hill coefficient (n_H)	~ 1.7
Inflection point	~ 3.5
Ra ($81^{1/h}$)	12
Inhibitors	GKRP and acyl-coenzyme A
Activators	GKA drugs and putative endogenous activators
Cellular localizations	Cytosol, nucleus, secretory granules, mitochondria
Tissue distributions	Pancreatic α and β cells, hepatocytes, enteroendocrine cells, hypothalamic neurons, pituitary cells

*Note that all kinetic constants were obtained at pH 7.4 and that the glucose $S_{0.5}$ rises as the pH falls.
ATP—adenosine triphosphate; GKA—glucokinase activator; GKRP—glucokinase regulatory protein; k_{cat}—turnover number; K_m—Michaelis constant.

the rate of the catalytic cycles increases as a function of glucose such that it becomes much faster than the transitions between the two forms, in effect changing the equilibrium between the two forms and resulting in sigmoidicity.

The new structural information and the classical ideas about GK kinetics are highly complementary and provide plausible explanations for the cooperative kinetics of the enzyme and also explain other kinetic features (*eg*, the nature of allosteric activation). There is some evidence that GK may interact with other cellular constituents. Examples are the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase [9], and the proapoptotic factor BAD [10]. The biological significance of such interactions remains to be proven.

Tissue Distribution and Expression Control of GK

Glucokinase is found in several tissues of vertebrates, perhaps most intensively studied in the rat [11]: hepatocytes, α and β cells of the pancreatic islets, enteroendocrine cells, hypothalamic neurons, and pituitary cells. The evidence for this wide distribution stems from actual enzyme activity measurements as convincing indication for a functional role (in hepatocytes, pancreatic islet cells, basal hypothalamus, and pituitary gland) from demonstration of GK mRNA by Northern blotting or by reverse transcriptase-polymerase-chain reaction, and from immunochemical data using histochemistry or Western blotting of extracts (for the rest of the sites).

The cellular concentrations of GK vary significantly, perhaps by as much as tenfold, with highest levels in liver of fed animals and much lower levels in other cells. Considering the sizes of the cell populations of these tissues and differences in cellular concentrations of the enzyme, the actual total amounts of GK in individual tissues have a tremendous range: the entire human pancreatic islet organ of approximately 1 g contains approximately 5 to 10 μg of GK, whereas the human liver has 50 to 100 mg of GK. The capacities for glucose phosphorylation of individual organs may thus be estimated. The human liver has the capacity to phosphorylate approximately 1 g of glucose per minute at 15 mM of glucose in portal blood. These facts by themselves may suggest that a regulatory GK system or network exists and, as discussed later, there is much evidence in support of such a concept. It is pertinent to point out in the present context that GK has highly specific intracellular distribution patterns differing from cell type to cell type [12,13]. This view is a considerable advance from an earlier simplistic concept that GK is primarily a soluble cytosolic protein. It has been reported that GK is bound to mitochondria or granules of the β cell and that hepatic GK shuttles back and forth from the nucleus to the cytosol, depending on the nutritional state of the animal [14–16]. These observations imply that there are distinct binding sites on the enzyme that allow such compartmentation to occur.

The GK expression control varies greatly from tissue to tissue, as originally discovered by comparing GK enzyme activities of liver and pancreatic islets in hyperinsulinemic and hypoglycemic insulinoma-bearing rats and also at different intervals after removal of the tumor [17]. Hepatic GK was greatly increased and islet GK was drastically reduced when insulin was very high and glucose very low, whereas the opposite was true when insulin was low and glucose high. These data suggested organ-specific differential expression control of GK. The cloning of a single GK gene and the discovery of two distinct promoters in this gene (an upstream neuroendocrine and a downstream hepatic promoter) have since substantiated this idea [18,19].

It should be noted that the intron and exon structures of the GK genes of several species are known and that differential, tissue-specific splicing is reasonably well understood. However, some confusion did originally arise from investigations of mRNA processing in pituitary cells, which resulted in the conclusion that the pituitary lacks enzymatically active GK [20,21]. Recent measurements with a specific and sensitive spectrometric NAD-coupled assay clearly demonstrated enzymatic GK activity in rat pituitary glands [22]. This finding raises many biologically significant questions and suggests a new model system for studying GK's role in endocrine cells other than the β cell.

Controversy still exists about the nature of GK expression control in pancreatic β cells. The classical view holds that the neuroendocrine promoter is constitutively active in these cells and that regulation is primarily post-transcriptional or even post-translational (including effects of insulin, cyclic AMP, biotin, retinoic acid, or substrate stabilization of the protein), whereas the hepatic promoter

is primarily insulin driven and regulation is almost entirely transcriptional [23–26]. A modern challenge to this view states that insulin, released by glucose stimulation of the β cell, serves as a paracrine, transcriptional regulator of GK expression in β cells analogous to the insulin-dependent mechanism in the liver [27]. It remains to be seen where the truth lies. Whatever the mechanism, glucose induction of GK in cultured islets shows a sigmoidal concentration dependency and may result in a six- to sevenfold change of enzyme levels, from a constitutive basal level of approximately 65 pmol/μg of protein per hour [28•]. The process of GK induction in liver is best illustrated using a fasting and refeeding paradigm in intact animals, demonstrating that mRNA and enzyme activity change in parallel with the fluctuations of the plasma insulin [29]. Using the same experimental conditions of fasting and refeeding, little if any change of islet GK activity or mRNA was observed, supporting the classical view of differential expression control in these two organs [29].

Distinct, Organ-specific Functional Roles of GK

It is now widely accepted that GK serves as a glucose sensor of the pancreatic β cell [30–32], and there is some evidence that the enzyme may play a similar role in hypothalamic neurons [33]. GK determines the rate of β-cell glucose metabolism and, thus, the rate by which metabolic coupling factors that connect intermediary metabolism with insulin release are generated. Note that other metabolic steps (*ie,* glucose transport, P-fructokinase, fructose-2,6-bisphosphate aldolase, pyruvate kinase, and so forth) are present far in excess and have a much lower control strength for metabolic flux than GK (*ie,* < 0.1 compared with > 0.8). The most important coupling factors are ATP^{4-} and $MgADP^{-}$ because the former inhibits and the latter activates K^{-} channels of the β-cell membrane, leading to glucose-dependent, graded depolarization of the cell membrane, the event that triggers the opening of voltage-dependent, threshold-operated Ca^{2+} channels. Note that it is primarily the drop of $MgADP^{-}$ resulting from glucose oxidation that inhibits K^{+} conductivity and depolarizes the cell. Ca^{++} enters the cell and causes insulin release by exocytosis, a process augmented by $MgATP^{2-}$, cyclic AMP, and activators of protein kinase C, all facilitated by increased glucose metabolism or elevated Ca^{++}.

It must be appreciated that GK, the adenine nucleotide-sensitive K^{+} channel, and the L-type Ca^{++} channel are operating in tandem and that each step is essential for coupling metabolism with secretion. Other nutrient stimuli of insulin release (*ie,* free fatty acids and amino acids) require glucose and GK to be effective. This is, incidentally, also true for the action of endocrine and neural transmitter secretagogues (*eg,* glucagon-like peptide-1, glucose-dependent insulinotropic peptide, or acetylcholine). Similar GK-based metabolic coupling may occur in hypothalamic neurons and pancreatic α cells. In GK-containing pituitary cells that are activated by hypothalamic-releasing hormones, one can imagine that altered glucose metabolism may change the efficacy of releasing hormones (*eg,* corticotropin-releasing hormone may be more or less effective in releasing adrenocorticotropic hormone when glucose is high).

The primary role of GK in the liver is fundamentally different from that in entero- and neuroendocrine cells. Liver GK functions as a high-capacity system (*ie,* approximately 1 g of glucose per minute may be removed from the blood stream sufficient to deal with a substantial postprandial glucose load). This is also the place to comment on the unique interplay between GK and the nuclear glucokinase regulatory protein (GKRP) in liver. GK, in part sequestered in the nuclear space by binding to GKRP, is released to the cytosol because the GK/GKRP complex is dissociated by glucose. Fructose, conversely, enhances glucose disposal by the liver because the GK/GKRP complex is disrupted by fructose-1-P, the product of the fructokinase reaction. The possibility needs to be considered that GK may also serve as a glucose sensor in hepatocytes themselves or in specialized fuel-sensing cells located in the hepatic vascular tree as part of the sentinel system, which informs the central nervous system about glucose availability in the splanchnic bed. This potentially important system remains to be fully characterized.

GK Disease

The prediction that GK may be a diabetes gene was confirmed by the finding of linkage between hyperglycemia and the GK locus in families with maturity-onset diabetes of the young (MODY) and by the discoveries of GK-linked permanent neonatal diabetes mellitus (PNDM-GK) or hyperinsulinemic hypoglycemia (HI-GK). Inactivating mutations of the enzyme cause MODY when only one allele is affected (MODY-2 or MODY-GK) and lead to PNDM when both alleles are affected, either by the same or different mutations. Activating mutations (so far discovered only in heterozygous cases) cause hyperinsulinemic hypoglycemia, which may be very severe and lead to seizures (HI-GK).

At least 200 GK mutations have been discovered to date [34]. All common forms of gene mutations have been identified in patients with GK disease (missense, nonsense, acceptor, and donor site mutations, as well as deletions and insertions have been observed). Of these, approximately 50 missense mutations were carefully analyzed using purified recombinant mutant GK–glutathione S-transferase (GST) fusion proteins, and the results have been sufficient to test a mathematical model of GK-based β-cell glucose sensing, which was found to predict fairly accurately the fasting blood glucose levels of carriers of such mutants [35••]. The mutants affect any or all basic

kinetic constants of the enzyme. In the case of inactivating mutations, the glucose $S_{0.5}$ may be increased, the k_{cat}, or turnover number, may be lowered, the ATP K_m may be increased, or a combination of all three defects may exist. The enzyme may also be thermally labile and that alone, if severe enough, can explain the diabetic phenotype. As the number of cases thus analyzed increased, mutants were found that did not show any obvious defect or showed paradoxic changes in a direction opposite to what was expected from the phenotype. Given convincing evidence that such mutants are disease causing (autosomal-dominant transmission in single or, if possible, several independent families with multiple generations affected), such mutants provide unique opportunities to discover new roles of GK not readily apparent from analyzing just the basic kinetic and biophysical characteristics. V62M, which was discovered in unrelated English and Italian families with MODY, is a case in point [36••]. V62M was found to be activated kinetically, and turned out to be moderately unstable, but exhibited profound defects of regulation because it was refractory to the action of GKRP and GKA drugs. The study led to the postulate that in the case of a GKA and GKRP refractory enzyme, even moderate instability may lead to enzyme deficiency because of limited protection by endogenous ligands. It also provided some support for the notion that an endogenous activator of GK may exist.

The elucidation of activating mutations that cause HI-GK has been particularly rewarding [37,38,39•]. First, it led to the recognition of a previously unknown allosteric activator site of GK. The enzyme is activated by the following spontaneous amino acid substitutions: V62M, T65I, W99R, M197I, Y214C, insertion of alanine at position 454, V455M, and A456V. Seven of these eight mutations are clustered in a well-defined region approximately 20 Angstrom distant from the substrate binding site. Only M197I is far remote from this activating region [40]. Expressed in terms of the relative activity index (the mutant activity index [$k_{cat}/S_{0.5}{}^{h} \times 2.5/2.5 + \text{ATP } K_m$] divided by the wild-type index), activations ranged from four times to forty times that of the wild type. However, a clear parallelism between the biochemical severity of the mutant and the hypoglycemic phenotype could not be established. It is possible that our analysis is still missing important aspects of GK biochemistry or that counter-regulation predominates in glucose homeostasis when the glucose falls below a critical level. Still, the substantial body of knowledge about GK disease has, in effect, proven the validity of the GK glucose sensor hypothesis.

System Analysis of the GK Network: Hierarchical Control or Balanced Network

The primary goal of studying the GK system in such great detail is to fully understand and perhaps therapeutically exploit the GK molecule's role in glucose homeostasis. Because there are at least a half dozen of GK-containing tissues to be considered, questions do arise whether these tissues function as a system or network, and if so what organization or order may exist in the system. Is there a top-down hierarchy with a master gland in charge, is regulation based on subtle balance between equal partners, or is there some other ordering principle at work? It seems to this author that the system is hierarchical and that the GK-containing pancreatic β-cell organ plays the predominant role because it determines the glucose set point of the body. The insulin-producing pancreatic β cell and the β-cell GK glucose sensor are thus central to glucose homeostasis according to this view. A small fraction of approximately one per 10,000 of the body's GK total complement of 50 to 100 mg has the critical power to control the system. This view is illustrated by the analysis of GK disease and is also supported by work with mouse models of tissue-specific alterations in GK gene expression. The pancreatic β-cell GK glucose sensor is the dominant determinant in the range of approximately 2.5 to 15 mM of glucose. Beyond these boundaries, other controls are more and more activated, particularly toward lower glucose levels involving counter-regulatory hormone and neurotransmitter release. In contrast, hyperglycemia elicits practically no counter-regulation. This hypothesis of a hierarchical organization of the GK system deserves to be tested further. For example, what would happen if the hypothalamic GK-containing neurons were to be selectively GK depleted or modified to overexpress the enzyme. Similar questions can be asked for other GK-containing tissues. An answer to this question has been obtained for the β cell and supports the view advanced in this assay. The recent demonstration of significant GK activity in the rat pituitary points to a unique opportunity to characterize another potentially important component of this system along the lines previously performed in great detail in β cells using transgenic mouse models.

GKAs as Promising Antidiabetic Drugs

In view of the preceding discussion, it should not come as a surprise that GK did emerge as an ideal candidate target for discovery programs in search of antidiabetic drugs. A team at Hoffmann La Roche, Basel, Switzerland, used GKRP-inhibited GST-GK fusion protein to screen for compounds that may activate the GK/GKRP complex by blocking the inhibitor [5••]. In the course of this work, direct rather than the expected indirect GKAs were discovered.

These early GKAs (derivatives of propionic amide) function like nonessential mixed activators that lower the glucose $S_{0.5}$ markedly and the Hill coefficient moderately, whereas the k_{cat} is increased by 1.5- to 2.0-fold. There is no significant effect on the ATP K_m when glucose is saturating. However, the ATP K_m is moderately increased when glucose is in the physiologic range. GKAs increased the protective action of glucose in GK thermolability experi-

ments but did not stabilize the enzyme in the absence of glucose. Reports about chemically different activators describe very similar actions [7••,41]. As expected from a drug that enhances glucose phosphorylation in the pancreatic β cells and in hepatocytes, it was found that GKAs enhance glucose metabolism and glucose-induced insulin release from isolated perifused pancreatic islets, and that they enhance hepatic glycogen synthesis and curb hepatic glucose production. The GKAs lower blood sugar in normal laboratory animals and also in a variety of animal models with type 2 diabetes mellitus (T2DM). GKAs prevent the development of hyperglycemia in diet-induced obesity. These characteristics suggest that GKAs could be of use in the treatment of T2DM in humans. Dual action in β cells and hepatocytes would make these compounds very attractive for drug treatment because both β cells and hepatocytes are defective in T2DM.

The discovery of GKAs intensified the attempts to crystallize GK, and these efforts were successful [5••,6,7••]. GK was crystallized independently by two groups both in the presence of α-D-glucose and activator and also in the absence of ligands. Both groups identified seven to nine contact sites of GKAs on GK: V62, R63, M210, I211, Y214, Y215, M235, V452, and V455. Many of these, or neighboring amino acids, had also been found to be mutated in GK that is activated and causes HI-GK. GKA binding sites and spontaneous activating mutants showed considerable overlap and delineated a previously unknown "allosteric" activator site of the enzyme. Therefore, it was of interest to study the effects of GKAs on mutants of GK, particularly on those that changed the contact sites of the drug. We found that many of the mutants were resistant to the drug, particularly those with amino acid changes at the contact of GKAs (*ie,* V62M, M210K, Y214C, V455M, and A456V). These studies with spontaneous activating mutants provided critical guidance to the pharmaceutical industry in their attempts to design optimal GKAs for treatment of diabetes.

In view of the wide variety of GK-containing cells, it will be of considerable importance to explore what effects, if any, GKAs have on the complex GK system. The GKAs were developed at a time when extrapancreatic and extrahepatic GK sites were practically unknown, perhaps fortunately, because that increased the prospect of developing a tissue-specific drug. The discovery of additional GK locales could present a problem because the danger of undesirable side effects of GKAs increases but may also be an advantage because concerted activation of the entire GK system may be beneficial in the diabetic condition.

Conclusions

In recent years, remarkable changes in emphasis have occurred in the studies and concepts concerning glucose homeostasis and the pathogenesis of T2DM, such that the historically predominant interest in major insulin target tissues of liver, muscle, and adipose tissue is now nearly matched by an equal interest in the endocrine pancreas, the entero- and neuroendocrine system, and the central nervous system and the autonomic nervous system as important players. This brief report reflects this broadening of the perspective in the field of GK research and it can be predicted that this trend will continue.

This situation presents unique challenges because the extrahepatic and extrapancreatic GK-containing cells are rare and often scattered throughout a particular tissue. Furthermore, the catalytic activities are probably on the low side, increasing the difficulty of biochemical and physiologic studies. However, many powerful biological tools are available and the newly discovered GKAs will greatly aid in this endeavor. The other hope is that the biochemical genetic research of GK disease will continue vigorously because this author believes that a meticulous analysis of spontaneous mutants in humans holds much promise for new discoveries about the biochemistry and biological role of the GK molecule. It can also be predicted that this work in human genetics will be greatly enhanced by the exploration of new mouse models of GK disease using N-ethylnitrosourea mutagenesis [42,43].

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