

Regulation of Pancreatic β -Cell Glucokinase

From Basics to Therapeutics

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Glucokinase (GK) serves as glucose sensor in pancreatic β -cells and in other glucose sensor cells in the body. Biochemical genetic studies have characterized many activating and inactivating GK mutants that have been discovered in patients with hyperinsulinemic hypoglycemia or diabetes, all inherited as autosomal dominant traits. Mathematical modeling of the kinetic data of recombinant human wild-type and mutant GK accurately predicts the effects of GK mutations on the threshold of glucose-stimulated insulin release and glucose homeostasis. Structure/function studies of the enzyme suggest the existence of a hitherto unknown allosteric activator site of the enzyme that has significant implications for the physiological chemistry of GK-containing cells, particularly the pancreatic β -cells. Glucose is the preeminent positive regulator of β -cell GK expression and involves molecular mechanisms that are still to be elucidated in detail, but seem to have a specific requirement for increased glucose metabolism. Pharmaceutical chemists, motivated by the clear tenets of the GK glucose-sensor paradigm, have searched for and have discovered a novel class of GK activator molecules. The therapeutic application of this basic discovery offers a new principle for drug therapy of diabetes. *Diabetes* 51 (Suppl. 3):S394–S404, 2002

The enzyme glucokinase (GK) serves as a glucose sensor in pancreatic β -cells and seems to play a similar role in selected endocrine enterocytes, hepatocytes, and specialized hypothalamic neurons (1). Collectively, these cells may be conceptualized as an integrated system of GK-containing cells. Further, it appears that glucose homeostasis requires the intactness of this complex GK-based signaling network (Fig. 1). It is therefore mandatory to learn as much as possible about the GK molecule itself and to understand how this enzyme

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BGPR, β -cell glucose phosphorylation rate; F1P, fructose-1-phosphate; F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; GK, glucokinase; GKB, GK content of the β -cell; GK-L, liver isoform of GK; GKRP, GK regulatory protein; GSIR, glucose-stimulated insulin release; PHHL-GK; GK-linked persistent hyperinsulinemic hypoglycemia; PNDM-GK, GK-linked permanent neonatal diabetes; MODY-2, GK-linked maturity-onset diabetes of the young; PL, placental lactogen.

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is regulated in the cells that comprise this signaling network. Important clues to this functional question are contained in the structure of the GK gene (2,3). It has two promoters, initially conceptualized as specific for pancreatic β -cells and hepatocytes, but used now to also explain differential expression in the broader system of diverse GK-containing cells. The current knowledge about kinetic features of GK (4,5), the interactions of this enzyme with its intracellular regulators (6), its subcellular distribution (7), and the functional consequences of numerous mutations that cause disease in humans (1,4) is substantial and greatly contributes to the firm scientific foundation of the GK glucose-sensor paradigm. Two isoforms of the enzyme, the liver and islet isoforms, have been identified as consistent with molecular genetic information (2,3). Significant information has been collected during the last 30 years in careful measurements of catalytic activities (8,9), mRNA levels (10,11), and protein contents (10–12) of GK and other phenomena related to expression regulation of the enzyme and has formed the basis of a viewpoint called here the “classic view of GK regulation” (13). According to this concept, insulin is the prime mover in GK induction in hepatocytes, and glucose is the critical factor for GK induction in pancreatic β -cells. (Note that the GK regulation in enteric and central nervous system GK-containing cells has not been carefully explored to date.) This view is challenged by more recent studies in which it is proposed that insulin drives GK induction in β -cells (14) as effectively as has been accepted to be true for hepatocytes (15). This evolving hypothesis is called the “modern view of GK regulation.”

The present study reiterates the accepted facts and functional significance of GK enzymology in light of “glucokinase disease” in humans and attempts to provide a perspective on the relative scientific merits of the older and newer concepts of GK regulation and how the problems might be resolved. The emphasis is on the regulation of GK in β -cells and not in hepatocytes. In more general terms, the study emphasizes the iterative process of scientific discovery in the dialectic chain of thesis, antithesis, and synthesis.

BASIC ENZYMOLOGY OF GLUCOKINASE AND GLUCOKINASE DISEASE

GK, one of the four members of the hexokinase family of enzymes (1,2,4,5), operates as a monomer and phosphorylates glucose on carbon 6 with MgATP as the second substrate to form glucose-6-phosphate (G6P). Glucose is its preferred substrate under physiological conditions, hence its widely accepted name glucokinase rather than

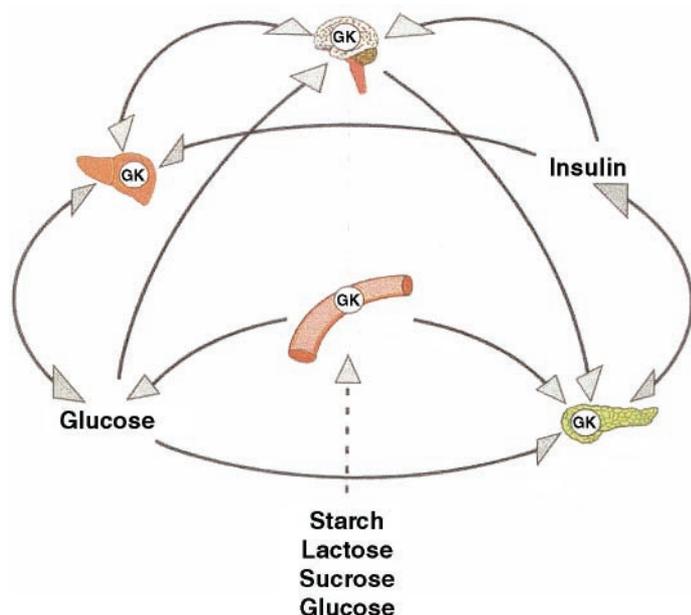


FIG. 1. GK-containing glucose sensor cells in tissues that are involved in glucose homeostasis. The glucose set point of 5 mmol/l for the system is determined by the glucose thresholds of the components, which are interconnected by glucose, hormones, and neurotransmitters. Depending on the nutritional situation, different individual components may assume the role of primary regulatory unit. Used with permission from Bell et al. (1).

hexokinase IV or D. There is little argument about the enzyme's basic kinetic constants and important other biochemical features: Its $S_{0.5}$ for glucose of about 8 mmol/l, its cooperativeness with the substrate glucose (nH ~ 1.7) extrapolating to an inflection point of ~ 4 mmol/l, and the lack of product inhibition by G6P enables the enzyme to govern the rate of glucose catabolism in GK-containing cells (1,4). These kinetic characteristics are also the basis of the enzyme's glucose sensor function in GK-containing cells, provided glucose transport is not rate limiting, a condition that seems to be fulfilled in the well-characterized glucose sensor cells and hepatocytes (1,4). In cells that contain GK regulatory protein (GKRP), the enzyme may be inhibited indirectly by fructose-6-phosphate (F6P), an effect that is competitive with glucose and reversed by fructose-1-phosphate (F1P) (6). This mechanism has been proven to operate in hepatocytes, but is of questionable significance in other GK-containing cells (16,17). The normal phenotype that was found in studies of GKRP knockout mice provides strong evidence for this interpretation. In vitro, GK is inhibited by palmityl-CoA and other long-chain acyl-CoA esters with a K_i of ~ 1 μ mol/l. It is doubtful that the effect is of physiological significance (4,5).

The strongest evidence for the GK glucose-sensor concept derives from the functional consequences of GK gene mutations in humans (1,4,18–24). To date, ~ 150 such mutations have been found (Fig. 2) that are manifest in at least three clearly distinguishable syndromes inherited in an autosomal dominant manner: 1) GK-linked persistent hyperinsulinemic hypoglycemia (PHHI-GK), caused by activating mutations of the enzyme (21,23); 2) GK-linked permanent neonatal diabetes (PNDM-GK), which develops in newborns who carry two alleles, both altered by inac-

tivating GK mutations (22); and 3) GK-linked maturity-onset diabetes of the young (MODY-GK, also called MODY-2), when the patient has one defective allele causing inactivation of the enzyme (18,25). These syndromes are plausibly explained by the glucose-sensor concept, which states that the catalytic capacity of GK in the β -cells is defined by its k_{cat} , and the affinities for its substrates is the predominant determinant of the glucose threshold for glucose-stimulated insulin release (GSIR) or the glucose set point of the organism (4,18). The normal glucose threshold for GSIR is precisely maintained close to 5 mmol/l in humans and in most laboratory rodents and is governed by GK in the context of the biophysical characteristics of potassium and calcium channels that respond to the rate of glucose metabolism via free ATP^{4-} and $MgADP^-$ (4). The β -cell glucose threshold for GSIR in PHHI-GK patients may be as low as 1.5 mmol/l because of the lowered glucose $S_{0.5}$ and/or increased k_{cat} ; in cases of PNDM-GK, when the newborn may have two alleles with totally inactivating deletions or point mutations, it may be infinitely high because of total inactivation; and in MODY-2 patients, it may be increased only moderately to ~ 7 mmol/l because of a single inactivating mutation.

A simple mathematical model may be used to predict the β -cell threshold for GSIR using the kinetic characteristics of the normal or mutated GK enzyme and by accepting a few plausible assumptions (18,26). This model states that the β -cell glucose phosphorylation rate at the threshold for GSIR is ~ 25 –30% of its physiological capacity, as shown in the following:

$$BGPR = 2 \frac{G^{hw} \times k_{cat}^w}{G^{hw} + G_{0.5}^{hw}} \times \frac{ATP}{ATP + ATP K_M^w} \times GK B^w \times E^w \times S^w \quad (1)$$

$$\frac{BGPR}{2GK B^w k_{cat}^w E^w S^w} = \frac{G^{hw}}{G^{hw} + G_{0.5}^{hw}} \times \frac{2.5}{2.5 + ATP K_M^w} \quad (2)$$

$$\frac{BGPR}{70.7} = \frac{G^{hm}}{G^{hm} + G_{0.5}^{hm}} \times \frac{k_{cat}^m}{k_{cat}^w} \times \frac{2.5}{2.5 + ATP K_M^m} \times \frac{E^{m(adapt)}}{0.57} \quad (3)$$

$$\frac{BGPR}{70.7} = \frac{0.5G^{hw}}{G^{hw} + G_{0.5}^{hw}} \times \frac{2.5}{2.5 + ATP K_M^w} \times \frac{E^{w(adapt)}}{0.57} + \frac{0.5G^{hm}}{G^{hm} + G_{0.5}^{hm}} \times \frac{k_{cat}^m}{k_{cat}^w} \times \frac{2.5}{2.5 + ATP K_M^m} \times \frac{E^{m(adapt)}}{0.57} \quad (4)$$

The β -cell glucose phosphorylation rate (BGPR) of the wild-type homozygote (w) is a function of the basic kinetic constants of GK, the GK content of the β -cell (GKB; defined to be unity), an enzyme expression coefficient E [$(5^{1.8} \times 2)/(5^{1.8} + 8.3^{1.8}) = 0.57$], and a stability coefficient S of the enzyme, also defined to be unity. To obtain the relative BGPR (Eq. 2), Eq. 1 is normalized by dividing it by $2GK B^w k_{cat}^w E^w S^w$. At the glucose (G) threshold for insulin release (GSIR) of 5 mmol/l, the relative BGPR is 0.26. The glucose threshold for GSIR for individuals with a homozygous GK mutant can be calculated using Eq. 3. This threshold is reached at a glucose level that supports a relative BGPR of 0.26 of wild-type. The modeling also needs to account for adaptation through GK induction using E, which is a function of the glucose $S_{0.5}$ of the mutant enzyme. When the enzyme is altered by activating

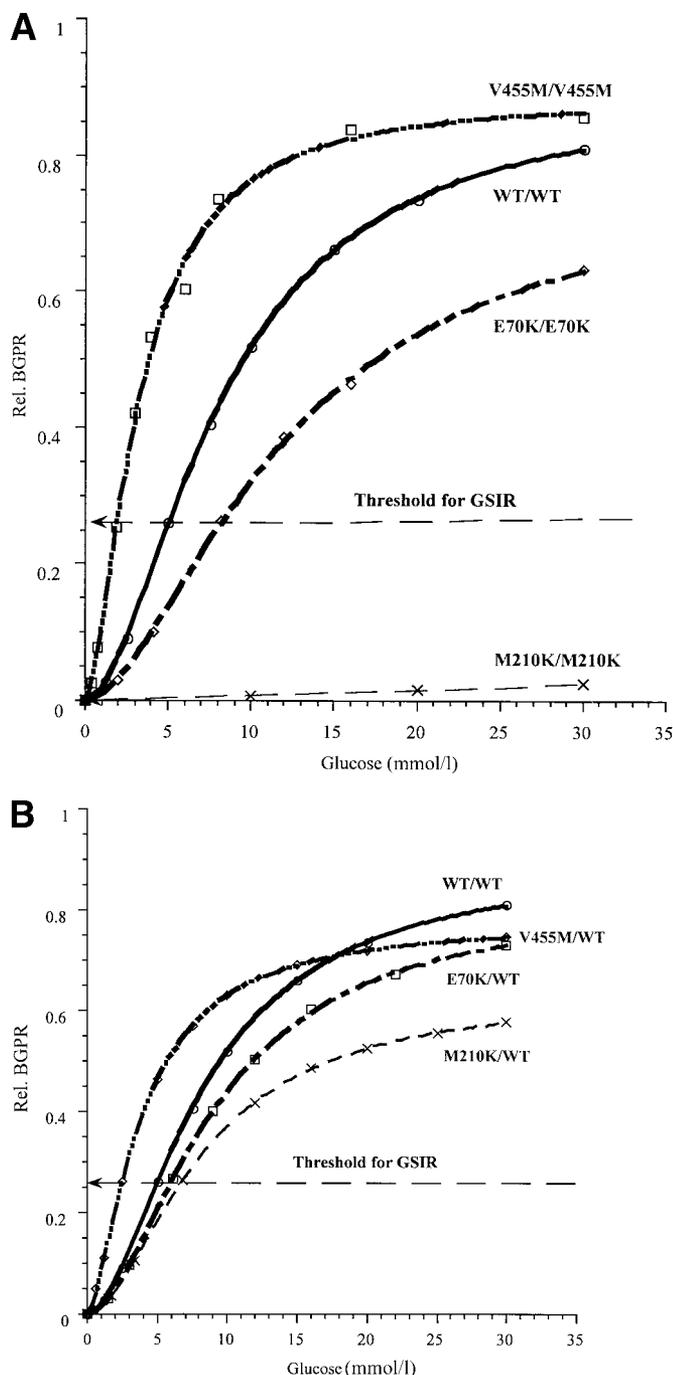


FIG. 3. A: Relative (Rel.) BGPR and glucose thresholds of GSIR in patients homozygous for activating (A456V) and inactivating (E70K and M210K) GK mutations (A). The GSIR thresholds for the adapted state were calculated using Eq. 3. **B:** The result from the corresponding heterozygous cases. WT, wild-type.

that is physiologically occupied by an endogenous activator molecule yet to be discovered (23). The location of this putative allosteric site may be delineated by several allosteric-activating mutations in a region of the GK structure opposite and spatially remote from the substrate binding site. These mutants are V62M, D158A, Y214A, V455M, and A456V (Fig. 4). The change of GK kinetics brought about by the five allosteric-activating mutants strikingly resemble those caused by the activator drugs. This suggests that such a molecular “on-off” switch of GK may have physio-

logical significance given the existence of the postulated endogenous activator(s).

THE CLASSIC OR OLD VIEW OF GLUCOKINASE REGULATION IN β -CELLS

According to the old view of cellular GK regulation, there are two types of GK-containing cells (13). One is exemplified by the hepatocytes (13,15), in which insulin induces GK (GK-L cell), and the other is exemplified by the β -cell (13,31,32), in which glucose is the critical inducer of the constitutively expressed enzyme (B-type GK cell). The experimental evidence for this view is quite extensive. A few striking illustrations are chosen to make the point. The argument for differential regulation of GK in β -cells and hepatocytes was first made in 1986 (13) in a publication that described the dramatic effects of hyperinsulinemic hypoglycemia (created by transplantable insulinomas in NEDH rats) and acute hypoinsulinemic hyperglycemia (caused by the excision of the insulinoma) on GK activity in hepatocytes and pancreatic islet cells (Fig. 5). Chronic hyperinsulinemia of ~ 10 ng insulin/ml serum caused severe hypoglycemia of 1–2 mmol/l blood glucose. Hepatic GK rose about fourfold, but islet GK fell to $\sim 25\%$ of controls. Islet insulin dropped to nearly undetectable levels, but islet glucagon rose somewhat. Excision of the insulinoma raised the blood glucose to ~ 20 mmol/l and caused serum insulin to plummet to 0.4 ng/ml. Islet GK returned to near normal within 24 h without changing islet insulin, and hepatic GK began to decline. Then 6 days after tumor removal, all parameters measured returned to normal. The data were explained by differential cell-specific expression regulation in β -cells and hepatocytes, driven primarily by glucose in one case and by insulin in the other.

Infusion of glucose into the hypoglycemic, markedly hyperinsulinemic animals, *resulting in marked hyperglycemia yet unchanged serum insulin*, also induced islet GK, consistent with this interpretation. This view is strongly corroborated by many other studies of GK expression in fasting/feeding transitions and in studies of glucose or insulin effects in cultured islets or β -cells (8–10). The fasting/feeding transition studies are particularly informative. After being fasted for 72 h, islet GK expression in experimental animals was found to be indistinguishable from that in controls based on immunodetection of GK protein and quantitation of GK mRNA by Northern blotting, whereas hepatic GK expression was markedly decreased in experimental animals, as indicated by both methods of analysis. Re-feeding did not influence islet GK expression, but induced hepatic GK mRNA and protein markedly. When the GK enzymatic activity, glucose usage, and insulin secretion with islets isolated from rats undergoing the fasting/refeeding transition were investigated, only minor changes of GK activity (i.e., 20–30%) were observed, in contrast to the marked changes of GSIR. It is important to note that the small enzyme drop and decrease of glucose usage was delayed compared to the change of insulin release and could thus not be a factor when shorter periods of fasting/feeding are considered.

When the question was further investigated with cultured isolated islets (11,31), it was found that high glucose was a very effective GK inducer, but that insulin when

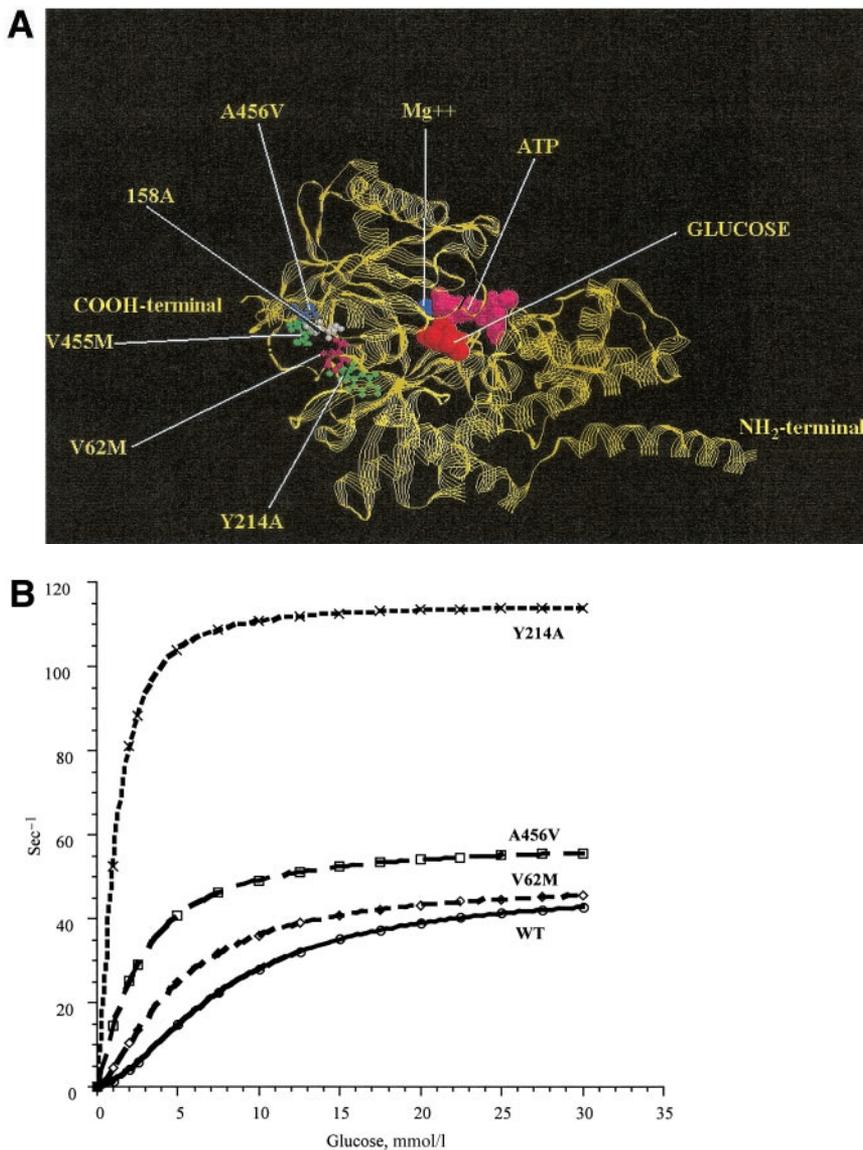


FIG. 4. A: Allosteric activation region of GK. **A:** The structural model of GK by Mahalingam et al. (53) was used. Glucose and MgATP, shown as specific filling structures, occupy the substrate site, and the activating mutations V62M, D158A, Y214A, V455M, and F456V are shown in ball and stick drawings. **B:** The glucose dependency curves for wild-type (WT), V62M, Y214A, and A456V are shown. G455M and D158A are intermediate between A456V and WT and are not shown to maintain clarity of the graph.

added to islets cultured in 3 mmol/l glucose had no impact on GK expression or GSIR (Fig. 6). Another careful study of this issue of GK regulation by glucose showed that enzymatic GK activity of islet tissue increased significantly at a time point when the protein level was not altered, thereby suggesting a posttranslational modification caused by exposure to high glucose (32,33).

Even though glucose seemed to be responsible for GK induction, either by direct action of the glucose molecule itself or through enhanced metabolism, the question of whether the process might be subject to modification by a variety of physiological factors other than fuels was explored. It was observed that this was indeed the case (34–37). Placental lactogen (PL) (34), cAMP (35), biotin (36), and retinoic acid (37) enhanced GK induction. The research with PL is quite detailed and includes whole animal studies, studies with cultured islets, and overexpression of PL in β -cells of transgenic mice. PL increases islet GK and glucose metabolism and decreases the glucose threshold for GSIR. These observations with agonists of diverse structures and functions demonstrate the involvement of a variety of modifying signaling pathways

impinging on the expression control of β -cell GK. The topic is so vast that discussing its molecular basis would require more space than is available here. Much of this information, however, is potentially very significant and needs to be independently confirmed, and the underlying mechanisms need to be firmly delineated.

To begin this exploration and to reevaluate the classic model of GK regulation, a new simple method was developed to allow precise measurements of GK kinetics in extracts of isolated islets. The assay was designed such that it provides reliable V_{max} data, glucose $S_{0.5}$ values, and Hill coefficients (Fig. 7). It was confirmed that exogenous insulin has no effect on the GK of islets cultured in 3 mmol/l glucose, but it was also observed that it practically reverses the inhibition that Ca^{2+} -channel blockers have on GK induction by high glucose. When islets are cultured in the presence of 10 mmol/l glutamine and 10 mmol/l leucine, insulin secretion is comparable to that with 3 mmol/l glucose present; GK is also very low. A Ca^{2+} -channel blocker decreases GK activity under these culture conditions, but exogenous insulin is not able to reverse the effect of lowering cytosolic Ca^{2+} . Adding extreme insulin levels comparable with those seen with 50

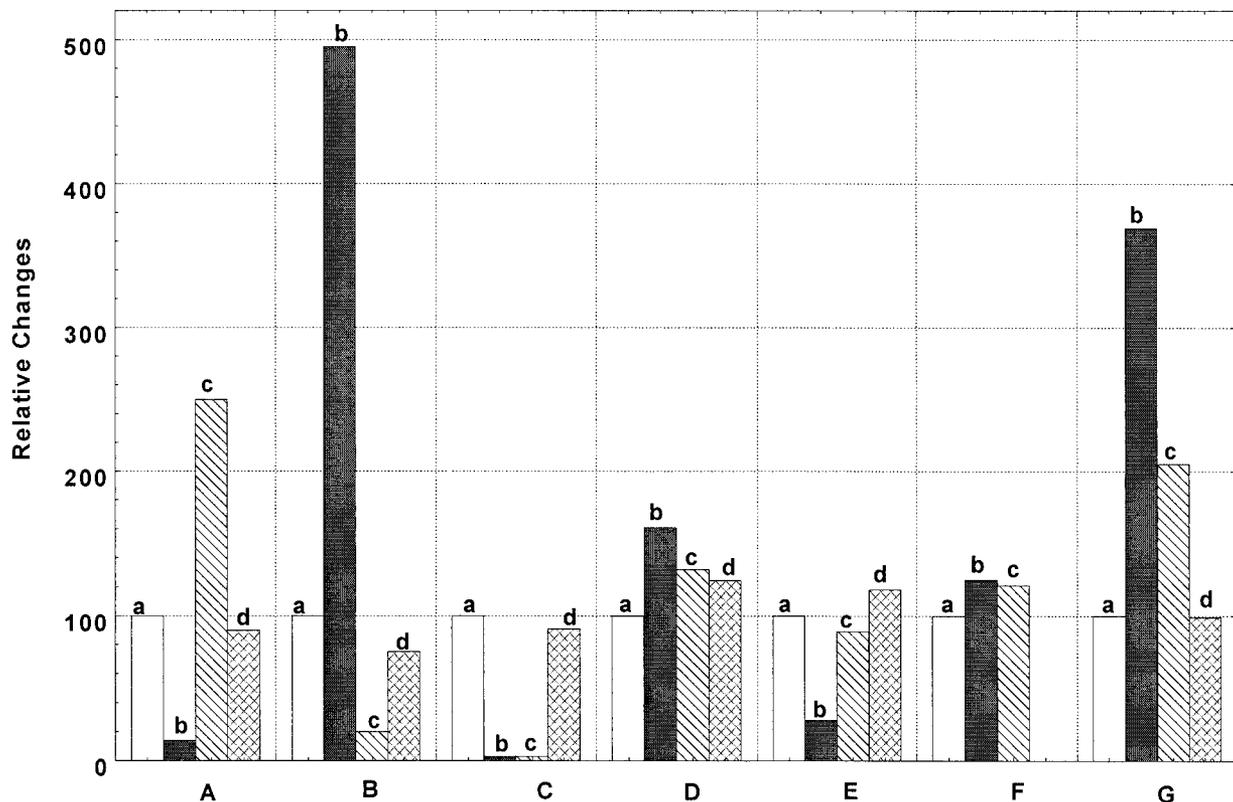


FIG. 5. Differential expression regulation of pancreatic islet and liver GK in insulinoma-bearing NEDH rats (13). Four conditions are shown: fed controls (a), hypoglycemic insulinoma-bearing rats (b), hyperglycemic rats 24 h after excision of the insulinoma (c), and normoglycemic rats 6 days after excision of the tumor (d). Blood glucose (A), serum insulin (B), islet insulin (C), islet glucagon (D), islet GK (E), islet hexokinase (F), and liver GK (G) are shown in relative terms. The corresponding control levels (100%) were 139 mg% for A, 2.0 ng/ml for B, 100 ng/ μ g dry weight for C, 4.1 ng/ μ g dry weight for D, 135 $\text{mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ at 30°C for E, 299 $\text{mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ at 37°C for F, and 1.52 $\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ at 30°C for G.

mmol/l glucose to islets incubated with high leucine and glutamine does not influence GK activity. These data confirm that glucose, rather than insulin, is the inducer of GK, but do not rule out a permissive role for the hormone. The data could also be interpreted to suggest a critical role of Ca^{2+} in GK expression.

THE NEW VIEW OF GLUCOKINASE REGULATION IN β -CELLS

Beginning with the discovery of insulin receptors in the β -cell plasma membrane (38), followed by the demonstration that the various types of insulin receptor substrates (e.g., IRS-1 and -2) exist in islet tissue (39,40), and capped by the observation that insulin itself may cause insulin release (41), the role of insulin in β -cell biology has been investigated with increasing intensity. A most striking example of this recent endeavor is the development of a gene knock-out mouse that lacks the insulin receptor of the pancreatic β -cells (the BIRKO mice) (14,42,43). These animals are normoglycemic at birth and remain so for nearly 2 months, even though β -cells lack insulin receptors. Serum insulin levels are also in the normal range during this early age. The animals become glucose intolerant by age 6 months as the islet cell mass decreases. Islets from these animals have been used to explore the role of insulin signaling in glucose induction of β -cell GK (14). These studies were done with islets taken from mice of unspecified age. It was demonstrated that insulin was

unable to augment GK mRNA production and did not enhance the synthesis of GK-eGF (GK tagged with enhanced green fluorescence marker) in contrast to the hormone's effectiveness in β -cells from control animals. Results from a series of detailed experiments using a variety of pharmacological interventions have suggested that high glucose induction of GK expression is insulin mediated. For example, high glucose induction was prevented by a Ca^{2+} -channel blocker, by the phosphatidylinositol 3-kinase inhibitor wortmannin and by an inhibitor of akt (or protein kinase B). Tolbutamide was as effective as high glucose. The data were interpreted to mean that glucose's effect on GK expression is indirect and is brought about by a specific insulin-signaling pathway. It was found that this signaling chain differs from a second insulin-signaling pathway that is involved in the glucose stimulation of insulin biosynthesis (42). The implications of these results are far reaching. Pertinent to the present discussion is that they do imply that the apparently normal insulin secretion during the early normoglycemic age of the BIRKO mice is GK independent. This would be a very striking hypothesis, if not a revolutionary one. It needs to be tested, therefore, whether islets from these animals do indeed lack GK activity, or more accurately whether they lack the increase of GK activity that is seen with elevated glucose.

The surprise about the normalcy of the neonatal phenotype of BIRKO mice was as great as that about the normal

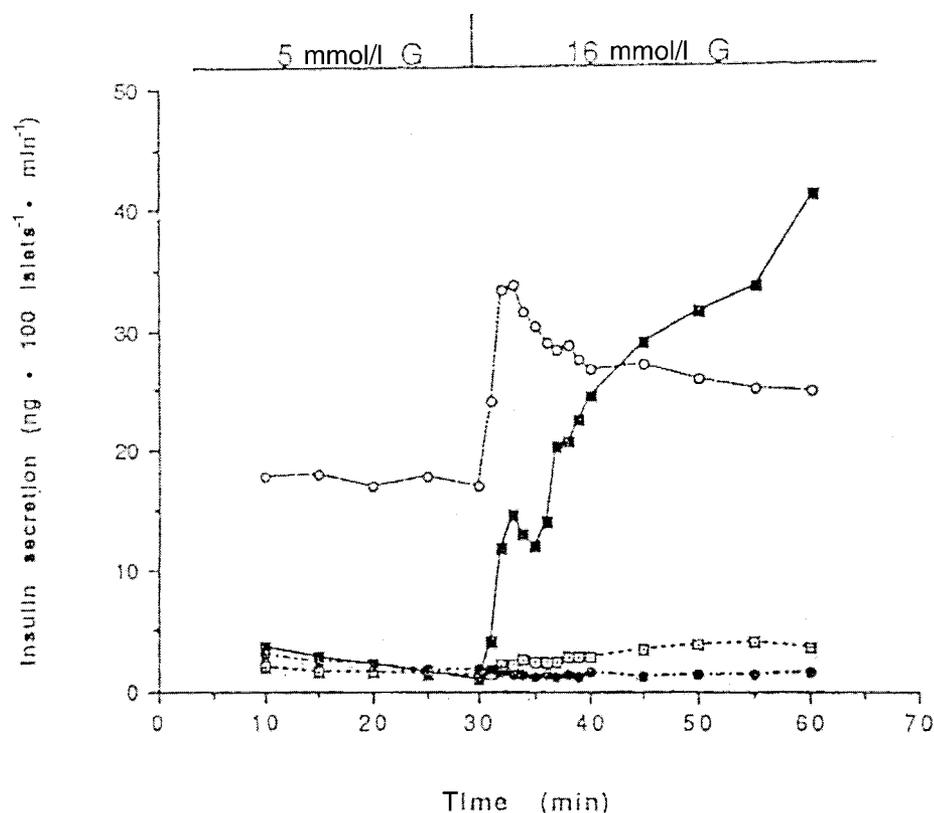


FIG. 6. Insulin secretion from perfused islets stimulated with glucose. The means of three perfusion experiments for each condition are recorded. Islets were perfused with Krebs-Ringer bicarbonate buffer containing 5 mmol/l glucose (G) for 30 min and then switched to perfusion medium with 16 mmol/l glucose. Islet DNA and insulin contents were indistinguishable for all conditions. The GK activities were as follows (in terms of mole glucose phosphorylated per kilogram DNA per hour): ■, freshly isolated islets, 5.22 ± 0.45 ; □, islets cultured for 7 days in medium containing 3 mmol/l glucose, 2.55 ± 0.47 ; ●, islets cultured for 7 days in medium containing 3 mmol/l glucose plus 350 ng/ml exogenous insulin, 2.63 ± 0.64 ; ○, islets cultured for 7 days in medium containing 30 mmol/l glucose, 12.32 ± 0.70 . GK mRNA levels were also the same at 3 and 30 mmol/l glucose (11). Used with permission from Liang et al. (31).

phenotype that was found in KIR-6.2 and SUR-1 knock-out mice (43,44). A variety of adaptive mechanisms may explain these outcomes, and their detailed characterization promises important insights into basic glucose homeostatic mechanisms.

The interpretation of the apparent insulin dependency of glucose-induced GK expression of β -cells is greatly complicated by additional recent observations important for our understanding of β -cell GK biochemistry. Several laboratories have observed that β -cell GK is associated with the insulin-containing secretory granules (45–48). The particulate fraction of GK amounts to $\sim 15\%$ according to one report; particulate GK rises *pari passu* with cytosolic GK, as has been shown in studies with glucose-stimulated isolated islets in culture (48). At a recent meeting, it was communicated that high glucose causes the release and activation of granular GK, and that this glucose effect is indirect and mediated by insulin (49). There is also new published evidence that islet tissue may contain GKRP (50), which would imply short-term regulation of GK by F6P and F1P. This would contradict the interpretations of earlier studies that showed that fructose does not modify glucose metabolism (16) and would be somewhat surprising because GKRP knock-out mice show normal GSIR (17).

There is experimental support for the view that the bifunctional enzyme 2-PFK/FBPase-2 is present in islet tissue (51), and it is thus tempting to extrapolate from most recent findings in hepatocytes (52) to the β -cell and suggest that the level of the bifunctional enzyme may positively influence GK expression in an insulin-independent manner. However, F26PB levels of the islets are much less responsive to glucose than those of hepatocytes.

ATTEMPT AT SYNTHESIS: MULTIFACTORIAL REGULATION OF β -CELL GLUCOKINASE EXPRESSION

Taking into account all experimental data and in view of the unique features of tissue-specific GK regulation and subcellular distribution, it is necessary to retain the classic dichotomy of two distinct models that describe GK regulation: the L-type GK-containing cells exemplified by hepatocytes and the B-type GK-containing cells exemplified by pancreatic β -cells (Fig. 8).

In L-type GK cells, insulin is the predominant inducer of GK (13,15). The insulin effect is seen in the virtual absence of or at very low glucose. GKRP determines the subcellular distribution of GK in the hepatocytes as a function of the F6P/F1P ratio, allowing nuclear sequestration of the enzyme when this ratio is high and release into the cytosol when it is low (6,7). This is illustrated by findings in GKRP knock-out mice that have only half as much hepatic GK as controls and where all the enzyme is found in the cytosol (17). The bifunctional enzyme PFK-2/FBPase-2 binds to GK and supports GK expression directly or indirectly in an insulin-independent manner (52). Because of enhanced GK activity, high glucose results in relatively high G6P levels that may then function as a metabolic coupling factor mediating the induction of lipogenic enzymes.

In B-type GK-containing cells, exemplified by pancreatic β -cells, glucose is the predominant inducer of GK (11,13,31). Glucose-driven GK expression is modified by many positive and negative factors: it is augmented by cAMP (35), biotin (36), retinoic acid (37), PL (34), and insulin (14), and it is decreased by Ca^{2+} -channel blockers—that is, by low cytosolic Ca^{2+} levels. Even though insulin is not sufficient to induce the enzyme, the hormone may still be required for optimal GK expression in B-type

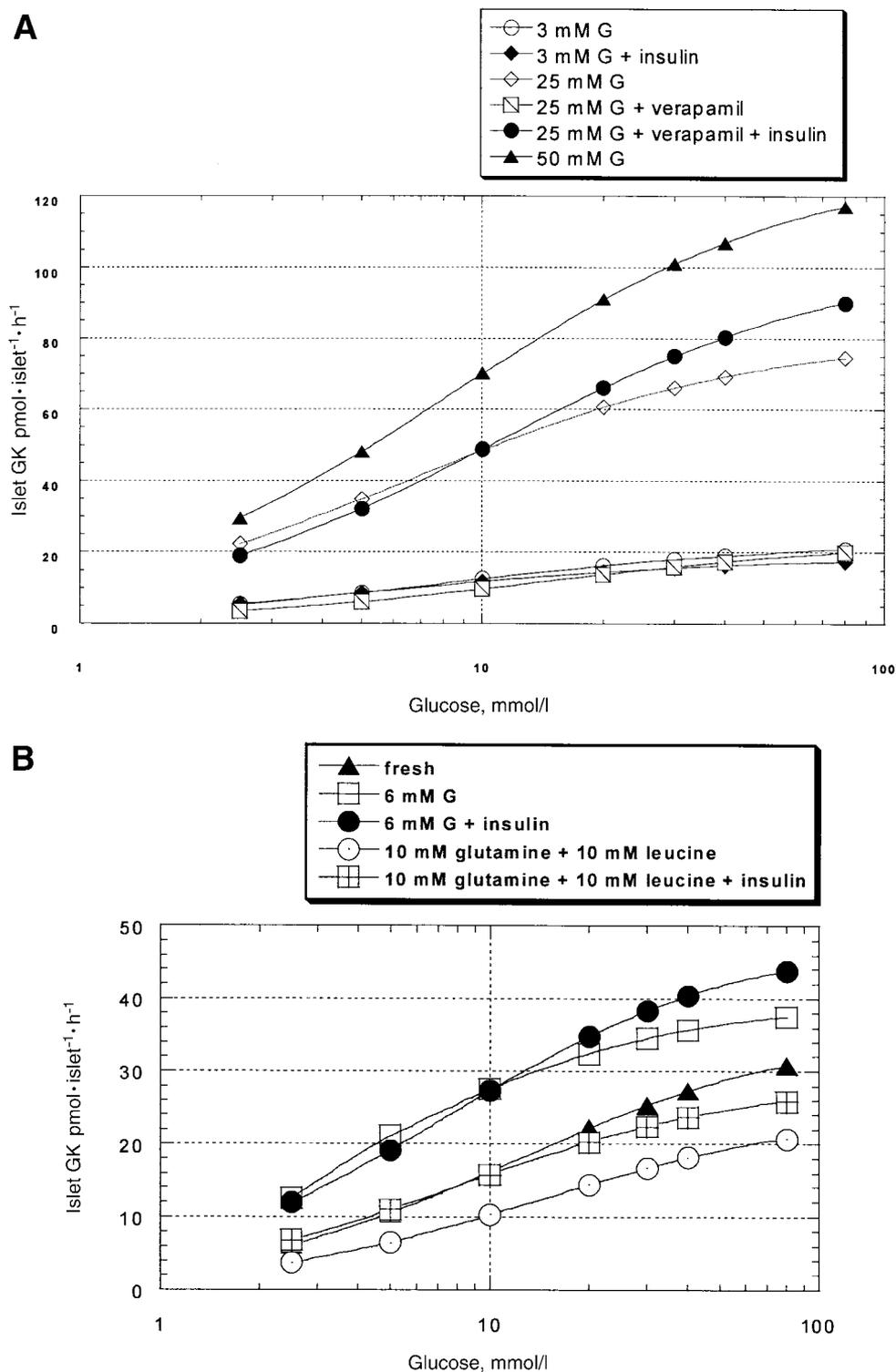


FIG. 7. Glucose, amino acids, and insulin as inducers of GK activity in pancreatic islets. GK activity was measured in the supernatant of islet homogenates. *A*: Rat islets were cultured for 4–5 days at 3, 10, 25, or 50 mmol/l glucose (G); verapamil was added at 100 μ mol/l and insulin at 250 ng/ml medium. *B*: Islets were freshly isolated or cultured for 4–5 days at 6 mmol/l glucose with and without 350 ng insulin/ml or at <1 mmol/l glucose and 10 mmol/l each of glutamine and leucine with or without 1 μ g insulin/ml of culture medium added equivalent to hormone levels accumulating in the culture medium of islets at 50 mmol/l glucose. All curves are best fits of single experiments representing the results of at least three similar experiments for each condition.

GK-containing cells. GKRP and the bifunctional enzyme PFK-2/FBPase-2 seem to be of little relevance in these cells (17,51). A cautionary note is indicated here, because it is very difficult in practice to test mechanisms of GK

induction in the total absence of extracellular insulin, for obvious reasons.

As is true for the majority of regulatory enzymes, the GK molecule is best understood as an allosteric enzyme that

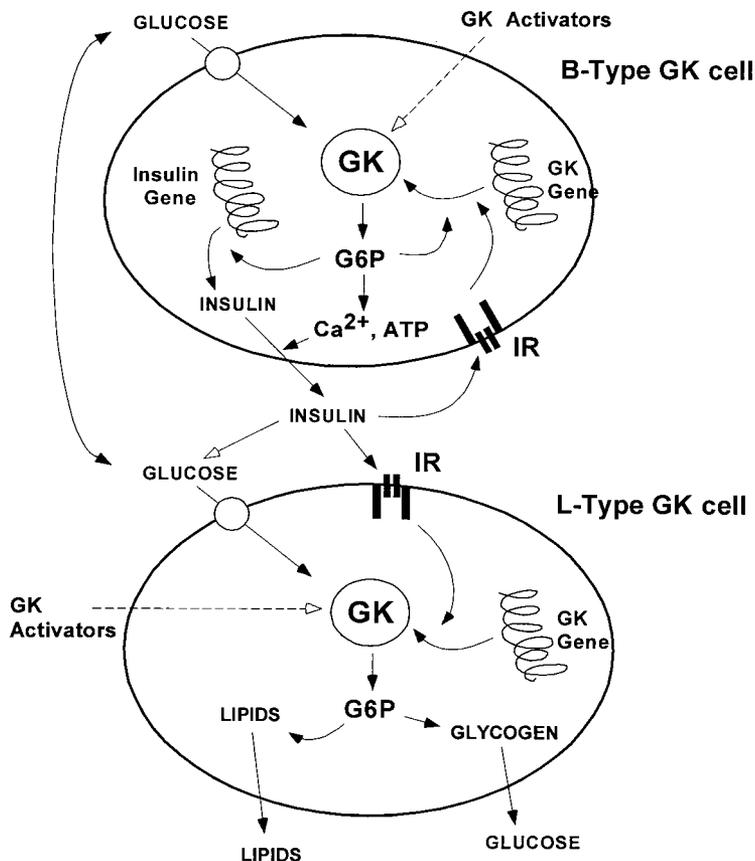


FIG. 8. Differential expression of regulation of GK in B-type and L-type GK cells. The B-type GK cell is primarily glucose driven, whereas the L-type is primarily insulin driven. GK expression is differentially controlled by cell-specific GK promoters, the upstream islet cell-specific promoter in B-type cells (B-promoters) and the downstream hepatocyte-specific promoter in L-type cells (L-promoters). Glucose metabolism and/or glucose itself govern GK expression primarily translationally and/or posttranslationally. Insulin governs GK expression primarily transcriptionally. The term "primarily" makes allowance for the fact that many other factors may modify the responses to glucose or insulin. Newly discovered GK activator drugs are equally effective in both cell types. This bare-bones model first presented in 1986 can now be expanded easily and be thoroughly tested based on the vast information of current knowledge about intracellular signaling.

responds to endogenous activators and inhibitors on a timescale of seconds. The enzyme has cooperative kinetics with its substrate glucose, as indicated by a Hill coefficient of 1.5–1.8. It is atypical, though, because it appears to operate as a monomer (4,5). However, it may form hetero-oligomers with GKR and/or PFK-2/FBPase-2 (6,52). Its rate is controlled by the F6P/F1P ratio via GKR (6,7). The higher this ratio, the stronger the inhibition. This regulation thus depends on two other genes: the *1-fructokinase* gene and the *GKR* gene. GK has a binding site for the PFK-2/FBPase-2 of unknown physiological or pathological significance (52). GK-B and GK-L differ markedly in their amino acid sequences of the NH₂-terminal stretches of 14 amino acids, a difference of unknown significance. GK is responsive to allosteric activation by single-point mutations of a circumscribed cluster of amino acids (23) and, consistent with this phenomenon, responds to a novel class of small molecules termed GK-activator drugs (29,30). The existence of endogenous GK activators is therefore postulated.

IMPLICATIONS OF GK RESEARCH FOR MOLECULAR PATHOGENESIS OF DIABETES

The implications for diabetes research emanating from the expanded GK glucose-sensor paradigm as described in this brief study are manifold and could be far reaching. It is now understood that a network of L- and B-type GK cells participates in glucose homeostasis. The pancreatic β -cell is the unquestionable leader of this group. Because GK activity and expression is regulated by a broad array of cell-specific factors, GK pathophysiology might be an important aspect of type 2 diabetes in general, even

without apparent linkage to the GK gene, a situation similar to that found to exist for the insulin receptor gene and intensively explored for years. The conceptualization of a complex of clinically striking GK-linked syndromes as "glucokinase disease" (i.e., PHHI-GK, PNDM-GK, and MODY-2) underscores the critical role of the GK enzyme for glucose homeostasis. The discovery of a relatively large percentage (~10%) of GK mutants discovered in patients with diabetes, but with apparently normal enzyme kinetics, clearly suggests molecular mechanisms that are not detected by current routine analysis. Such mechanisms might involve lack of allosteric activation (e.g., V62M) or an inability of the enzyme for binding to the insulin granule or regulatory proteins, to mention the most obvious possibilities. "Kinetically normal" GK mutants causing GK disease need to be investigated vigorously using new biochemical biological tools. Continued research heeding the ideas emanating from the present synthesis is mandatory. The newly discovered GK activator drug has particularly great promise for exploring the role of GK in diabetes, and it is hoped that its great potential for a new drug therapy of type 2 diabetes will be realized in the near future.

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