Perspectives in Diabetes

The Network of Glucokinase-Expressing Cells in Glucose Homeostasis and the Potential of Glucokinase Activators for Diabetes Therapy

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The glucose-phosphorylating enzyme glucokinase has structural, kinetic, and molecular genetic features that are ideal for its primary role as glucose sensor in a network of neuro/endocrine sentinel cells that maintain glucose homeostasis in many vertebrates including humans. The glucokinase-containing, insulin-producing β-cells of the pancreas take the prominent lead in this network, functioning in the aggregate as the master gland. The β-cells are also conceptualized as the prototype for all other glucose sensor cells, which determines our current understanding of many extrapancreatic glucose sensors. About 99% of the enzyme resides, however, in the hepato-parenchymal cells and serves its second role in a high-capacity process of blood glucose clearance. Two examples strikingly illustrate how pivotal a position glucokinase has in the regulation of glucose metabolism: *1*) activating and inactivating mutations of the enzyme cause hypo- and hyperglycemia syndromes in humans described collectively as "glucokinase disease" and fully explained by the glucose sensor paradigm, and *2*) glucokinase activator drugs (GKAs) have been discovered that bind to an allosteric site and increase the kcat and lower the glucose $S_{0.5}$ of the enzyme. GKAs enhance glucose-stimulated insulin release from pancreatic islets and glucose disposition by the liver. They are now intensively explored to develop a novel treatment for diabetes. Future biophysical, molecular, genetic, and pharmacological studies hold much promise to unravel the evolving complexity of the glucokinase glucose sensor system. *Diabetes* 55:1–12, 2006

INTRODUCTION AND HISTORICAL BACKGROUND

Glucokinase (ATP: D-glucose 6-phosphotransferase, EC 2.7.1.1), also known as hexokinase IV or D, is one of four glucose-phosphorylating enzymes found in vertebrate tissues (1–4). It catalyzes the phosphorylation of various hexoses by ATP according to the following reaction: $R - CH_2OH + MgATP^{2-} \rightarrow R - CH2 - O - PO^{2-} + MgADP^{-} + H^{+}$. (Note that calling the enzyme glucokinase rather than hexokinase IV or D is preferred by physiologists and human biochemical geneticists because its physiological substrate is indeed D-glucose.)

Glucokinase was discovered ~40 years ago independently in three laboratories, and tissue surveys at the time indicated that the enzyme was liver specific, serving a critical role in postprandial glucose clearing from the circulation (5–8). Shortly thereafter in 1968, Matschinsky and Ellerman (9) reported on their demonstration of glucokinase in pancreatic islet tissue and proposed that glucokinase functions in a dual role: as the molecular glucose sensor element in the insulin-producing pancreatic β-cells in addition to its established function as the high-capacity enzymatic step initiating the storage of glucose in the form of glycogen in the liver. Glucokinase was eventually measured in islet tissue dissected from freeze-dried sections of human pancreas (10). This was possible because an ultrasensitive radiometric oil-well method had been developed (11). A central role of glucokinase in glucose homeostasis was thus established, and the possibility was considered that a glucokinase defect could cause diabetes in humans. The recognition in 1986 that glucokinase expression in pancreatic β-cells and hepatocytes is differentially controlled was another milestone (12), soon confirmed and followed by the observation that endocrine and hepatic glucokinase are encoded by a single gene with two distinct cell-specific promoters: an upstream promoter for the β-cell and a downstream promoter for the hepatocyte (13,14). Glucokinase has since been found in other tissues including certain neurons of the hypothalamus and the brainstem, in the pituitary and entero-endocrine K and L cells, leading to the formulation of the hypothesis that a network of glucokinase-containing sentinel glucose sensor cells may be essential for maintaining glucose homeostasis (15,16). Of relevance to the present discussion is a considerable body of evidence demonstrating that glucokinase expression in all extrapancreatic cells, i.e., neurons and endocrine cells, is controlled by the upstream glucokinase promoter, now aptly called the neuro-endocrine promoter to distinguish it from the hepatic promoter (17–20). There is also ample evidence that insulin is the primary regulator for the hepatic promoter, whereas the neuro-endocrine promoter is regulated in a complex fashion with a prominent role for glucose as inducer of the enzyme (as clearly demonstrated in the case of pancreatic β-cells). There is no doubt that the glucokinase-containing β-cells of the microscopic pancreatic islets of Langerhans function in a concerted fashion and constitute (in the aggregate) an endocrine master

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GKA, glucokinase activator drug; GKRP, glucokinase regulatory protein; GSIR, glucose-stimulated insulin release; MODY, maturity-onset diabetes of the young; OCR, oxygen consumption rate.

gland for maintaining glucose homeostasis. The proposed glucose sensor role for glucokinase in other neuro-endocrine cells is less well established. Lacking are critical experiments to test the now fashionable hypothesis that the involvement of these cells is indeed crucial for maintaining optimal glucose homeostasis and that such a role is dependent on glucokinase. For example, what is the consequence for the otherwise intact organism of selectively eliminating the enzyme from extrapancreatic glucokinase-containing cell types, one by one or in combination, or of activating the enzyme cell specifically? Until such or similar tests have been accomplished and have resulted in definitive answers, it needs to be acknowledged and stressed how speculative these ideas really are.

It is a demanding task to develop a clear sense of the significance that single molecules may have for glucose homeostasis, considering the complexity of the system. Insulin is perhaps the exception, and glucokinase is a case in point! In 2005, after nearly 40 years of intensive work and often fighting an uphill battle against strong resistance toward the glucokinase glucose sensor concept, most would probably agree that glucokinase is a leading player with only a few other molecules of comparable significance for the regulation of blood glucose levels. Two developments prominently contributed to this change in perspective. First, throughout the 1990s, glucokinase disease encompassing glucokinase-linked hypo- and hyperglycemia syndromes was recognized in extensive family studies and carefully characterized both genetically and biochemically (16,21–23). Second, during the same period, glucokinase activator drugs were developed, which increase the maximal activity and glucose affinity of the enzyme and lower blood glucose in normal and diabetic animals by increasing insulin release and curbing glucose production by the liver (24,25). The availability of glucokinase activator drugs also facilitated the successful crystallization and structural analysis of the enzyme, which immeasurably increased the understanding of enzyme function and provided new research opportunities (24,26,27). These striking developments in human genetics and drug research of the last 15 years have demonstrated the practical medical significance of the concepts that had been invented, tested, and proven experimentally by basic science during the 1960s, 1970s, and 1980s. Reports about comprehensive studies of mouse models of glucokinase disease published in the mid-1990s support the glucokinase glucose sensor paradigm in every respect (28), and intensive continuation of the work using such models to explore the role of glucokinase in glucose homeostasis in even greater depth holds great promise for the future (29–31).

A FEW BASIC FACTS ABOUT GLUCOKINASE KINETICS AND STRUCTURE

The glucokinase glucose sensor paradigm, glucokinase disease, and the potential of glucokinase activator drugs for diabetes therapy as discussed in this article can be fully comprehended only if the unique biochemical and structural features of the enzyme are appreciated. Table 1 contains a list of widely accepted kinetic constants and other relevant biochemical information that is for the most part self explanatory (see also the monographs by Cardenas et al. [1] and Matschinsky and Magnuson [32]). It should be remembered that the hepatic and the neuroendocrine forms of the enzyme (differing merely in the composition of the NH_2-terminal sequence of 14 amino acids) are functionally indistinguishable, and it is emphasized that the enzyme shows cooperative kinetics with regard to glucose as expressed by a Hill coefficient of 1.7, an inflection point of 3.5 mmol/l, and a cooperativity index R_a of 12 compared with 81 for a hyperbolic enzyme (33). Only few physiological regulators of glucokinase are known. Glucokinase regulatory protein (GKRP), found in the hepatic nucleus, is a well-established inhibitor, enhanced by fructose-6-P and counteracted by glucose and fructose-1-P (hence the stimulation of hepatic glycolysis by low fructose, the substrate of fructokinase-1) (34). Long-chain fatty acyl-CoAs have been shown to inhibit the purified enzyme (35), but the physiological relevance of this observation remains questionable (as are many other experimental effects of acyl-CoA on various enzymes, ion channels, and pumps). However, in contrast to the other hexokinases, glucokinase is not directly inhibited by metabolites of glucose (e.g., glucose-6-P or glucose-1,6-P_2), and its rate is therefore entirely determined by the intracellular glucose levels since ATP is saturating. Recent reports (36–39) stress the possibility that glucokinase may be associated with various macromolecules or intracellular structures that could have important implications for this enzyme's function. Examples of proteins that form complexes with glucokinase are GKRP (as discussed), the proapoptotic factor BAD, and the bifunctional enzyme fructose-6-P/2-P-kinase/2-phosphatase. Also to be considered are subcellular structures that appear to bind glucokinase, such as insulin granules and mitochondria.

TABLE 1
Characteristics of human glucokinase*

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

*All kinetic constants were obtained at pH 7.4, and the glucose $S_{0.5}$ rises as the pH falls.

Stunning advances have been made in recent years in our understanding of the structure of glucokinase by homology modeling and by crystallography. Tentative glucokinase structures based on homology modeling using brain hexokinase had provided the first useful information on the nature of the glucose and ATP binding sites (40,41) and also on the location and composition of an allosteric activator site, which was ultimately delineated in detail by solving the glucokinase/activator cocrystal structure and resulted in the further exploration and understanding of numerous mutants that cause hypo- and hyperglycemia due to glucokinase disease (24–27). The glucose binding site of the enzyme involves eight amino acids (S151, T168, K169, N204, D205, N231, E256, and Q287). To form the ATP

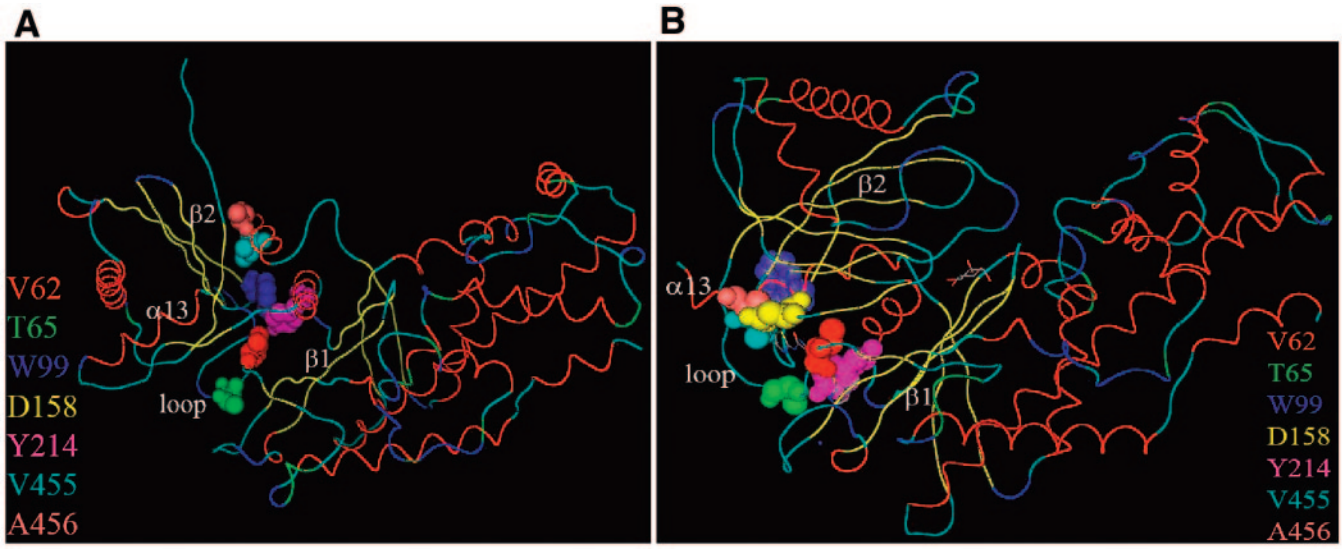

FIG. 1. Location of V62, T65, W99, D158, Y214, V455, and A456 in the open (*A*) (Protein Data Bank entry 1V4S) and closed (*B*) (Protein Data Bank entry 1V4S) conformation. Note that D158's coordinates are not available in the open conformation. α Helixes in red, β sheets in yellow, and the loop region spanning the GKA binding site in white. The NH_2-terminal (in green) and the COOH-terminal (in blue) are indicated. Closure of the substrate-binding cleft is accomplished by a massive movement of the small left lobe without much change of the larger right lobe.

binding pocket, seven amino acids are needed (T82, R85, T228, K296, S336, S411, and K414). Many of these have been found to be changed in cases of inactivating mutants that cause maturity-onset diabetes of the young (MODY)-2. The existence of an allosteric activator site was inferred from the fact that activating mutants V62M, T65I, W99R, D158A, Y214A, V455M, and A456V, many of them detected in hypoglycemic patients, were clustered in a region of the enzyme clearly separate from the substrate binding site (42,43). The enzyme has now been crystallized in both the absence and presence of a small molecule activator, providing confirmation that these amino acids reside in the glucokinase activator (GKA) binding pocket (Fig. 1) (25–27). When crystallized in the presence of a GKA and α-D-glucose, the closed form is observed, which shows a narrowing of the substrate binding cleft and an opening of the activator site. The conformational transition from the ligand-free open form to the liganded closed form is associated with marked positional changes of loops connecting the large and the small lobes of the molecule (in particular of loop I stretching from V62 to G72 and linking β-1 with β-2) and involves an extended motion of the COOH-terminal α-13 helix (compare Fig. 1*A* and *B*). These glucose-induced structural changes may be conveniently studied spectrophotometrically by monitoring the enzyme's slow transition kinetics or by observing ligand-induced changes of tryptophan fluorescence (44,45). The results of these structural studies and functional biophysical analysis, a line of inquiry that has tremendous potential for future research, provide insight into the mechanisms by which a monomeric enzyme with a single glucose binding site shows kinetic cooperativity, a characteristic critical for the precise sensor function of glucokinase. The demonstration of an open and closed structure support the two state "mnemonic" and the "slow transition" models of glucokinase action previously advanced by enzyme kineticists (46). The current knowledge about this enzyme can be summarized by stating that glucokinase is one of the best characterized key elements involved in glucose homeostasis, a situation greatly benefiting basic science and diabetes-related research, including drug discovery and therapeutic endeavors. Greatest advances in the understanding of the basic enzymology of glucokinase can probably be expected from crystallography and fluorescence spectroscopy, particularly if combined with mutational and pharmacological manipulations of the enzyme.

THE ROLE OF GLUCOKINASE IN PANCREATIC B-CELLS AS MODEL GLUCOSE SENSOR CELLS

The insulin-producing β-cell functions as a glucostat and is perhaps the most prominent in a cast of many well-known players participating in maintaining glucose homeostasis (47–50). The β-cell may also be conceptualized as a model cell for other auxiliary glucose sensor cells that are found distributed throughout the vertebrate body. The β-cells are located in microscopic cell clusters of the islets of Langerhans (~1,000,000 of them scattered throughout the human pancreas and amounting to ~1 g of tissue). They are associated with other endocrine cells (α pancreatic polypeptide, D, and ghrelin cells) and are richly innervated by the automomic nervous system and intensely capillarized. They serve as fuel sensor cells responding to glucose, amino acids, and fatty acids. These responses may be positively and negatively modified in a complex manner by the neuro-endocrine system. Glucose is physiologically the most important stimulus in humans and common laboratory rodents, and the action of most other physiological fuel stimuli and neuro-endocrine modifiers is claimed to be strictly glucose dependent. The glucose-induced activation of intermediary metabolism (i.e., glycolysis, the pentose-P shunt, the citric acid cycle, alterations of amino acid and lipid catabolism, and synthesis) results in the generation of so-called "metabolic coupling factors" that mediate insulin release (Fig. 2*A* and *B*). The cytosolic levels of ATP and ADP are outstanding examples and represent probably the most important coupling factors. The glucose-induced increase of ATP^{4-} (even though it may be small, amounting to just a few perecnt) and the lowering of $MgADP^{1-}$ (a parameter exhibiting larger negative changes of 25–50%) lead in combination to a reduction of K^{1+} efflux

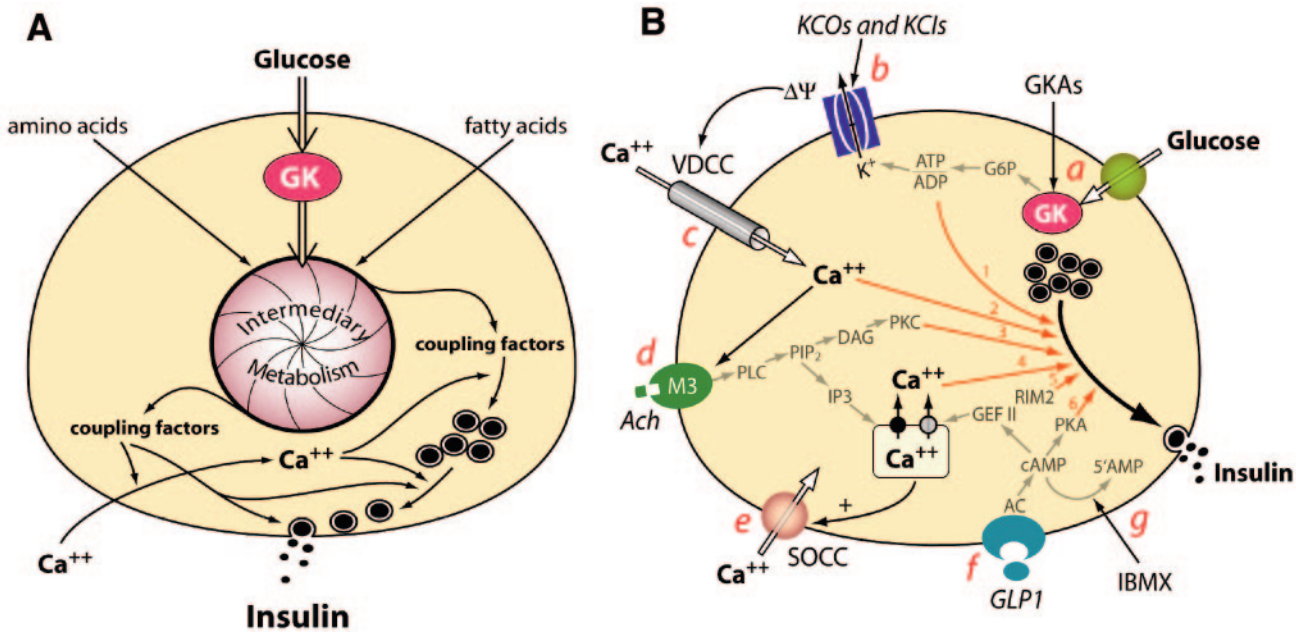

FIG. 2. Glucokinase-dependent mechanisms in fuel stimulation of insulin release and their modification by neuro-endocrine factors or pharmacological agents. *A*: Minimalistic scheme of fuel sensing by the β-cell. Glucokinase (GK) is central to the process, and intermediary metabolism results in the generation of coupling factors that cause insulin release with Ca^{2+} as a major player. *B*: Depicts modifying pathways that impinge on insulin release and also lists a number of hormones, transmitters, and drugs that activate or inhibit these pathways. Many abbreviations are self-explanatory but others, less common, are defined as follows: AC, adenylate cyclase; cAMP, cyclic AMP; PKC, protein kinase C; PKA, protein kinase A; DAG, diacylglyceride; IP_3, inositol triphosphate; GEFII, cAMP-guanidine nucleotide exchange factor (or Epac2); ΔΨ, change of cell membrane potential; RIM2, exocytosis-regulating protein; VDCC, voltage-dependent calcium channel; SOCC, store-operated calcium channel; KCO, potassium channel opener; KCI, potassium channel inhibitor. *B* is a modified version of a previously published figure (51).

from the cell by inhibiting the SUR-1/Kir6.2 K-channel complex, depolarizing the cell and opening voltage-sensitive Ca-channels, which then causes the cytosolic Ca^{2+} levels to rise, almost certainly the most important single event in the complex process of stimulus secretion coupling. Other metabolic coupling factors have been proposed to play an auxiliary or even essential role (NAD(P)H, malonyl-CoA, acyl-CoA, diacylglycerol, glutamate, glutamine, and others), but no compelling case comparable with that for ATP and ADP has been made for any one of these. Note that glucose also promotes insulin synthesis via factors arising from its metabolism. Physiological levels of amino acids and fatty acids greatly enhance glucose action but are usually thought to be ineffective on their own (Fig. 2*A* is a minimalistic depiction of our current understanding). The neuro-endocrine modification of fuel-stimulated insulin secretion is quantitatively significant and physiologically critical (Fig. 2*B*). The entero-pancreatic axis (via glucagon-like peptide-1, gastric inhibitory peptide, and perhaps also cholecystokinin) and the vagal input via acetylcholine are activated by feeding (51). These hormones and transmitters enhance the fuel response through G-protein–coupled receptors and phospholipase C, which then activate a whole set of adenylate cyclases, stimulate protein kinases (protein kinases A and C), and also mobilize Ca^{2+} from intracellular stores, processes that combined result in a profound augmentation of the primary fuel-triggered response. Physiologically speaking, this results in a left shift and augmentation of the fuel-induced dose-response curves. Our own studies with isolated islets from SUR-1 knockout mice can be used to illustrate the distinction between fuel-induced triggering and neuro-endocrine augmentation or amplification of release (Fig. 3) (51). The β-cells of K-channel–deficient islets have high basal Ca^{2+} levels (at least twice the levels of controls) and show a greatly enhanced basal insulin release (i.e., the process of insulin release has been triggered by depolarization due to deleting the K-channels), but little or no augmentation of secretion is demonstrable when high glucose is the sole stimulus. This observation runs counter to the predictions of the current dogma, which holds that glucose-stimulated insulin release (GSIR) has a triggering component (referred to as ATP channel dependent) and a quantitatively comparable augmentation component (defined as ATP channel independent) (50). The restitution of defective glucose responsiveness by acetylcholine/glucagon-like peptide-1 as observed in SUR-1–null islets implies as yet undefined interactions between glucose and amino acid metabolism and these other signaling pathways. It is attractive to speculate that a high P potential resulting from increased oxidation of metabolic substrate as apparent from stimulation of respiration when fuel molecules are introduced is the crucial common factor that facilitates neuro-endocrine potentiation. The experiment depicted in Fig. 3 also demonstrates that the rates of insulin release and of energy production (assumed to be equal to energy consumption) as manifested by the oxygen consumption rate (OCR) profiles are not tightly coupled, contrary to what might be expected. The energy cost of exocytosis is apparently negligible since baseline OCRs are the same in the two conditions in spite of greatly different insulin secretion rates, and acetylcholine has practically no effect on the OCR even though it markedly enhances hormone release.

What are then the functions of glucokinase in the β-cell?

- Glucokinase is required for GSIR in the physiological range of 4–8 mmol/l.

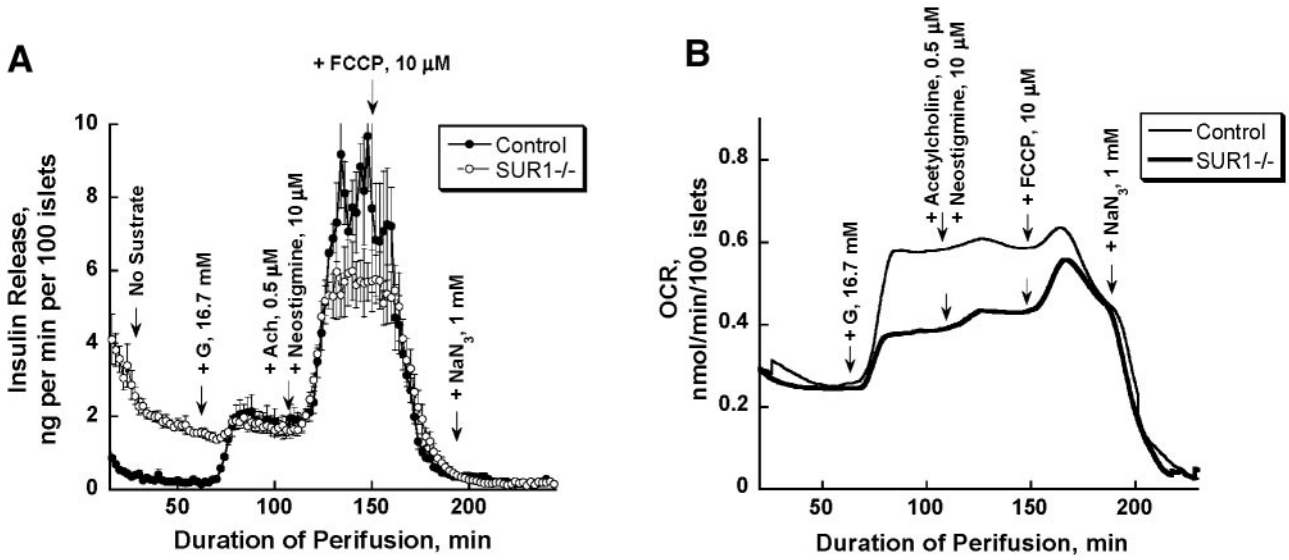

FIG. 3. Glucose and acetylcholine stimulation of insulin release and respiration of isolated perifused islets of normal and SUR-l–null mice. *A*: Insulin release profiles of control and SUR-1–null islets in response to glucose, acetylcholine, the uncoupler carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP), and the respiration inhibitor Na-azide. *B*: Corresponding rates of respiration (OCR). The means of four experiments are given.

- Glucokinase is needed for amino acid and fatty acid stimulation of insulin release.
- Glucokinase permits the enhancement of insulin release by enteric hormones and the vagal transmitter acetylcholine.
- Glucokinase controls glucose stimulation of insulin biosynthesis and storage.
- Glucokinase mediates the growth-promoting effect of glucose.

To understand the role of glucokinase in β-cells, one needs to appreciate that the enzyme operates unchecked by feedback control and that it imparts a graded, sigmoidal concentration dependency on the primary metabolic effects of elevated glucose in the range of 0–25 mmol/l but that physiological cellular responses (in particular the elevation of cytosolic Ca^{2+} and GSIR) are threshold controlled at 5 mmol/l glucose. This transduction of graded-to-threshold–based processes is the result of the operation of glucokinase in tandem with the adenine nucleotide–controlled K channel and the voltage-regulated Ca channel, which all cooperate in a finely tuned manner. Resetting any one of the three essential parts of the system by physiological, pharmacological, or mutationally induced means may alter the threshold for GSIR. This physiological threshold lies at 5 mmol/l glucose, provided glucose is the sole stimulus, and is reached when glucokinase operates at 25–30% of its capacity under standard conditions. The threshold is significantly lower when glucose is used in combination with other fuels or neuroendocrine modifiers. Lowering the glucose $S_{0.5}$, the Hill number, increasing the k_{cat}, or enhancing glucokinase expression in β-cells leads to a left shift of the threshold and enlarged maximal response, whereas modifications in the opposite direction including an increase of the K_m for ATP cause a right shift and reduced magnitude of GSIR. Minimal mathematical modeling has been used to explore the prominent role of glucokinase in the triad of regulatory players (52). A clear relationship was found to exist between a measure of glucokinase activity (i.e., the glucokinase activity index: $(k_{cat}/S_{0.5}{}^{nH}) \times [2.5/(2.5 + \text{ATP } K_m)]$) and the threshold for GSIR (Fig. 6 and the paragraph below on glucokinase disease).

The regulation of glucokinase expression deserves special attention. It seems that the enzyme is expressed constitutively and minimally regulated in all glucokinase-based glucose sensor cells and that it is under the control of the neuroendocrine promoter of the gene, perhaps because of the high physiological impact of neuro-endocrine cells, particularly the pancreatic β-cells (13,14,16,17). In the β-cell, it is induced by supraphysiological glucose levels as much as five- to sevenfold from a capacity of ~60 to ~350 nmol · mg protein^{-1} · h^{-1} (53). Physiological changes of glucose concentrations from 5 to 7 mmol/l cause barely detectable increases of the enzyme, if any. This relative constancy of glucokinase is also apparent from fasting/refeeding transitions, which show practically no changes in glucokinase mRNA or activity (54). Controversy exists about the biochemical basis of this glucokinase induction by glucose. One view holds that the glucose effect is primarily posttranscriptional and results in part from glucose binding, which stabilizes the enzyme, decreases turnover, and elevates cellular contents (12,53,55). The other view states that the effect of glucose is indirect and is mediated by insulin via activation of the β-cell insulin receptor, which then signals augmented transcription (56). The controversy has not been resolved satisfactorily because it remains an unmet challenge to dissociate GSIR from glucose induction of glucokinase expression. The problem is however of fundamental significance for our understanding of the glucokinase glucose sensor function in health and disease. Whatever the biochemical basis, the glucose induction of glucokinase is very effective and shows sigmoidal glucose dependency with a glucose $S_{0.5}$ of ~8.0 mmol/l, as demonstrated in experiments with isolated cultured rat islets (53). Under these experimental conditions, media insulin is very high, perhaps hyperphysiologic (between 300 and 3,000 ng/ml). Exogenous insulin added at levels from 250 to 2,500 ng/ml does not cause induction in the absence of glucose even though ample

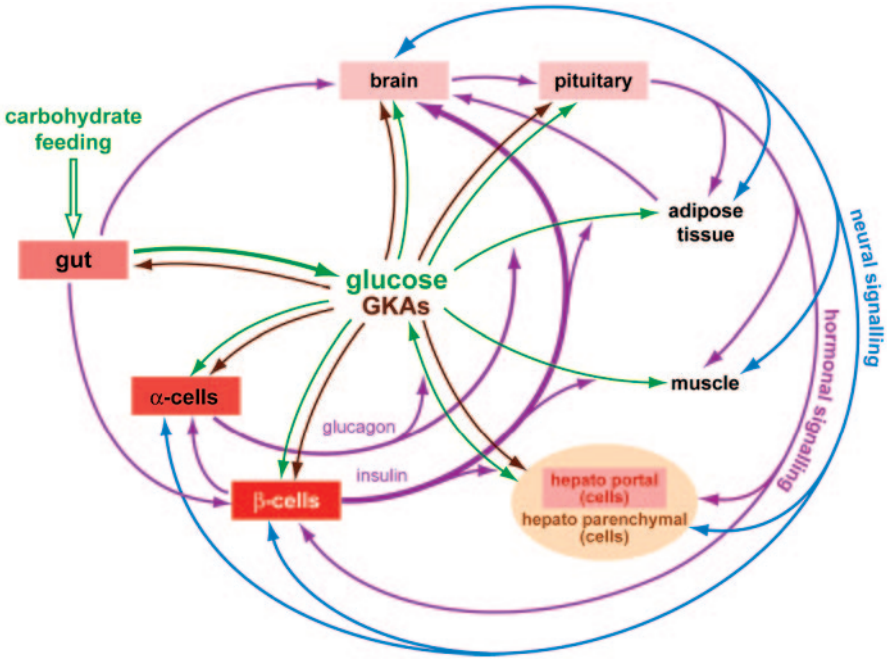

FIG. 4. Glucose and GKA actions on network of sentinel glucokinase cells and parenchymal hepatocytes in glucose homeostasis. Glucose alone or enhanced by GKAs act on glucokinase in a wide variety of glucose sensor cells, which results in secondary signaling throughout the network via hormones or the autonomic nervous system impacting on glucose homeostasis. GKAs and glucose also affect the metabolism of hepato-parenchymal cells, resulting in enhanced clearance of blood glucose and glycogen synthesis. It should be appreciated that glucose or GKAs may result in stimulatory or inhibitory effects, directly or indirectly. For example, they stimulate β-cells but inhibit α-cells, directly or indirectly. For reasons of clarity, ± signs indicating such actions are omitted.

fuel is present (i.e., glutamine and leucine at 10 mmol/l each). Furthermore, using similar conditions, glucokinase may be induced by mannoheptulose, a competitive inhibitor that is not metabolized, alone or in combination with glucose, which speaks against a critical role of metabolism and insulin release in the induction process (53). This induction can occur without demonstrable changes of the glucokinase mRNA levels. Still, these experiments do not eliminate a physiological process in glucose induction of the enzyme, which might be dependent on or might be facilitated by insulin, but they demonstrate the basic principle that the enzyme can be induced very effectively by the mere binding of a nonmetabolizable analogue to the glucose binding site without concomitant enhancement of glucose metabolism. There is also some evidence in the literature that induction may be facilitated by various factors including prolactin, cAMP-dependent agonists, retinoic acid, and biotin (57). The brief account indicates that much work needs to be done to develop a clear understanding of this cardinal problem of β-cell biochemistry and regulation.

Changes of glucokinase induction are probably significant when the β-cell adapts to altered mean blood glucose levels during extended exposure as it occurs in diabetes or hypoglycemia. Enzyme induction may compensate, in part at least, for defective β-cell function in diabetes, and persistent hypoglycemia would probably downregulate the glucose sensor. Such changes, although speculative at this point, may be of relevance for the new therapeutic approach proposing the use of glucokinase activator drugs (see below).

THE NETWORK OF SENTINEL GLUCOKINASE GLUCOSE SENSOR CELLS

There are many known glucokinase-containing cell and tissue types (e.g., hepato-parenchymal and hepato-portal cells, pancreatic α- and β-cells, entero-endocrine K- and L-cells, pituitary cells, and neurons of various hypothalamic nuclei, of the tractus solitarious of the vagus, and of raphe nuclei), and it is hypothesized that they operate as a network that has the purpose of fine tuning glucose homeostasis (Fig. 4) (15,16,19,20). It is also hypothesized that the pancreatic β-cells serve as models for glucose sensor cells activated by glucose and that the presence of the triad of glucokinase, ATP/ADP-controlled K channels, and voltage-gated L-type Ca channels is an essential requirement to qualify for inclusion in this category. The presence of Glut-2, the glucose transporter found in β-cells and hepatocytes, should, however, not be considered as an indicator to qualify as a glucose sensor cell. Based on these considerations, it can be argued that the hepatic parenchymal cells that, parenthetically, contain at least 99% of the body's glucokinase complement do not actually participate directly in this signaling network. In fact, there is compelling evidence from physiological studies with conscious dogs and work with knockout and transgenic mice that specific glucose sensor cells of unknown identity exist in the hepato-portal vasculature, project to the central nervous system through vagal afferents, and modify metabolism via the automomic nervous system (58). The proposed role of glucokinase as glucose sensor of entero-endocrine, pancreatic α, and pituitary cells also remains to be fully established. In some of these cases (e.g., pancreatic α cells and certain hypothalamic neurons), glucose inhibits cellular function, and a mechanism differing from that accepted to operate in glucose sensing of pancreatic β-cells has to be postulated. For example, enhanced glucose metabolism might enhance an electrogenic Na^+ pump or augment an hyperpolarizing K^+ current, to mention two possibilities. Whatever the case may be, it is required that enzymatic activity of glucokinase be demonstrated in candidate cells because routine mRNA or immunochemical measurements could be misleading. Recent measurements of pituitary extracts are shown here as an example, where earlier studies had concluded that glucokinase activity was absent (15,59–61) despite glucokinase gene expression in anterior pituicytes, principally corticotrophes (Fig. 5). The ability to precisely determine the characteristic kinetic constants and to show responsiveness to specific glucokinase activators were critical for making a convincing case that pituitary cells do indeed contain significant levels of functional enzyme. Although ACTH-expressing

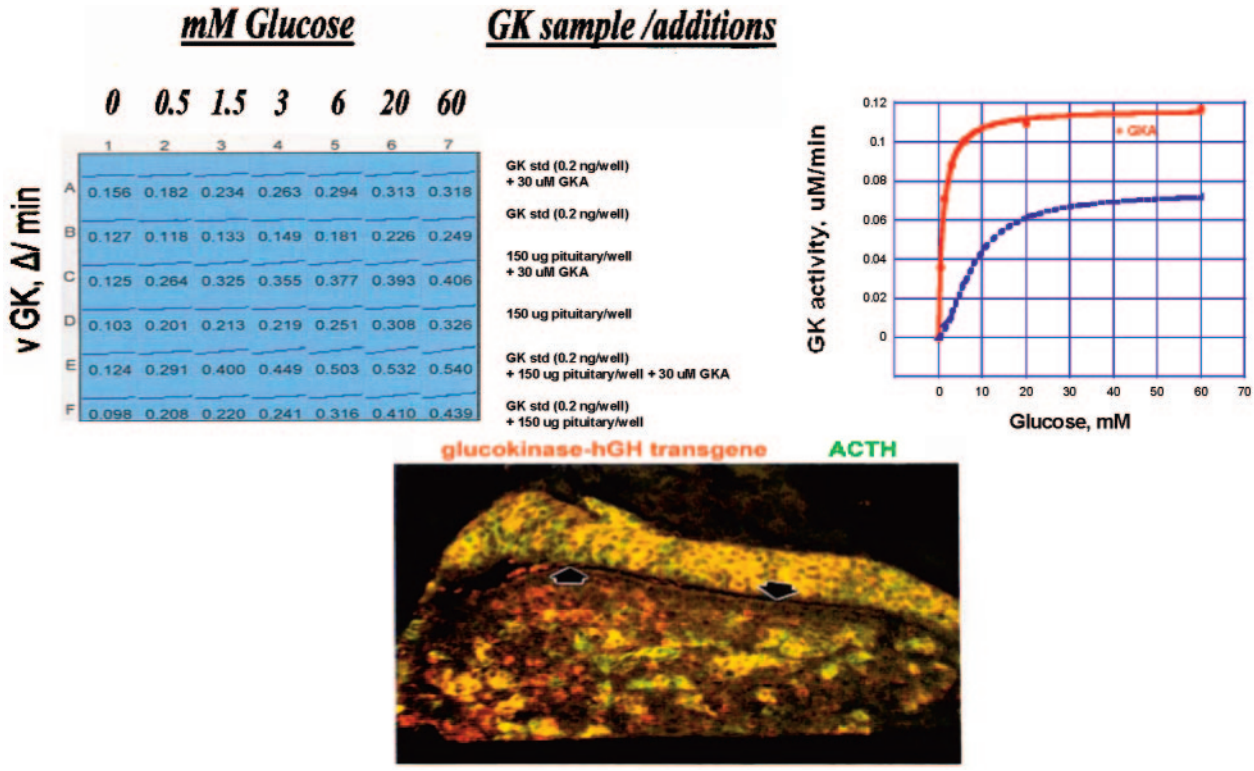

FIG. 5. Demonstration of functional glucokinase in rat pituitary extract. The results of a typical multiwell plate reader essay of glucokinase in pituitary extracts is shown. The left panel records the progress curves of NADH formation with recombinant glucokinase standards, the pituitary sample, and a combination of the two (to assess recovery or interference by the crude extract), both in the presence and absence of GKA (to ascertain that glucokinase is being measured). The glucose concentrations ranged from 0 to 60 mmol/l. The *right panel* shows the glucose concentration dependencies in the presence and absence of GKA. Note the sigmoidal shape of the curve in the absence of activator. The *lower panel* shows a representative transgenic mouse pituitary gland midsagittal section stained for a glucokinase transgene (glucokinase-hGH; orange) and ACTH (green) to mark corticotrophes. Arrows point to the cleft between intermediate (*top*) and anterior (*bottom*) lobe. Costaining cells emit a yellowish color. The *lower panel* represents a different example of a previously published result (15). Note added in proof: Immunohistochemical studies have since shown that corticotrophes do not contain glucokinase (F.M.M., M.A.M., D.Z., T.L.J., N.D., Y.H., R.T., J.G., unpublished data). The identity of glucokinase-positive pituitary cells remains to be determined.

cells appear to be the predominant glucokinase-expressing cells in the pituitary, it remains to be established which other cell types may also express glucokinase and what the physiological implications might be. Then, there is the nature of glucose transport of these cells to be considered. It must be appreciated however that any high-capacity glucose transport process would suffice because glucokinase can serve as a glucose sensor in cells that have intracellular glucose in the low range of 2–3 mmol/l approaching blood glucose levels and exhibiting rapid equilibration between outside and inside water spaces. It remains to be demonstrated for the majority of these cells that this requirement is fulfilled. However, it has been shown that the pancreatic α-cells that lack Glut-2 maintain intracellular glucose between 1/2 and 2/3 of the serum level, sufficient to engage glucokinase as glucose sensor (62). Pituitary cells seem to be devoid of Glut-2 (59,61), and the nature of glucose transport into K- and L-cells appears to be insufficiently characterized (as far as these authors can tell). Finally, glucokinase-positive neurons may or may not have Glut-2 but show other critical features that indicate their involvement in glucose recognition and signaling (19,20). In trying to develop a sense of the overall biological importance of this hypothetical glucokinase-based sentinel glucose sensor system, one should remember that patients with permanent diabetes due to total glucokinase deficiency and thus lacking functional glucose sensor cells as here defined can be controlled with insulin treatment equally well as type 1 diabetic subjects who do retain their extrapancreatic glucokinase-based glucose sensor system. It seems then that the role of this network in glucose homeostasis is a subtle one, even though it may prove to be highly important biologically. The diagram of Fig. 4 and the concepts it represents should be considered as a framework for thought and future experimentation regarding this crucial subject matter.

The hepato-parenchymal cells, where the bulk of the body's glucokinase resides, deserve additional comment. Glucokinase is critical for the operation of the direct pathway of glycogen synthesis in the liver. Its expression is transcriptionally controlled primarily by insulin, as indicated by a marked decline of glucokinase mRNA and protein in starvation and diabetes (17,18,54). Hepatocyte activity is also regulated on a short-term basis by GKRP, which is inhibitory and resides in the nucleus of the hepatocyte (34,63). GKRP complexes with glucokinase and sequesters the enzyme in the nucleus, a process facilitated by fructose-6-P and counteracted by fructose-1-P and physiological levels of glucose. Enhanced glucose phosphorylation also results in a reduction of net hepatic glucose output, in part explained by elevated levels of fructose-2,6-P_2. The significance of hepatic glucokinase for glucose homeostasis is illustrated by the hypoglycemic effect of organ-specific overexpression of the enzyme (28). Hepatic parenchymal glucokinase is thus part of a high-capacity glucose disposal system contrasting with the low-capacity regulatory glucose sensor system based on glucokinase. The high-capacity hepato-parenchymal cells contain, in addition to high levels of glucokinase, the

entire enzymatic machinery for glycogen synthesis and mobilization including glucose-6-phospatase and the glucose transporter Glut-2. It is also noteworthy that glucokinase expression is tightly regulated by insulin. In contrast, low-capacity glucokinase-expressing neuro-endocrine cells appear to store little glycogen, and glucose uptake may be mediated by transporters other than Glut-2. Furthermore, glucokinase expression appears to be constitutive and minimally regulated by hormones. It is almost certain that this distinction between the two cell types will be critical for understanding the pharmacology of glucokinase activator drugs to be discussed below.

GLUCOKINASE-LINKED HYPO- AND HYPERGLYCEMIA SYNDROMES

In view of the preeminent role of glucokinase in the regulatory glucose sensor system and the hepato-parenchymal high-capacity glucose disposal system, it is perhaps not surprising that mutations of the glucokinase gene have a profound influence on glucose homeostasis in humans as predicted far in advance of the actual discovery of such mutants. Early linkage studies published in 1992 by Froguel et al. (21) and by Hattersley et al. (22) demonstrated that certain autosomal dominant cases of MODY were associated with the glucokinase gene. Somewhere in excess of 200 glucokinase mutations have subsequently been discovered that lead to activation or inactivation of the enzyme and cause hypo- and hyperglycemia syndromes, respectively. These mutations are collectively described as glucokinase disease (23,42,43,52,64). A mild form of hyperglycemia is the result when only one allele is affected by an inactivating mutation (MODY-2), but severe life-threatening permanent diabetes of the newborn ensues when two alleles with inhibitory mutations are involved. An activating mutation affecting a single allele is sufficient to cause severe hypoglycemia. Note that cases with homozygous or compound heterozygous activating mutations have not yet been found. The systematic biochemical analysis of ~50 different recombinant mutant enzymes has resulted in a clear molecular understanding of glucokinase disease (52). It is fortunate and of great practical advantage that the 75-kD recombinant glutathione S-transferase–glucokinase fusion protein of wild-type glucokinase is kinetically indistinguishable from the purified native 50-kD enzyme, allowing the comprehensive characterization of a critically large number of such mutant enzymes (based on the reasonable assumption that the kinetics and biophysics of the mutant enzymes are also not modified by the glutathione S-transferase attachment). The biochemical analysis uncovered many molecular defects in mutated enzymes: the k_{cat}, glucose $S_{0.5}$, ATP K_m, Hill number, responsiveness to GKRP, and the newly discovered GKAs (see below), and thermal stability of the protein were found to be altered in isolation or, as was more often the case, in combination. The biochemical data were then used for mathematical modeling studies (52). The modeling is based on a few plausible assumptions about β-cell glucose metabolism and metabolic coupling and allows a correlation to be made between a predicted threshold for GSIR and the glucokinase activity index defined above. It is critical in this modeling to correct for altered glucokinase expression governed by ambient glucose levels, which explains why most MODY-2 patients have an average fasting blood glucose of ~7 mmol/l (by definition equivalent to the threshold for GSIR) instead of 10–12 mmol/l, which is predicted to be seen when enzyme induction is discounted (52). Details of the modeling procedure and practical examples can be found in the literature. Figure 6 demonstrates the marked impact of glucokinase induction on the threshold for GSIR.

These biochemical genetic studies of glucokinase disease were also instrumental in the discovery of a hitherto unknown allosteric activator site of the enzyme (42,43,52,64,65). The mutants V62M, T65I, W99R, Y214C, V455M, and A456V (also a very recently recognized insertion of alanine at position 454 [66]) all activate the enzyme by influencing the kinetic constants such that the relative activity index rises significantly above unity, which then explains the lowering of the threshold for GSIR in these hypoglycemia cases (Fig. 6). It is striking that all these mutants are clustered in a clearly confined region of the enzyme's structure distal from the substrate binding site by ~20 angstroms (Figs. 1 and 7). The existence of an allosteric activator site suggests that an endogenous activator might be present in certain or all glucokinase-positive cells to regulate the enzyme (See below about the fact that glucokinase activator drugs bind to the same site.) It can be expected that the painstaking biochemical genetic analysis of mutants that cause glucokinase disease will continue to augment our understanding of this important molecule. A striking example of such studies has recently been published in the case of the V62M mutation, which is paradoxically activating (relative activity index >2.50) but causes MODY (67). The enzyme was refractory to inhibition by GKRP and activation by glucokinase activators and showed moderate thermal instablity. The data were taken as evidence that diabetes developed because of defective regulation of glucokinase. In the course of studying large numbers of mutants, we also found a significant percentage of enzymes that showed no or apparently inconsequential changes so that an explanation of the clinical phenotype of the index cases remains elusive (e.g., A53S, G264S, R275C, and V367M) (52). These mutants may offer new opportunities to discover glucokinase functions not addressed by the array of laboratory tests currently applied. One possible way to explore the underlying molecular defect that alters glucose homeostasis in such cases is to use these rare mutant genes as total replacement for the normal gene of the mouse and test the effect of this manipulation on glucose homeostasis of the intact animal. Proof of principle of this approach has been accomplished by generating mice that are heterozygous for E414K (a MODY mutant) or A456V (a PHHI mutant). The animals were hyper- or hypoglycemic as expected, and the glucose thresholds for GSIR were shifted in the predicted manner when assessed with isolated perifused pancreatic islets (31).

GKAs HOLD SIGNIFICANT PROMISE FOR A NEW DIABETES THERAPY

The potential of glucokinase as a drug target was recognized early on by the lead author and researchers at Hoffmann-La Roche, and in the early 1990s the company embarked on a drug discovery program to explore this opportunity. The strategy was to find an antagonist to the physiological inhibitor GKRP to indirectly activate glucokinase. (In effect, a search was made for a fructose-1-P mimetic.) The project resulted in the discovery of a lead compound (a substituted phenylacetic acid derivative) that directly activated the enzyme while also counteract-

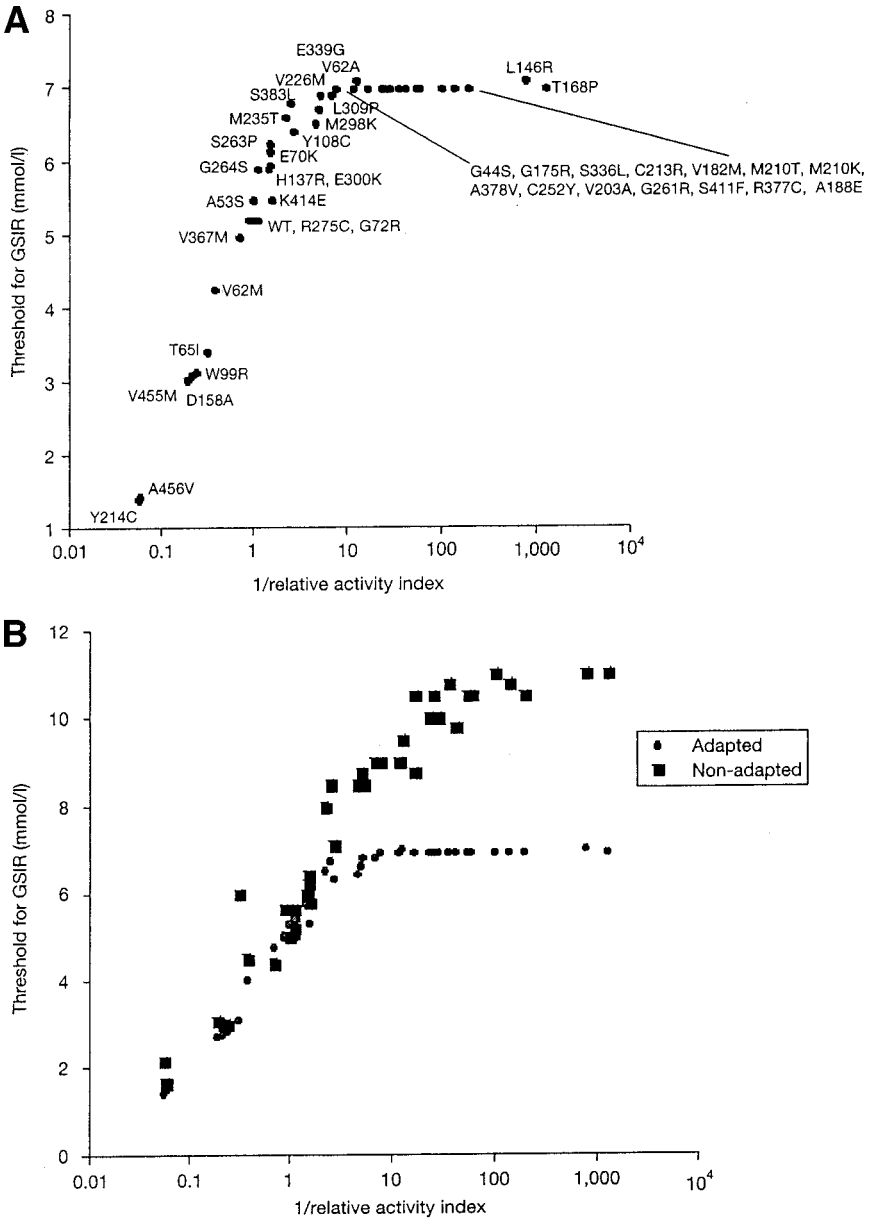

FIG. 6. The threshold for GSIR as a function of the glucokinase activity index (results of modeling studies). *A*: Results of modeling that predicts the thresholds for glucose-stimulated insulin release (equivalent to the fasting blood glucose) of patients with glucokinase disease as a function of the activity index of many activating and inactivating mutants of glucokinase. Note that only one allele is affected in this selection of cases. The glucose threshold of the wild-type (WT) control is by definition 5 mmol/l. *B*: Impact that adaptation of β-cell glucokinase on the glucose threshold in this series of cases (with permission from [52]). The terms "adaptation" or "adapted" are used here in a very special sense and refer to the phenomenon of altered expression of glucokinase as a function of the fasting blood glucose explained by posttranslational mechanisms independent of glucose metabolism (52). This "adaptive" change is entirely restricted to the glucokinase molecule, but the two alleles are affected differentially as a result of mutations. β-Cell glucose metabolism is therefore practically indistinguishable at the thresholds for GSIR for all these cases even though threshold glucose levels have a wide range between 1.5 and 7.0 mmol/l. This figure is reproduced, with permission, from an article by Gloyn et al (52).

ing GKRP inhibition (24,25,68–70). Chemical optimization resulted in a series of molecules, termed glucokinase activators (or GKAs), that lower the glucose $S_{0.5}$ and increase the k_{cat} of normal and certain mutant glucokinases (Fig. 7*B*). At high drug concentrations, the Hill number may be decreased, and at low glucose (less than the $S_{0.5}$) the ATP K_m is increased. GKAs also increase glucose stabilization of the enzyme in a thermo-lability test. As might be predicted, it was found that GKAs enhanced the effect of glucose on insulin release from isolated pancreatic islets of rodents and humans resulting in a left shift of the glucose concentration dependency curve owing to the stimulation of glucose metabolism. GKAs enhance glucose induction of pancreatic islet glucokinase as shown in isolated cultured rat islets. In the intact animal, GKAs also stimulated insulin release and were found to enhance glycogen synthesis and to curb hepatic glucose production in pancreatic clamp studies. Consequently, GKAs lowered the blood glucose in normal rodents in a dose-dependent manner. GKAs also lowered or normalized blood glucose in various animal models of type 2 diabetes. They furthermore prevented the development of diabetes during diet-induced obesity in mice. These basic findings are consistent with our understanding of the role that glucokinase has in glucose homeostasis, as discussed in detail in this essay. The availability of the GKAs greatly facilitated the crystallization of the enzyme (24,26,27,71). It was discovered by crystallography that in the presence of glucose, the drug binds to a site that is separate from the substrate binding site and involves amino acids that were found to be mutated in cases of glucokinase-linked hypoglycemia. The following amino acids were identified to form the drug binding site: V62, R63, M210, I211, Y214, Y215, M235, V452, and M455 (Fig. 7*A*). Amino acid substitutions at these sites, including double and triple mutations at several of these locations that are frequently activating by themselves, lower the efficacy or totally block the action of GKAs (Fig. 7*C*). These compelling results of pharmacological and structural studies have enticed many pharmaceutical firms to launch intensive drug discovery programs to test and exploit the potential of this new diabetes treatment para-

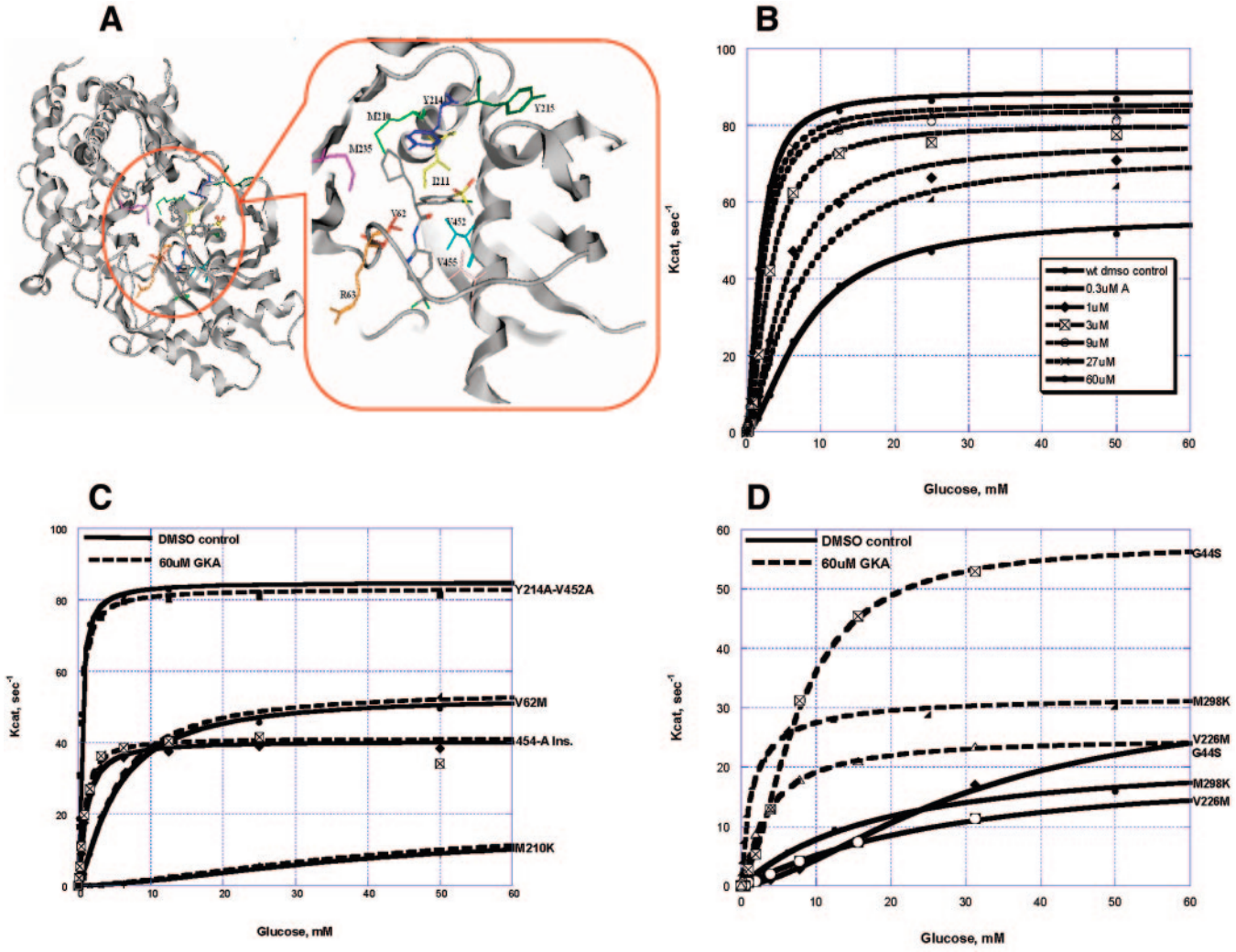

FIG. 7. Structure of allosteric GKA binding site of glucokinase and examples of GKA effects on normal and mutant enzymes. *A*: An impression of the structure of the allosteric activator site of glucokinase and contact amino acids for GKAs. *B*: Concentration-dependent effect of a GKA on glucokinase activity as a function of glucose in the essay. Note that the relative effect of the activator is highest at the lowest glucose concentrations. C: Examples of mutants that are refractory to the action of GKAs. *D*: Effectiveness of GKAs in reversing the inhibition resulting from inactivating mutations.

digm. Such a broad involvement of industry is fortunate for science and medicine and promises to generate a wealth of new insights. The challenges to be faced on the way to produce an effective drug for diabetic individuals are undoubtedly formidable, and it remains to be seen whether GKAs are beneficial for diabetic individuals in the long run. Possible problems that might arise from pursuing this therapeutic strategy have been pointed out but are not discussed here (72). It seemed more important for this perspective to speculate how the complexity of the multicomponent glucose sensor system as discussed above might manifest itself when GKAs are eventually used in the treatment of diabetes. The analysis of this perspective led to the distinction of low-capacity regulatory and high-capacity disposal components in glucokinase-dependent glucose metabolism. It is predicted here that simultaneous activation of the various parts of the regulatory component with GKAs will result in an increase of insulin release, a decreased secretion of counterregulatory hormones, and a modification of the automomic nervous system with its wide-ranging peripheral effects (Fig. 4). These multiple actions will synergize with the direct stimulation of the high-capacity hepato-parenchymal glucokinase component to enhance glucose clearing from the blood and curb glucose production. It is postulated that GKAs would thereby reactivate a broad array of physiological mechanisms geared to maintain glucose homeostasis but defective in the diabetic organism.

FUTURE OUTLOOK

This brief 2005 perspective about the role of glucokinase in glucose homeostasis provides also an indication on what can be expected from future research on this topic. Front and center will be results on clinical trials using GKAs in the treatment of diabetes in humans. Second, interest in the biochemical genetic characterization of activating and inactivating mutants causing glucokinase disease will be maintained at a high level. Third, physiological exploration of glucokinase-expressing glucose sensor cells utilizing GKAs to enhance detection limits will continue vigorously. Fourth, crystallography and other biophysical approaches will contribute to a deeper understanding of the glucokinase molecule itself. It is certain that industry and academia will continue their active

partnership in glucokinase related studies as one of the highly promising endeavors of current diabetes research.

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